Vol. 1. No. 1. MARCH 1946

BRITISH JOURNAL OF PHARMACOLOGY AND CHEMOTHERAPY

EDITED FOR

THE BRITISH PHARMACOLOGICAL SOCIETY

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In co-operation with the EDITOR of the British Medical Journal

LONDON

BRITISH MEDICAL ASSOCIATION

TAVISTOCK SQUARE, W.C.1

YEARLY SUBSCRIPTION (4 NUMBERS) 25s. U.S.A. $6.00 SINGLE NUMBER 7s. 6d.

FOREWORD

The first issue of a *British Journal of Pharmacology and Chemotherapy* is a notable event. It is not, of course, to be supposed that the growing volume and importance of pharmacological publication which have justified it, which, indeed, have created the need for it, represent a movement which is special to Britain ; the phenomenon is to be seen throughout the scientific world. Pharmacology, we should recognize, has rapidly risen to major rank among the group of scientific disciplines which come within the scope of experimental medicine. This promotion has obviously been accelerated by the growing influence in practical therapeutics which Pharmacology has acquired through the recent development of its vigorous offspring, Chemotherapy—a fact fittingly recognized in the new Journal's title.

The event is one which naturally revives memories of the foundation, some 37 years ago, of the *Journal of Pharmacology and Experimental Therapeutics* by the late J. J. Abel, whose leadership and personal inspiration had already done so much to establish a vigorous school of Pharmacology in the United States of America. At that time, though Scotland had Chairs of Pharmacology at Edinburgh, Glasgow, Aberdeen and Dundee, with distinguished incumbents, England had, so far, recognized a need for only one full-time Chair, which had been created four years earlier at University College, London. A. R. Cushny, who had been a pupil of Cash at Aberdeen, and later of Schmiedeberg, and had been Abel's successor at Ann Arbor, Michigan, had come back in 1905 to be the first holder of the new Chair at University College. W. E. Dixon, though still holding a position of minor academic rank, was then already creating a centre of lively interest and experimental activity in Pharmacology at Cambridge, and R. B. Wilde, though largely occupied in clinical work, was giving a regular course in Pharmacology from a Chair in Liverpool. For the rest of England, those who found their way into Pharmacology had done so largely by natural interest and their own unguided exploration.

To all these the launching of the *Journal of Pharmacology* by Abel had provided a much-needed outlet for pharmacological papers in the English

language. The earlier volumes of that Journal bear witness to the fact that, from the outset, it offered effective hospitality to papers by British workers. Their opportunities of publication had otherwise been almost limited to the weekly medical journals and the *Journal of Physiology*; and the editor of the latter, the late J. N. Langley, was showing a steadily increasing reluctance to accept papers which could be regarded as pharmacological. I well remember going in 1911 to consult Cushny about a difficulty created for me by Langley's refusal of a paper of mine, for the reason that Pharmacology was encroaching unduly on his space. I suggested to Cushny that, while we were all grateful to Abel's Journal for what it was already doing for us, we should feel happier if, though still published at Baltimore, it could be recognized as having a wider, English-speaking interest and editorial responsibility. The upshot was a friendly negotiation leading to a joint editorial control of the Journal by Abel and Cushny, assisted by advisers of whom several were now to be British. The arrangement lasted in that form till Cushny's premature death in 1926, and then, with successors in due course to Abel as well as to Cushny, till the present time. It has served British Pharmacology well, and we have abundant reason to be grateful to our American colleagues for these 35 years of association and shared responsibility. We may regard it, perhaps, as an early, spontaneous and limited example of that wider collaboration between the scientists of the English-speaking nations which in the recent war became so intimate and so efficiently organized.

On the maintenance of that full and friendly collaboration in science much may depend for the future of the world, and we must hope to make it even stronger and closer in Pharmacology than it has been. On the other hand, it is necessary to watch developments which the demands of war itself have accelerated, and to recognize the likelihood of a rapidly and healthily growing demand for space to publish papers dealing with Pharmacology and Chemotherapy. On both sides of the Atlantic the intensive and organized researches of the war period must have caused a serious accumulation of such matter. Pharmacologists, too, of countries which have suffered aggression and spoliation in war may well be seeking opportunity for publication, of which their own countries cannot yet offer a prospect. It would not be fair to expect a single journal to cope with these heavy arrears, as well as with the expanded output which the wartime development is certain to leave as a lasting consequence, and, at the same time, to ensure promptitude of publication for important new discoveries in this widening field, from Britain and British Dominions as well as from the United States of America. The British Pharmacological Society, which had come into existence long after Abel's *Journal of Pharmacology* was founded, accordingly reviewed the position and came to the conclusion that they could best serve the common interest, and best show their lasting gratitude for the friendly help and collaboration they have received from their American colleagues, by starting a separate Journal, edited and published in Britain, and thus lightening

the prospective load on the one in which they have, for so many years, been generously allowed to have a share of interest and control. The British Medical Association has offered to sponsor the new *British Journal of Pharmacology* and to be responsible for the technical aspects of its publication, leaving the editorial responsibility entirely in the hands of a Board appointed by the British Pharmacological Society. It is certainly the hope of everybody concerned with the venture, that there will still be abundant opportunities of co-operation and friendly interchange between the new British Journal and the *Journal of Pharmacology and Experimental Therapeutics*, which has so long and so well served British as well as American achievements in this field.

March 12, 1946. H. H. D.

Brit. J. Pharmacol. (1946), **1**, 4–14.

ANALGESIC ACTION OF PETHIDINE DERIVATIVES AND RELATED COMPOUNDS

BY

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(Received August 17, 1945)

Comprehensive and up-to-date reviews on the actions and evaluation of analgesics have been published in America by Small, Eddy, Mosettig and Himmelsbach (1938), and by the Committee on Drug Addiction under the chairmanship of White (1941). Fourneau (1938) has discussed much of the older work on the relationship of chemical constitution to analgesic efficiency. Schaumann (1940) has reported on the synthetic compounds derived from 4-phenyl-piperidines synthesized by Eisleb (1941), and by his analysis has illuminated our conceptions of the structural essentials for analgesic activity. He concluded that of the compounds he examined an optimum was reached in ethyl-4-phenyl-l-methyl-piperidine-4-carboxylate hydrochloride (pethidine, demerol, dolantin)—a compound which has received the recognition of an approved name, pethidine hydrochloride, in the 7th Addendum to the *British Pharmacopoeia* (1944). Woolfe and Macdonald (1944) have attempted an evaluation of the analgesic activity of this drug, using a simple technique for the measurement of such action in mice. Pethidine already has an extensive clinical literature, and there is reasonable agreement between estimates of its efficiency, relative to other drugs, on mice and men.

This paper describes the application of the experimental technique to a large number of pethidine derivatives and related compounds, most of which have been synthesized for the first time by Bergel *et al.* (1944). Very few of these have been investigated by Schaumann. While no immediate claim for clinical importance can be made for any of the active compounds, except perhaps for *iso*-pethidine (C 21) (cf. Glazebrook and Branwood, 1945), our pharmacological experience

with them adds to the confidence with which one can attempt to relate constitution to analgesic activity.

EXPERIMENTAL

In our studies of these drugs, analgesic activity was assessed by a hot-plate technique (see Woolfe and Macdonald, 1944). The mouse under observation was placed on a smooth metal surface kept at 55° C. for 30 secs., or less if it appeared to suffer discomfort as indicated by movements of the hind-limbs during such exposure. In the absence of obvious hind-limb movement, it was assumed that analgesia had been obtained.

All drugs were given hypodermically, and all were tested against one sample of pethidine hydrochloride to determine relative activity, using mice from one batch, of approximately the same age and weight, in each comparison. The compounds were usually supplied and tested as hydrochlorides, but some were bases and were dissolved in a minimum of hydrochloric acid. A few other salts were occasionally used and such are indicated in the tables. None was written off as inactive on fewer than eight mice, and all showing analgesic activity were tested on not less than thirty animals. For some, over one hundred mice were used. The figure given for the relative activity was obtained from the comparison of the weights of the drugs, calculated as base, giving freedom from obvious discomfort lasting for 30 mins. An illustrative experiment on the stereoisomers of nor-*iso*-pethidine is summarized in the protocol.

PROTOCOL: NOR-*iso*-PETHIDINE ISOMERS

(In this batch of mice, the figure for the dose of pethidine base required to produce analgesia for 30 min. was 30 mg./kg.)

Optical Form	Dose (mg./kg.)	Proportion of Animals in which Analgesia occurred	Average Duration of Analgesia (A)	Standard Deviation of (A)	Dose to produce Analgesia lasting 30 min.	Analgesic Activity (Pethidine = 1)
dl. C. 23	50	5/10	17·6	8·0	123	1/4
	100	7/10	28·9	8·8		
	150	7/10	31·6	9·7		
l. C. 24	30·4	3/10	—		58	1/2
	45·6	5/10	26·4	9·9		
	60·8	6/10	30·9	13·8		
	76·0	6/10	36·8	15·1		
	91·2	7/10	40·3	14·7		
d. C. 25	60·8	0/10	—		—	0
	91·2	2/10	Convulsions in 1/10			
	121·6	4/10	Convulsions in 5/10		—	

RESULTS

(1) *Certain Salts of Pethidine* (Table I).—It will be seen that the hydrochloride is reasonably stable for six months in aqueous solution (C 1a). The hydriodide (C 2) is equally effective, though less soluble.

TABLE I

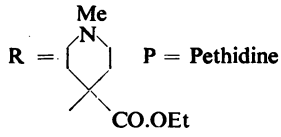
Compound	Name	Relative Activity (Pethidine = 1)	Remarks
C 1	Pethidine hydrochloride (Formula II, p. 12)	1	LD_{50} : 250 mg./kg.
C 1a	The same after 6 months in solution in ampoules	1	Reasonably stable
C 2	Pethidine hydriodide	1	,, ,,

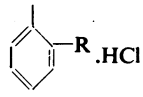
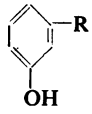
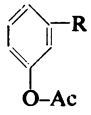
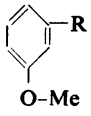
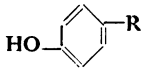
(2) *Variations in the Phenyl Ring of Pethidine* (Table II).—Our observations on over 150 animals indicated that ethyl 4-(*o*-tolyl)-1-methylpiperidine-4-carboxylate or 2′-methyl-pethidine, as hydrochloride (C 3), is a more potent and longer-lasting analgesic than pethidine itself, although as hydriodide or tartrate (C 4, C 5) its activity is of the order of that of pethidine hydrochloride.

Ethyl 4-(3′-hydroxyphenyl)-1-methylpiperidine-4-carboxylate or 3′-hydroxy-pethidine (C 6) we found substantially equal to pethidine itself. Constitutionally, it is nearer to morphine. The corresponding acetoxy-compound (C 7) also had activity of the same order. This is in agreement with Fourneau's view of the uselessness of acetylation of the phenolic hydroxyl. The methoxy- (C 8) had less activity than the hydroxy-compound (C 6), which shows a relationship similar to that of codeine to morphine and heroin. The greater stability of the methoxy-group to hydrolysis *in vivo* as compared with that of the acetyl-group would account for this phenomenon. Schaumann reported that the corresponding 4′-hydroxy-compound (C 9) had only one-fifth of the activity of pethidine, which we have confirmed. We did not examine other 4′-substituted derivatives.

(3) *Variations in the Ester Group* (Table III).—It is clear that the alcohol component of the ester group profoundly affects activity. In sub-lethal doses, the methyl- (C 11), butyl-, cyclohexyl- and glycol-esters and the amides showed no significant analgesic activity. Some of these observations confirm those of Schaumann (1940). *All* deviations from the ethyl-ester group which we have tried resulted in considerable loss of analgesic value. Moderate activity is retained in the *iso*-propyl- (C 12) and allyl-ester (C 13), but even less in the *n*-propyl-ester (C 14). Although the ketones (C 15, 16, 17), as shown by Schaumann, are quite active, the secondary alcohol (C 18) obtained by reduction of the methyl ketone (C 15) and its O-acetyl derivative showed no analgesic action in doses up to 80 mg./kg.

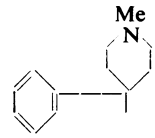
TABLE II



Compound No.	Formula	Name	Relative Activity (Pethidine = 1)	Remarks
C 3		2′–methyl–P.	1½	Rapidly loses activity on standing at room temperature at *p*H 5·4. LD_{50} = 200 mg./kg.
C 4	Same, as hydriodide		1	Retains activity for 5 days + in solution. In higher doses shows more prolonged action than corresponding doses of pethidine (C 1)
C 5	Same, as tartrate		1	,, ,,
C 6		3′–hydroxy–P.	1	
C 7		3′–acetoxy–P.	1	
C 8		3′–methoxy–P.	1/2	
C 9		4′–hydroxy–P.	1/5	(Confirms Schaumann)

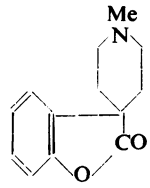
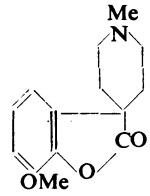
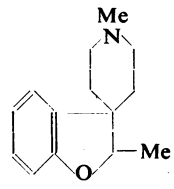
(4) *Combination of Features of 2 and 3* (Table IV).—The lactone of 4-(2′-hydroxyphenyl)-1-methylpiperidine-4-carboxylic acid (C 19) acts weakly. The corresponding 3′-methoxy-lactone (C 19a) in doses up to 120 mg./kg. and the related 2-methyl-3-4′-spiro-(l′-methylpiperidine)-coumaran (C 20) up to 80 mg./kg. showed no analgesic activity, in spite of the fact that they possess additional features of the morphine nucleus.

TABLE III

R′ = 

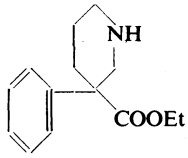
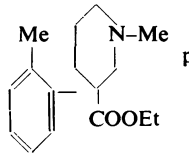
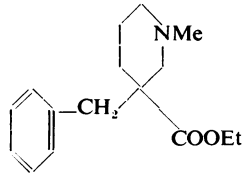
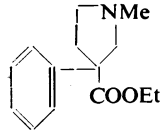
Compound No.	Formula	Relative Activity (Pethidine = 1)	Remarks
C 10	R′.CO_2H	0	Tested at 160 mg./kg.
C 11	R′.CO_2Me	<1/6	Depressant
C 12	R′.CO_2CHMe_2	1/2	Higher doses produce great depression, including that of respiration
C 13	R′.$CO_2CH_2.CH:CH_2$	1/2	
C 14	R′.CO_2.Pr.	1/3	Extreme depression
C 15	R′.CO.Me	1/3	
C 16	R′.CO.Et	1/3	
C 17	R′.CO.Pr	1/2	Excitement followed by depression
C 18	R′.CHOH.Me (and R′.CH(OAc)Me)	0	Tested at 80 mg./kg.

TABLE IV

Compound No.	Formula	Relative Activity (Pethidine = 1)	Remarks
C 19		1/4	Active only in rather toxic doses
C 19a		0	Tested at 120 mg./kg.
C 20		0	Tested at 80 mg./kg.

(5) *3-Phenyl-piperidine or* iso-*pethidine Series* (Table V).—Ethyl-3-phenyl-1-methylpiperidine-3-carboxylate or *iso*-pethidine (C 21) showed pronounced and prolonged though somewhat irregular activity. As all these compounds were synthesized as racemates, it seemed desirable to investigate some of the pure optical isomers. In the case of nor-*iso*-pethidine (C 22), it seems that pharmaco-

TABLE V

Compound No.	Formula	Relative Activity (Pethidine = 1)	Remarks
C 21	*Iso*–pethidine as hydrochloride (Formula III, p. 12)	1/2	Irregular; long-lasting in some, little or none in others
C 21a	Same, as tartrate	1/2	
C 21b	Tartrate after four months' storage in solution	1/2	
C 22	 dl–nor–*iso*–pethidine in hydrochloric acid solution	1/4	Rather irregular and rather toxic
C 23	Same, in tartaric acid solution	1/4	,, ,,
C 24	*l*–nor–*iso*–pethidine acid tartrate	1/2	Lethal at 200 mg./kg.
C 25	*d*–nor–*iso*–pethidine acid tartrate	Nil	Convulsions at 300 mg./kg.
C 26	 2′–methyl–*iso*–pethidine hydrochloride	3/4	Action delayed but prolonged
C 26a	Same, as hydriodide mixed with pethidine HCl, 25 mg./kg.	1	Reliable and prolonged action
C 27		0	Tested at 120 mg./kg.
C 28		0	Tested at 200 mg./kg.

logical activity is, as usual, found in the laevo form (C 24), and the dextro-form (C 25) is practically inactive.

In this as in the pethidine series activity appears to be enhanced by introducing a 2′-methyl group into the phenyl ring (C 26). The specificity of the ethyl-ester group is here even more pronounced, as the methyl-, *n*-propyl- and *iso*propyl-

TABLE VI

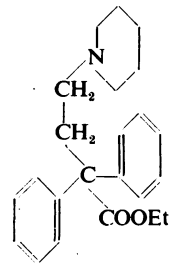
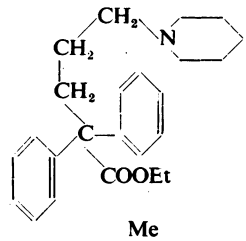
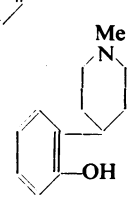
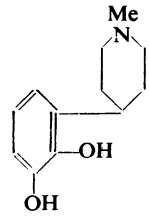
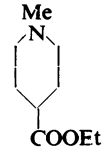
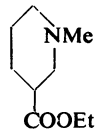
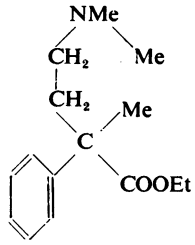
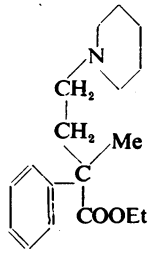
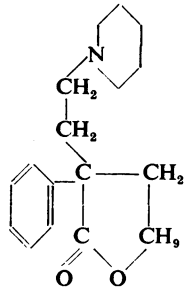
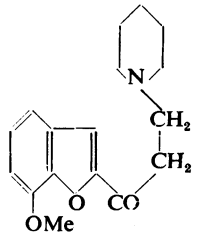
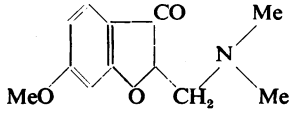
Compound No.	Formula	Relative Activity (Pethidine = 1)	Remarks
C 29		1/2	Wide side actions
C 29a		1/6	Strongly depressant
C 30		0	Tested at 120 mg./kg.
C 31		0	Tested at 120 mg./kg.
C 32		0	Tested at 120 mg./kg.

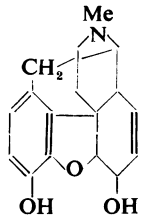
TABLE VI—*continued*

C 33		0	Tested at 120 mg./kg.
C 34		1/6	Very irregular
C 35		0	Tested at 120 mg./kg.
C 36		0	Tested at 120 mg./kg.
C 37		0	Tested up to 125 mg./kg. by Hoffmann-La Roche Laboratories, Basle, on the guinea pig
C 38		0	Tested up to 125 mg./kg. by Hoffmann-La Roche Laboratories, Basle, on the guinea pig

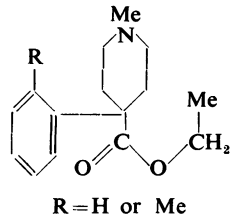
esters have no significant analgesic action in doses up to 120 mg./kg. Replacement of the phenyl- by a benzyl-group (C 27) or of the piperidine ring by a pyrrolidine ring (C 28) also led to complete loss of analgesic activity.

(6) *Miscellaneous Compounds* (Table VI).—Systematic testing of compounds C 29–C 36 revealed activity only in the compound C 29, thus confirming previous observations. The homologous compound C 29a, which bears the same relation to C 29 in respect to the position of the N-atom as *iso*-pethidine does to pethidine, showed only slight activity. Complete removal of either the ester- or the phenyl-group (C 30–C 33) resulted in total loss of activity in doses up to 120 mg./kg. body weight. Eisleb (1942) similarly found that 4-phenyl-1-methylpiperidine was inactive. In compounds C 34 and C 35, in which the original piperidine ring of pethidine has been opened, very slight or no analgesic action was observed. More drastic alterations in the structure as in C 36, 37, 38 (the last two tested by the Research Laboratories of Messrs. Hoffmann-La Roche, Basle) led again to loss of analgesic activity.

DISCUSSION

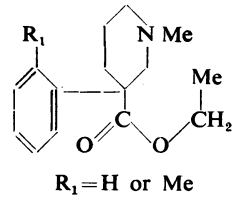


Morphine
I



R=H or Me

Pethidine series
II



R1=H or Me

Iso-pethidine series
III

Schaumann (1940) drew attention to the structural similarity of pethidine to morphine, though the synthetic drug lacks a number of the features of the natural product. His emphasis on the unimportance of the phenanthrene skeleton may, in our opinion, be exaggerated, especially since the 2′-methyl-pethidine (C 3, 4 and 5) showed in our experiments an increase in potency which may be related to its closer approximation in shape to the phenanthrene feature of morphine (cf. I and II (R = Me)).

It is interesting to find that 3′-hydroxy-pethidine (C 6) is not less active than pethidine, as Schaumann found to be the case with the 4′-hydroxy compound (C 9). The loss of activity following methylation (C 8), but not acetylation (C 7), of 3′-hydroxy-pethidine, may be due to the same causes as the similar loss of activity following methylation of the phenolic hydroxyl of morphine.

How can we account for the relative specificity of the ethyl-ester ? Apart from the *iso*-propyl- (C 12), allyl- (C 13) and, to a lesser degree, propyl-esters (C 14), all other esters show no or insignificant activity ; so, according to

Schaumann, do the amides. Ketones, on the other hand, are active, but here the length of the ketone chain seems less critical than that of the ester group. It is worth while mentioning here that Jensen *et al.* (1943) found that acyl derivatives of 4-phenyl-4-hydroxyl-1-methyl-piperidine have considerable activity, the maximum of which is possessed by the propionic acid derivative. Compounds C 19, an *iso*-coumaranone derivative, C 19a, its 7-methoxy-homologue, and C 20, a 2-methyl-coumaran derivative, show hardly any activity, although their structure bears a greater resemblance to the benzofuran feature of morphine than any compounds of the true pethidine series.

Accepting Schaumann's hypothesis, which is supported by our own results, that the phenyl-piperidine structure is essential for the optimal activity in this group of synthetic analgesics, we should now like to speculate on other aspects of the problem:

(1) As to the hydroaromatic rings in the morphine molecule, the one carrying the secondary alcohol group and the double bond is replaceable by an open chain consisting either of an esterified 4-carboxyl-group, a 4-acyl-group or a 4-hydroxy-acyl derivative. The fact that there exists an optimal length of the open chain for maximum analgesic activity, which is identical in each case and consists of 4 atoms, either carbon or carbon and oxygen, leaves little doubt as to the part played by these groupings in "mimicking" this hydroaromatic ring. The inactivity of the secondary alcohols corresponding to the ketones suggests that the carbonyl groups in these chains may subserve a function similar to that of the oxygen bridge in the morphine molecule.

(2) In the nitrogen ring system, the change from the 4-phenyl- (II) to 3-phenyl-piperidine (III) does not greatly reduce analgesic power. Indeed, some of these *iso*-compounds, though somewhat irregular in their effects, have produced analgesia of long duration. Again, the specificity of the ethyl ester group is dominant.

In the case of compound C 22, we have demonstrated that pharmacological action is associated, as is usual, with the l-form, but many more quantitative and comparative assays are required before conclusions can safely be drawn.

(3) If the piperidine ring of the pethidine series is opened, serious loss of activity occurs (C 34 and 35). But if a second phenyl-group is introduced into such a system as in C 29, analgesic power is restored to a certain extent. Recent reports, moreover (cf. Report on Pharmaceuticals: I. G. Farbenindustrie, 1945), indicate that replacement of the carbethoxy-group in such substances by appropriate acyl-groups leads to compounds of considerable activity. However, in the absence of full data it would be futile to speculate on the relationship between such compounds and those of the pethidine or *iso*-pethidine series.

(4) The results obtained in this study seem to indicate that the shape or fit of the molecule as a whole is more important in determining its analgesic value than any precise duplication of any one fraction of the morphine structure.

SUMMARY

(1) Some 50 synthetic compounds of the pethidine type were examined for analgesic activity using mice and a hot-plate technique.

(2) Some of these appear to have a stronger action than pethidine—notably the 2′-methyl-pethidine, and some have a longer action—notably the laevo-isomers of 3-phenyl-piperidine or *iso*-pethidine derivatives.

(3) Many inactive compounds were tested and some " rules " governing loss of potency are suggested.

(4) The nearer the compound approaches to morphine in the shape of its molecule, the more likely it is to be a good analgesic.

(5) Extensions of this work are planned in the hope that facts will emerge which will provide for a maturer judgment on the relationship of chemical constitution to analgesic value.

We gratefully acknowledge help from Drs. J. W. Haworth and N. C. Hindley and Miss M. Koenigstein, who have prepared some of the compounds used in this work.

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Brit. J. Pharmacol. (1946), **1**, 15–20.

CHEMOTHERAPEUTIC ACTION OF DYES IN TYPHUS INFECTION OF MICE

BY

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(Received November 1, 1945)

Several papers published during 1944 revealed that the rickettsiae of typhus fever are susceptible to attack by chemotherapeutic agents. Such activity has been shown in experimentally infected mice by Andrewes, King, van den Ende and Walker (1944), using *p*-sulphonamidobenzamidine (V147) and the corresponding amidoxime (V 186); by Yeomans, Snyder, Murray, Zarafonetis and Ecke (1944), using *p*-aminobenzoic acid; by Moragues, Pinkerton and Greiff (1944) with penicillin; by Peterson (1944) with forbisen and toluidine-blue and by Kikuth and Schilling (1944) with methylene-blue. No good results in experimental animals, other than mice, have been reported. The compounds, V147 and V186 and penicillin have proved unpromising in human typhus (van den Ende, Stuart-Harris, Fulton and Niven, 1946).

This paper records the results of extending Peterson's observations on toluidine-blue by studying the action of other dyes, particularly those chemically related to it.

TECHNIQUE

STUDIES OF *in vivo* ACTION IN MICE

Most of the workers, other than ourselves, mentioned above, have used the intraperitoneal route for infecting the mice in their studies. This has the disadvantage that it is available only for murine typhus, not for epidemic strains; nor is it so well adapted for quantitative comparison of drugs as the intranasal technique we employed. This method we have continued to use. Suspensions of rickettsiae, kept at −76° C., will maintain their potency unchanged for many months, and suitable dilutions inoculated intranasally into mice will produce isolated foci on the lungs. The foci can be counted with satisfactorily reproducible results, and the reduction in count used to judge the effect of drugs. Groups of six mice were used for each drug, with an appropriate control group of six mice. Details are given in the earlier paper (Andrewes *et al.* 1944). All intranasal inoculations were carried out in the special inoculation box (van den Ende, 1943), without the aid of which, we are convinced, the intranasal technique will almost certainly lead to accidental laboratory infections.

In routine testing of many chemotherapeutic agents over two years ago we failed to detect any activity on the part of toluidine-blue or methylene-blue. This was doubtless

because these drugs are very poorly tolerated by the intraperitoneal route which we were using. For further work with dyes we used one of three methods:

(a) Drugs were mixed in a known percentage with the moist food-mash. This method was labour-saving, but did not readily disclose the actual amount of drug taken, particularly as sick mice ate poorly.

(b) Drugs were administered into the oesophagus by means of a 1 cc. syringe and wide needle, of which the point had been cut off and the end blunted. This was done, as in our earlier intraperitoneal tests, two hours before infection, twice daily on the two following days, and on the morning of the third following day.

(c) The drugs were given subcutaneously according to the same time schedule as under *(b)*. Many caused local oedematous reactions and consequently each of the six injections was made at a different site.

Studies of *in vitro* Action and Tests on Rabbits' Skins

Peterson reported that toluidine-blue inactivated murine typhus rickettsiae *in vitro*. We accordingly included in our studies tests of such action. Suspensions of murine rickettsiae were held in contact with aqueous solutions of various dyes for 45 minutes at room temperature, and then inoculated in 0.1 cc. quantities intradermally into skins of shaved rabbits. Several dilutions of each of four or more dyes could be tested on the skin of one rabbit. Staining or inflammatory reactions caused by the dyes themselves rarely caused trouble in the dilute solutions in which we used them. Specific raised erythematous lesions, such as have been described by Giroud (1938), appeared within two or three days whenever the rickettsiae had not been inactivated; they reached their maximal intensity after about five days. Since the texture of the rabbits' skin may affect the size and nature of a rickettsial lesion, several control inocula of rickettsiae alone were always made in different positions into the animal's skin. The number of rickettsiae in the inoculum did not materially affect the results of drug tests over a wide range (1 : 100 to 1 : 10,000 of stock suspension); so we used throughout a 1 : 100 dilution of a stock suspension, 0.05 cc. of which, when diluted $1 : 10^5$, was capable of producing 40 lesions in a mouse's lungs. The time of contact of rickettsiae and dye did not greatly affect the issue, though slightly better inactivation occurred when contact was for one hour at 37° C. Very probably some action of dyes on the organisms continued after the mixture had been injected intradermally.

Results

Mouse Tests.—We readily confirmed Peterson's finding that toluidine-blue had a chemotherapeutic action when given mixed with the food. Table I shows that it wholly suppressed lung lesions when given in a 1.5 per cent concentration, that 0.5 per cent was less effective and 0.1 per cent useless. Oral administration with a syringe suppressed lesions wholly when 5 mg. were given on six occasions; results were less striking when the dose was reduced. The drug given subcutaneously in doses of 6×1 mg. or 3×2 mg. was also completely effective; these doses, however, produced considerable oedematous swellings at the sites of inoculation. We confirmed our earlier findings that toluidine-blue was ineffective when given in the maximal dose which was tolerated intraperitoneally (1 mg.).

Table I shows that methylene-blue was equally effective. Possibly it was a little more so, but the differences were not significant. Similar results were obtained in a feeding test with medicinally pure zinc-free methylene-blue. These experiments were carried out with murine typhus (Wilmington strain). In two

experiments toluidine-blue was shown to act also in epidemic typhus infection, though rather less effectively. Six doses of 5 mg. *per os* reduced the lung spot-count from 56 to 14.

Of other dyes tested only three (selenium-methylene-blue, new methylene-blue and 3-diethylamino-7-di-*n*-butylaminophenazthionium chloride) had any

TABLE I

CHEMOTHERAPEUTIC EFFECT OF METHYLENE-BLUE ANALOGUES AGAINST MURINE TYPHUS IN MICE

Drug	How given	Dose	Lung Spot Count (average of 6 mice)	Lung Spot Count in un-inoculated mice (average of 6)
Toluidine-blue	Mixed with food	1·5%	0	34
,, ,,	,, ,, ,,	0·5%	2	44
,, ,,	,, ,, ,,	0.5%	4.5	28
,, ,,	,, ,, ,,	0·1%	28	28
Toluidine-blue	*Per os* (syringe) twice daily (6 doses in all)	5 mg.	0	42
,, ,,	,, ,, ,,	2·5 mg.	1·5	42
,, ,,	,, ,, ,,	2 mg.	13	47
,, ,,	*Per os* (syringe) once daily (3 doses in all)	5 mg.	2	27
Toluidine-blue	Subcut. 6 doses	1 mg.	0	24
,, ,,	,, 3 ,,	2 mg.	0	27
Methylene-blue	Mixed with food	1·5%	0	44
,, ,,	,, ,, ,,	0·5%	0	28
,, ,,	,, ,, ,,	0·1%	10	28
,, ,,	,, ,, ,,	0·1%	17	24
Methylene-blue	*Per os* (syringe) (6 doses in all)	2 mg.	12	47
Methylene-blue	Subcut. 6 doses	1 mg.	0	24
,, ,,	,, 3 ,,	0·5 mg.	1	30
New-methylene-blue	Subcut. 6 doses	1 mg.	2	45
3-Diethylamino-7-di-*n*-butylaminophenazthionium chloride hydrochloride	Subcut. 3 doses	1 mg.	0	12
,, ,,	,, 3 ,,	0·25 mg.	20	30
Selenium-methylene-blue	Subcut. 3 doses	2 mg.	3·5	30
,, ,,	,, 3 ,,	0·5 mg.	14	30

action at all *in vivo*. The first two of these were apparently slightly less active than, the third about equal to, methylene and toluidine-blues. With the other dyes, no significant differences occurred between the spot-counts of treated and control mice. They were given at their maximal tolerated dose as shown in

TABLE II

DETAILS OF TESTS OF DYES AGAINST TYPHUS IN RABBITS AND MICE

Group to which dye belongs	Dye	Route of inoculation and dose (m.t.d.) used for mouse tests —in mg. unless otherwise stated	Activity in mouse test (see Table I)	Effective dilution *in vitro* as shown by rabbit test
Acridines	Proflavine	0·37 i.p.	0	Ineffective at 1:2,000
	Acriflavine	0·06 i.p.		
	2:7-Diaminoacridine	8 i.p.		
	2:8-Tetramethyldiamino-10-methyl-acridinium methosulphate	no test		
	Atebrin	2 s.c.; also tested 0·5%		
Oxazines	Brilliant-cresyl-blue	2 i.p.	0	
	Cresyl-fast-violet 2B	0·5 s.c.		
	Capri-blue G.O.N.	0·2 s.c.		
	Nile-blue B	0·5 s.c.		
Phenazines	Janus-green	0·05 i.p.	0	
	Neutral-red	1 s.c.; also tested 0·1% in diet		
	Safranine	0·5 s.c.		Ineffective at 1:4,000 (stronger solution not tested)
Azo-dyes	Trypan-red	0·5 s.c.	0	
	Trypan-blue	0·25 s.c.		
	Congo-red	1 s.c.		
Thiazines	Thionine	2-4 s.c. also tested 0·5% in diet	0	Ineffective at 1:2,000 (no test in rabbit)
	Thiodiphenylamine	1% in diet		
Pyronine	Pyronine B	No test	0	1:5,000
Phenazine	Methylene-violet 3RA	0·25 s.c.	0	1:25,000
Thiazine	1-Methyl-3-diethylamino-7 (mono)-*n*-butylamino-P*	0·25 s.c.	0	1:4,000
Oxazine	Meldola-blue	0·1 s.c.	0	1:50,000
Thiazine	Methylene-blue	1 s.c. (also tested in diet)	+	1:500,000
,,	Toluidine-blue	1-2 s.c. (also tested in diet)	+	1:500,000
,,	Methylene-green	1 s.c.	0	1:125,000
,,	1-Methyl-3-diethylamino-7-di-*n*-butylamino-P*	0·25	0	1:500,000
,,	1-Methyl-3-diethylamino-7-di-*n*-propylamino-P*	0·25	0	1:500,000
,,	1-Methyl-3:7-*bis* (diethylamino)-P*	0·25	0	1:500,000
,,	New-methylene-blue	1 s.c.	+	1:500,000
,,	3-Diethylamino-7-di-*n*-butylamino-P*	1 s.c.	+	1:500,000
Selenazine	Selenium-methylene-blue	2 s.c.	+	1:500,000

*P = phenazthionium chloride hydrochloride.

Table II—mostly subcutaneously. Forbisen, which Peterson reported to have some activity, was by our technique weakly active. When given by mouth in 6×25 mg. doses, it reduced the average spot-count from 26 to 5 in one test, and from 47 to 17 in another.

Rabbit Tests.—The last column of Table II shows the final concentration of dye which would inactivate rickettsiae *in vitro* when tested as described earlier. It is apparent that a few drugs, those of the last section in the table, are very active in the *in vitro* tests, their effective concentrations being about 250 times less than that of most of the dyes tested. All but four of the drugs we examined could by this test be readily classified as either " very active " or " useless."

It will be noticed that three of the thiazines were as active as methylene-blue in the rabbit, but were not active in the mouse test; this is presumably because they were more toxic and could only be given in 0.25 mg. doses.

Discussion

All the dyes in Table II, with the exception of the azo-dyes represented by trypan-red, trypan-blue and congo-red are similarly constituted and belong to the class of vital stains. They fall into the ortho-quinonoid group of dyes to which Ehrlich attached special significance. Presumably they are all taken up to a greater or lesser extent by the rickettsiae, but one half of them, as shown by the *in vitro* test, are comparatively harmless. The other half inactivate the rickettsiae for the most part at relatively high dilutions. In the mouse test, where one is dealing with a more complex chemotherapeutical system, the dyes have not only to be tolerated by the host but have to enter the cells harbouring the rickettsiae. Nevertheless the two tests do show a rough correlation.

The results in the absence of further evidence are capable of more than one interpretation, but an acceptable view of the chemotherapeutic process would be to suppose that the vital dyes are adsorbed or absorbed by the rickettsiae in either form of the test and the specific fixation of the dye interferes with some vital function necessary for reproduction of the parasite.

The action of the active thiazines seems unlikely to be necessarily dependent on a photodynamic effect, since dye introduced subcutaneously or *per os* is effective on rickettsiae within the lungs.

Comparison of the results of the mouse tests and rabbit tests shows that the latter may have some value as a screening method before resort to the direct test in mice, if allowance is made for toxicity. The five compounds effective in mice are amongst those which are most active in the rabbit test. Such correspondence as there is between the two tests must not be taken as necessarily applying to compounds chemically unrelated to these dyes: the drugs V147 and V186 and related compounds, which are more effective *in vivo* than any of the dyes tested (Andrewes *et al.*, 1944), fail to show any *in vitro* killing power when mixtures are tested in the rabbit's skin.

SUMMARY

Dyes have been tested for activity against typhus rickettsiae (a) by holding mixtures of organisms and dye in contact *in vitro* and inoculating into the rabbit's skin to test whether infectivity has been destroyed and (b) by a direct *in vivo* chemotherapeutic test in mice. The results of the two tests, so far as dyes were concerned, were closely parallel. Activity was found only in thiazines and in one selenazine.

We extend our thanks to Mrs. Frances Hamilton for her help with some of the inoculations and to Drs. L. Hellermann and O. L. Peterson, and to Imperial Chemical (Pharmaceuticals), Ltd., for their kindness in supplying us with compounds for test.

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Brit. J. Pharmacol. (1946), **1**, 21–37.

AMIDINES, GUANIDINES AND ADRENALINE INACTIVATION IN THE LIVER

BY

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(Received December 5, 1945)

In 1897 Langlois showed that injection of suprarenal extract into the mesenteric vein of a dog was less effective in causing a rise of blood pressure than injection of the same quantity of extract into the jugular vein. This difference between the two modes of administration was less when large amounts of the extract were used, and Langlois concluded "Il est facile de supposer que la foie ne peut neutralizer qu'une partie de la substance et que si l'injection est trop forte, un excès passe au debut dans la circulation générale." Since the difference persisted even when the injection was made very slowly, it could not be attributed to retardation of the release of the extract into the general circulation. Carnot and Josserand (1902) demonstrated that adrenaline injected into the portal vein of a dog caused a smaller rise of blood pressure than when injected into the saphenous, femoral or jugular veins. This experiment was confirmed by Elliott (1905), and by many subsequent workers on different species. Bacq (1937) also showed that 0.5 μg. adrenaline injected into the saphenous vein of the cat under dial anaesthesia caused the same relaxation of the non-pregnant uterus and contraction of the nictitating membrane as 2 μg. adrenaline injected into the portal vein. Markowitz and Mann (1929) criticized the conclusion that adrenaline was inactivated during its passage through the liver, believing that the smaller pressor effect after injection into the portal circulation was due to its reaching the general circulation in a very much lower concentration. Yet Battelli (1902) showed that the isolated rabbit's liver perfused with defibrinated blood by the portal vein destroyed adrenaline, an observation which was confirmed by Elliott (1905) and by Pak (1926). Philpot and Cantoni (1941) investigated the ratio of equipressor doses of various substances injected into the portal circulation (from a cannula in the splenic vein) and into the jugular vein in the spinal cat. They found the average ratio for adrenaline was 4.7; that is to say, the dose injected into the splenic vein had to be 4.7 times that injected into the jugular in order to obtain the same pressor effect. Since the pressor action of pituitrin was not modified by passage through the liver, they concluded that the criticism offered by Markowitz and Mann was untenable, and that in the liver *in vivo* the main instrument of adrenaline destruction was amine oxidase. They were supported in this conclusion by the observation that methylene blue, which inhibited the destruction of

adrenaline *in vitro* by a liver suspension, greatly augmented the pressor effect of adrenaline injected into the portal vein.

A study of the pharmacological action of various amidines (Dawes, 1945), and the observation by Blaschko and Duthie (1944) that pentamidine and propamidine could, like methylene blue, inhibit the destruction of tyramine by a suspension of rabbit liver in a concentration of 10^{-6}, both led to a review of the problem by the method used by Philpot and Cantoni. In this way a number of amidine and guanidine derivatives has been found to increase the pharmacological action of adrenaline and of other sympathomimetic amines injected into the portal circulation. However, not all of these compounds are able to inhibit the action of amine oxidase *in vitro*.

I am indebted to Dr. H. King, Dr. A. J. Ewins and Mr. W. A. Broom for many of the substances used in these experiments. The V substances and marfanil were obtained through the kindness of Dr. J. Walker. For the Synthalin B (dodecamethylene diguanidine dihydrochloride) I am indebted to Dr. E. M. Lourie, and for the diphenyl guanidine hydrochloride and S-methyl *iso*thiourea iodide to Dr. H. R. Ing.

Pentamidine (*p* : *p'*-diamidino-1 : 5-diphenoxypentane) was used throughout as the di-isethionate. The serial number V147 is used in place of the chemical description *p*-sulphonamidobenzamidine hydrochloride.

METHODS

Most of the experiments were performed in spinal cats prepared by section of the spinal cord at the level of the second cervical vertebra. In order to inject solutions into the portal vein, a cannula was tied either into a small mesenteric vein or into the splenic vein, and the abdomen was closed around the cannula.

RESULTS

In confirmation of Langlois (1897, 1898) it was observed that the relation between doses of adrenaline producing equal rises of blood pressure by the jugular and by the portal routes differed according to the amount injected. This is shown in Table I, where it is seen that the ratio was 1 : 5 when the dose injected into the

TABLE I

DOSES OF ADRENALINE PRODUCING EQUAL RISES OF BLOOD PRESSURE

Intrajugular injection μg.	Intraportal injection μg.	Blood Pressure rise mm. Hg
4	20	54
9	40	78
28	80	110
72	160	134

portal vein was 20 μg. adrenaline, and 1 : 2.2 when the dose injected was 160 μg. What does this difference indicate ? One explanation is that the liver destroys adrenaline, and that the rise of pressure after intraportal injection depends on the amount of adrenaline escaping into the general circulation through the hepatic veins. If the dose injected into the portal vein is large, a greater proportion will reach the general circulation, and there will be proportionately less difference between the doses injected into the portal and jugular veins which cause the same rise of blood pressure.

The Potentiation of Adrenaline Injected into the Portal Circulation

The pressor action of adrenaline injected into the portal vein of a spinal cat is both increased and prolonged by the simultaneous injection of aromatic

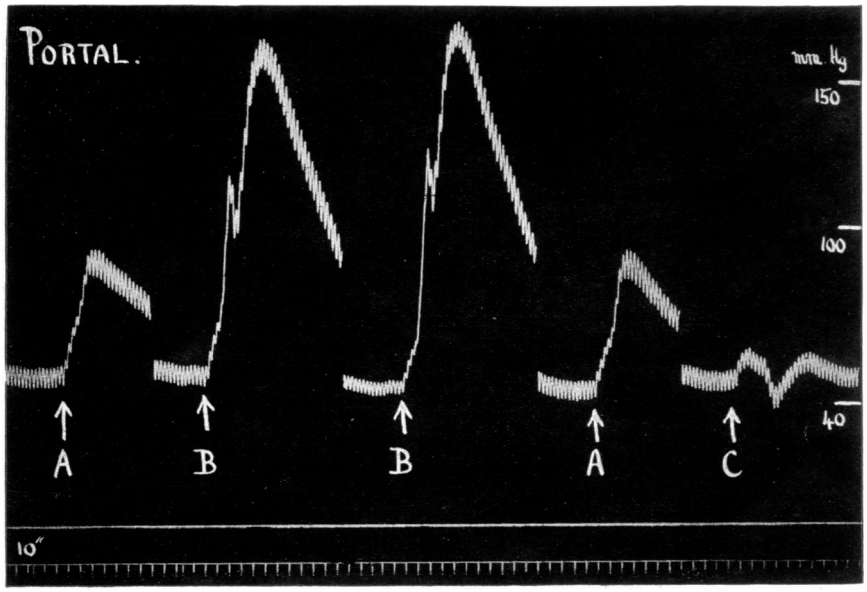

FIG. 1.—Spinal cat. 3.5 kg. Blood pressure record. All injections were made from a cannula in the splenic vein, so that the injected fluid entered the portal circulation, at 8 minute intervals. The injection of 40μg. adrenaline + 1 mg. pentamidine isethionate mixed in the same syringe (at B) caused a larger rise of blood pressure than 40 μg. adrenaline (at A). 1 mg. pentamidine isethionate alone (at C) has an insignificant effect.

diamidines and monoamidines, of aliphatic diguanidines, diamidines and monoamidines, and of guanidine itself. Fig. 1 shows the potentiation of 40 μg. adrenaline injected into the portal vein by 1 mg. pentamidine. In the same animal 2 mg. pentamidine injected into the jugular vein caused a prolonged reduction in the pressor action of 2 μg. adrenaline (Fig. 2). There is, therefore, a striking difference between the action of pentamidine on adrenaline injected by

the portal vein and by the jugular vein, in contrast to cocaine, ephedrine and tyramine, which potentiate the action of adrenaline by both routes. In order to investigate this difference, a closer study was made of the action of amidines on intrajugular and intra-arterial injection.

The Action of Amidines on Intrajugular and Intra-arterial Injection

Both aromatic diamidines (Fig. 2 and Wien, 1943) and aromatic monoamidines (Dawes, 1945) reduce the pressor action of adrenaline when injected into the jugular vein of a spinal cat in large doses. This reduction occurs mainly in the muscle vessels. Fig. 3 shows that pentamidine greatly reduces the vasoconstrictor action of adrenaline in the dog's hindlimb, perfused with defibrinated blood from a Dale-Schuster pump. Similarly Wien (1943) produced evidence of the reduction of the vasoconstrictor action of adrenaline by propamidine, in

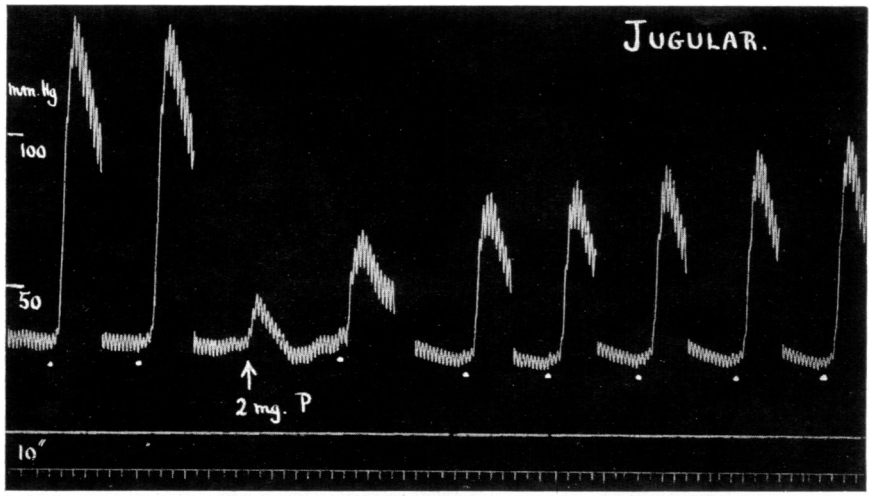

FIG. 2.—Same cat as in Fig. 1. All injections made into the jugular vein. The pressor action of 8 μg. adrenaline (injected at 4 minute intervals) is greatly reduced by 2 mg. pentamidine isethionate (P).

the cat's hindlimb perfused with haemoglobin-Ringer solution. In the dog heart-lung preparation, and in the isolated rabbit auricle (suspended in oxygenated Ringer-Locke at 28° C.), neither pentamidine nor V147 (*p*-sulphonamidobenzamidine hydrochloride) appreciably modified the action of adrenaline.

These experiments demonstrate that the main site of action of pentamidine in the peripheral circulation is on the vessels rather than on the heart. Now it is well known (Carnot and Josserand, 1902 ; Livon, 1904 ; Elliott, 1905) that injection of adrenaline into the femoral artery causes a much smaller rise of blood pressure than intravenous injection. This has been attributed to the slow libera-

tion of adrenaline from the tightly constricted vessels (Markowitz and Mann, 1929), combined with rapid inactivation. The situation bears an obvious resemblance to that following injection of adrenaline into the portal vein. Yet, although pentamidine and V147 greatly potentiate the pressor action of adrenaline injected into the portal vein, they produce no such striking effects on intra-arterial injection. Thus in spinal cats with a cannula in the external iliac artery, so that the injected solution passed into the vessels of the opposite leg, 16–20 μg. adrenaline were required on intra-arterial injection to cause the same rise of blood pressure as 2 μg. adrenaline by the jugular vein. In two such

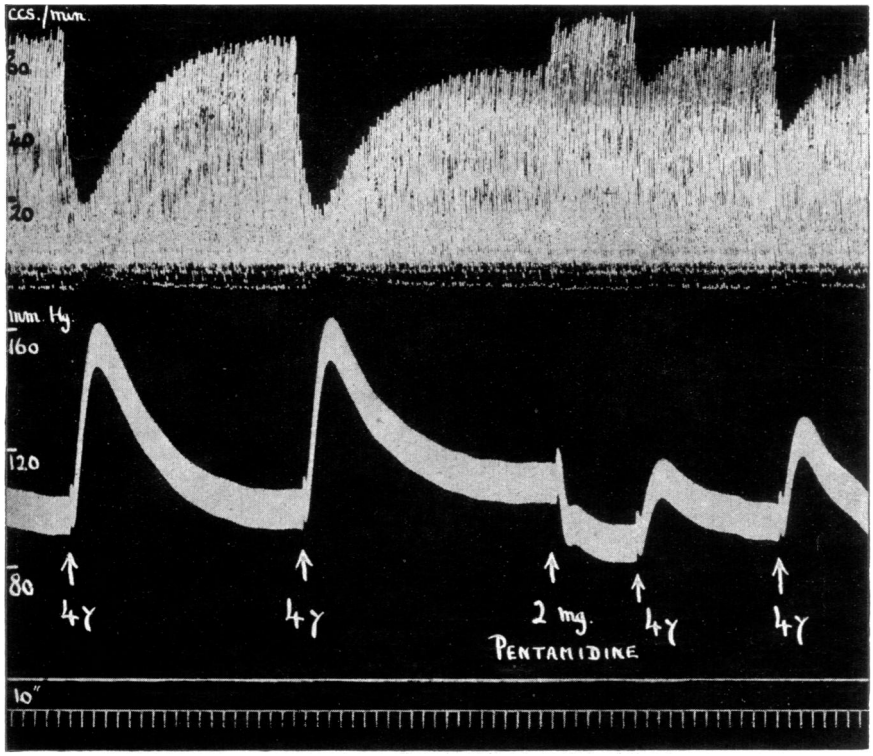

FIG. 3.—Dog's hindlimb perfused with defibrinated blood. Above: outflow record. Below: pressure in arterial cannula. The injection of 2 mg. pentamidine isethionate greatly reduces the vasoconstrictor action of 4 μg. adrenaline.

preparations the simultaneous injection of 0.5–2.0 mg. pentamidine or 1–5 mg. V147 caused only a tiny increase in the pressor action of adrenaline, whereas the same doses of these compounds more than doubled the response when injected with adrenaline into the portal vein. This difference between intra-arterial and intraportal injection is the more surprising when it is considered that both pentamidine and V147 not only greatly diminish the vasoconstrictor

action of adrenaline in the dog's hindlimb, but themselves have a far greater vasodilator action in the presence of an adrenaline infusion. It would be expected that administration of either of these drugs would allow more adrenaline to escape into the general circulation after intra-arterial injection. The fact that this does not occur leads to the conclusion that there must be some fundamental difference between the reactions of the liver and of the muscle to adrenaline and these amidine derivatives.

The Action of Amidines on Intraportal Injection

What is happening in the liver when pentamidine or V147 is injected together with adrenaline ? The answer to this question must be the key to the difference between intraportal and intrajugular injection. The potentiation of adrenaline on intraportal (but not on intrajugular) injection has been observed

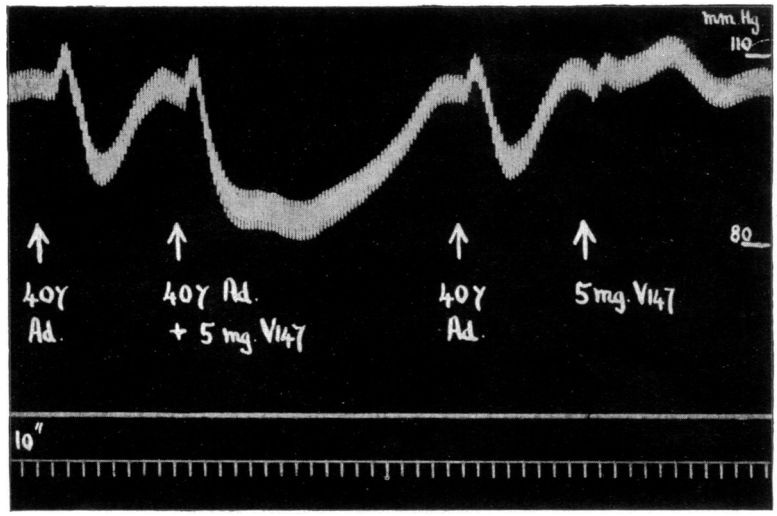

FIG. 4.—Spinal cat. Blood pressure record. All injections into the portal circulation. 3.5 mg. ergotamine tartrate had been injected previously to cause reversal of the pressor action of adrenaline. The injection of 5 mg. V147 increases and prolongs the depressor action of 40 μg. adrenaline.

in the rabbit under urethane, and in the dog under chloralose anaesthesia, as well as in the spinal cat, and consequently the phenomenon is not confined to one species. Moreover, not only the pressor action of adrenaline, but also the depressor action of small doses (in a cat under ether anaesthesia) is increased by amidines. The actions of adrenaline in causing contraction of the spleen, and of the innervated or denervated nictitating membrane, are also increased. Fig. 4 shows the potentiation by V147 of the depressor action of adrenaline injected into the portal vein of a spinal cat, after full ergotamine reversal. These experiments very strongly suggest that less adrenaline is being inactivated during its

passage through the liver in the presence of an amidine derivative, so that more adrenaline reaches the general circulation.

In no instance in which adrenaline and either pentamidine or V147 were injected into the portal circulation has the ensuing pressor response been as great as that caused by the injection of an equal quantity of adrenaline alone into the jugular vein of a spinal cat. The potentiation of the pressor action by these substances is therefore due entirely to a reduction in the effect of the liver upon adrenaline. Comparatively small doses of the drugs are required, and some potentiation of adrenaline has been observed with as little as 0.1 mg. V147 or pentamidine injected into the portal vein of a spinal cat weighing 2–3 kg. If a short interval is left between the injection of 1–2 mg. pentamidine and that of adrenaline, the potentiation is less, and it disappears altogether if the interval is as long as 10 minutes; by this time the greater proportion of the pentamidine absorbed by the liver has been inactivated. There is other evidence that pentamidine itself (as well as adrenaline) is rapidly absorbed from the blood stream during its passage through the liver. For instance 2–4 mg. pentamidine injected into the jugular vein of a spinal cat reduced by more than half the pressor action of 10–20 μg. adrenaline, whereas 2–4 mg. pentamidine injected into the portal vein scarcely affected the pressor action of adrenaline injected into the jugular vein.

The reduction in the inactivation of adrenaline during its passage through the liver, caused by the simultaneous administration of amidine derivatives, might be due to inhibition of the enzyme systems responsible for the inactivation of adrenaline, or to interference with the uptake of adrenaline by the liver cells. An alternative explanation might be that these drugs derange the hepatic circulation (by vasodilatation, for instance) and so allow the adrenaline to pass more rapidly through the liver. This last explanation, while it cannot be entirely excluded, is rendered unlikely for a variety of reasons. For instance, if the vasodilatation caused by some of these drugs were the principal cause of the potentiation in the liver, it might be expected that a similar potentiation would be observed after intra-arterial injection into muscle, in which this vasodilatation has been demonstrated (Fig. 3) : in fact no such potentiation is seen in muscle. Secondly, some difference might be expected in the interval between the injection of the solution and, say, the peak response in blood pressure and heart rate. As Fig. 5 shows, no difference is observed, whether adrenaline be injected alone or with V147 into the portal vein, in the time characteristics of the initial rise of blood pressure. A close inspection of such tracings also fails to reveal any difference in the time which elapses before the heart rate increases.

Many authors have shown that adrenaline causes a rise in portal pressure after injection into the portal vein of a cat or dog. The subject was extensively reviewed by Bauer, Dale, Poulsson and Richards (1932), and the observations of McMichael (1932) are also relevant. The conclusion that this rise in portal pressure is due to constriction of the portal venous radicles is well substantiated.

Yet, when V147 is injected together with adrenaline from a cannula tied into a small mesenteric vein of a spinal cat, the rise in lateral portal pressure (measured from the splenic vein from a cannula containing Ringer solution and heparin) is, if anything, slightly greater; i.e. V147 does not reduce the constrictor action of adrenaline under these circumstances. Similarly, as Fig. 4 shows, V147 still potentiates adrenaline after full ergotamine reversal, when the vasoconstrictor action of adrenaline upon the peripheral circulation has been abolished. The

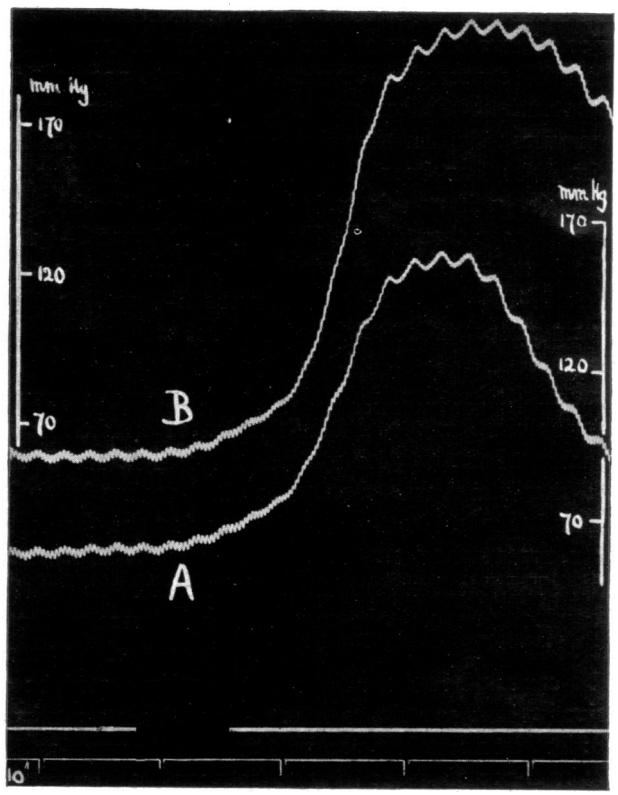

FIG. 5.—Spinal cat. Blood pressure records of two consecutive observations superimposed. Injections into the portal circulation were accurately timed during the interruption of the upper signal marker. The lower record A was obtained after injection of 20 μg. adrenaline and the upper record B after 20 μg. adrenaline +5 mg. V147.

method of Bauer, Dale, Poulsson and Richards (1932) as modified by Chakravarti and Tripod (1940) was used to perfuse the isolated dog's liver with defibrinated blood. In four such preparations, 4–20 mg. V147 injected into the blood entering the portal vein did not appreciably alter the portal pressure, hepatic arterial pressure, liver volume or venous outflow, even during the infusion of adrenaline.

In the same way, various drugs which cause vasodilatation in the peripheral circulation, such as sodium nitrite (2–10 mg.) and adenosine (0.2–1.0 mg.), do not increase the pressor effect of adrenaline after intraportal injection into spinal cats. Theophylline sodium acetate (10 mg.) causes an almost insignificant potentiation. Histamine (2–20 μg.) is quite inactive. Conversely guanidine and methylguanidine, which cause a rise of blood pressure in the intact animal owing to peripheral vasoconstriction, and which in high concentrations (1 : 100) cause contraction of isolated arterial strips (Lewis and Koessler, 1927), both potentiate the pressor action of adrenaline after intraportal injection. It therefore seems very improbable that guanidines and amidines reduce the inactivation of adrenaline in the liver simply by causing vasodilatation.

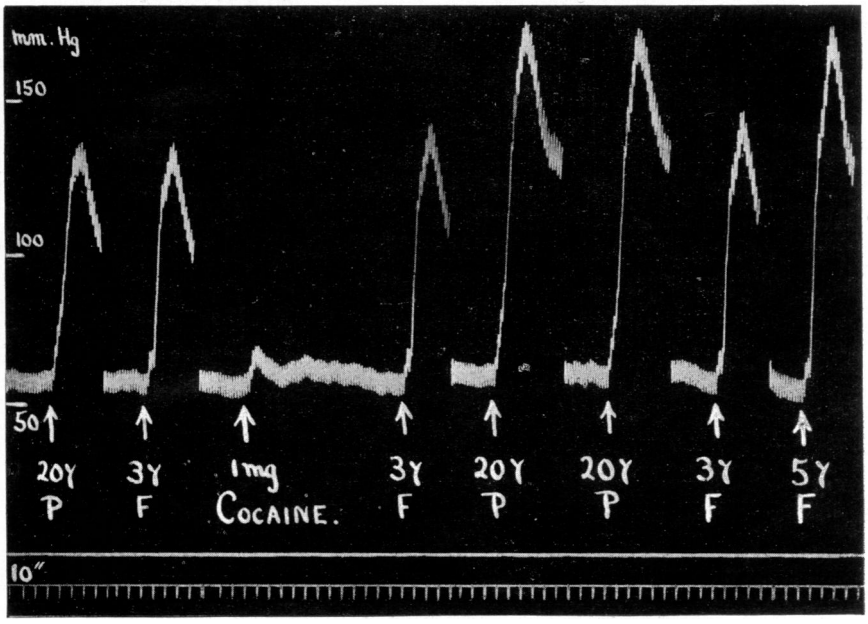

FIG. 6.—Spinal cat. Blood pressure record. Before injection of cocaine 20 μg. adrenaline injected into the portal vein (P) caused the same rise of blood pressure as 3 μg. into the femoral vein (F). After 1 mg. cocaine hydrochloride injected into the portal vein, 20 μg. adrenaline intraportally caused the same rise of blood pressure as 5 μg. adrenaline intrafemorally.

Sympathomimetic Amines and Cocaine.—It has already been mentioned that cocaine, ephedrine and tyramine potentiate the pressor action of adrenaline in the spinal cat, after injection into the portal vein. Fig. 6 illustrates this for cocaine. 20 μg. adrenaline injected into the portal vein caused the same rise of blood pressure as 3 μg. injected into the femoral vein ; after 1 mg. cocaine hydrochloride the pressor action of 20 μg. adrenaline intraportally was equal to

that of 5 μg. intrafemorally. (In this experiment the cocaine was injected into the portal circulation to emphasize as far as possible its action on the liver.) Ephedrine and tyramine have the same effect, though that of tyramine is more transient.

Amidine derivatives such as pentamidine, V147 and even guanidine itself, on intraportal injection into a spinal cat, increase the pressor action not only of adrenaline, but also (and equally well) that of others of the more active sympathomimetic amines, such as corbasil, epinine and noradrenaline. Less active amines such as *l-m*-sympatol, α-methyl adrenaline, N-methyl adrenaline and adrenalone, which have to be injected in doses of 0.1–1.0 mg. into the portal

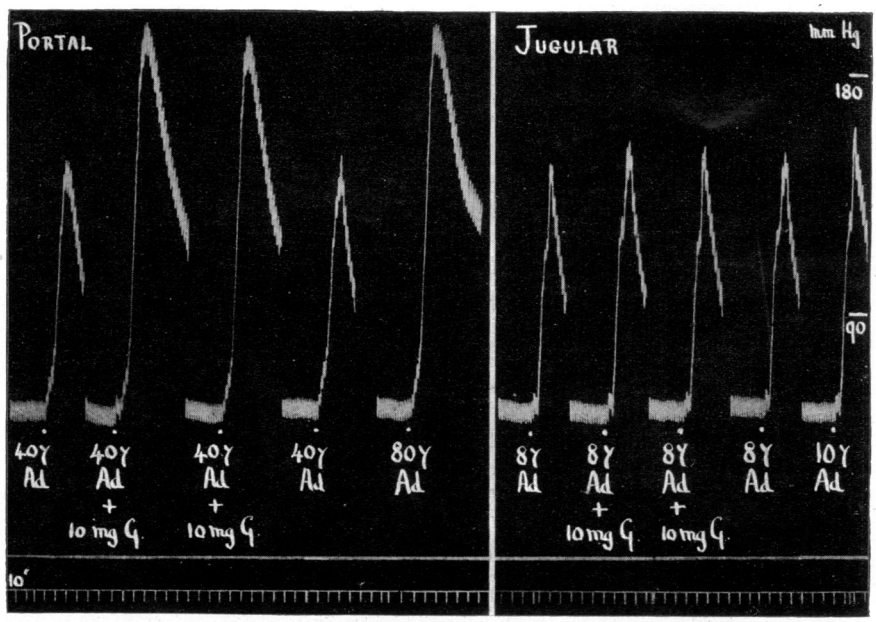

FIG. 7.—Spinal cat. Blood pressure record. 10 mg. guanidine hydrochloride greatly increases the pressure action of 40 μg. adrenaline injected into the portal vein, but scarcely affects the pressor action of 8 μg. adrenaline injected into the jugular vein.

vein to cause a rise of blood pressure, show a much smaller potentiation (relative to that of adrenaline) when the injection is accompanied by an amidine derivative.

Other Amidine Derivatives.—This investigation started with a study of the action of pentamidine and V147 on intraportal injection, but the property of potentiating the action of adrenaline under these circumstances alone is by no means confined to these two drugs. Guanidine hydrochloride itself in a dose of 10 mg. will double the response to adrenaline injected into the portal vein, but not via the jugular vein (Fig. 7), and the minimal effective dose of guanidine is about 0.5 mg./kg. Both methylguanidine hydrochloride and diphenylguanidine

hydrochloride cause a considerable potentiation of adrenaline on intraportal injection into a spinal cat in a dose of 1 mg.; they are slightly more active than guanidine.

Monoamidines of the type: $CH_3.(CH_2)_n.(:NH)NH_2$.

Five representatives of this group were studied, *viz.* those in which n was 3 and 4 (sulphates), 8, 10 and 14 (hydrochlorides). The method used for estimating the relative activity of these compounds was to inject adrenaline together with 0.25–0.5 ml. of the substance in 0.01M solution into the portal vein, and compare each member of the series with that immediately above and below it. The type of record produced is illustrated in Fig. 8, which shows the relative activity of

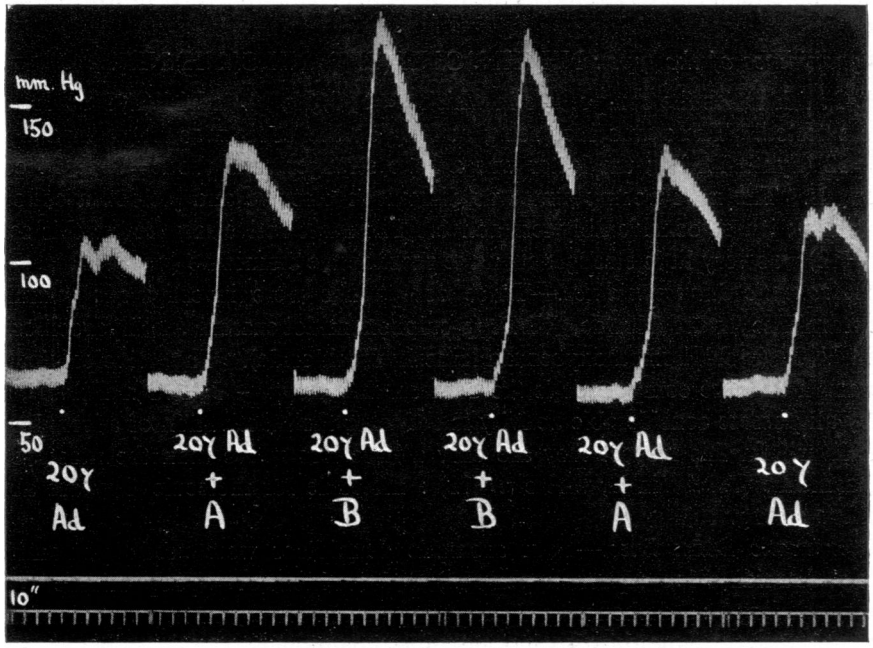

FIG. 8.—Spinal cat. All injections into the portal circulation. To show the relative increase of the pressor action of 20 μg. adrenaline by A, 0.25 ml. M/100 lauramidine and by B, 0.25 ml. M/100 *n*-decane-1 : 10-diamidine.

n-decane-1 : 10-diamidine and the corresponding monoamidine (lauramidine) where $n=10$. At least two cats were used for each comparison and the point of maximum activity in each series of compounds was confirmed on at least four cats.

In this series of monoamidines, all of which show activity, the strongest was that in which $n=4$. That in which $n=3$ was considerably less active and there was also a progressive decline in activity as the aliphatic chain was lengthened to

8, 10 and 14. The last member of the series ($n=14$) was difficult to dissolve, and produced a soapy colloidal solution. There is therefore a maximum in this series somewhere about $n=4-7$, probably nearer 4 than 7. The intermediate members of the group were unfortunately not available.

Diamidines of the type : $NH_2(HN:)C.(CH_2)_n.C(:NH)NH_2$.

In this series five compounds were examined, *viz.* those in which $n=7$ (sulphate) 10, 12, 14 and 16 (hydrochlorides). There was a progressive increase in activity as the chain was lengthened up to $n=14$; the compound in which $n=14$ was more active than that in which $n=12$ or 16. The most active of the series of monoamidines (that in which $n=4$) was not quite as strong (somewhat more than half as strong) as the most active diamidine (that in which $n=14$).

Diguanidines of the type : $NH_2(HN:)C.NH.(CH_2)_n.NH.C(:NH).NH_2$.

Of this series the compounds in which $n=5$, 8, 12, 14 and 16 (hydrochlorides) were tested. There was increasing activity as the chain was lengthened up to $n=12$ (Synthalin B) ; the latter was a little stronger than the $n=8$ or 14 compounds. The $n=16$ compound was difficult to dissolve and yielded a colloidal solution ; it was considerably less active. The most active member of this series ($n=12$) was very little stronger than the most active diamidine ($n=14$).

Aromatic Monoamidines.—Three compounds were investigated: *p*-aminobenzamidine hydrochloride, V147 (*p*-sulphonamidobenzamidine hydrochloride) and V187 (*p*-sulphomethoxybenzamidine hydrochloride). The principal feature common to these compounds is the benzamidine group $-C_6H_4.C(:NH)NH_2$, and they all possess considerable activity in reducing the inactivation of adrenaline in the liver. In this respect they are only a little less active than the most active diguanidine (Synthalin B, where $n=12$) : molecule for molecule they are about half as strong.

Aromatic Diamidines.—Four aromatic diamidines were used: pentamidine (*p* : *p'*-diamidino-1 : 5-diphenoxypentane) di-isethionate, propamidine (the corresponding 1 : 3-diphenoxypropane compound) di-isethionate, *p* : *p'*-diamidino-1 : 2-diphenoxyethane dihydrochloride and stilbamidine (*p* : *p'*-diamidinostilbene) di-isethionate. All four were about twice as active as the aromatic monoamidines, and therefore of the same order of activity as Synthalin B. Molecule for molecule pentamidine and Synthalin B. are approximately 50 times as active as guanidine.

The Effect of Changes in the Amidine Group.—The amidine group $-C(:NH)NH_2$ was found to be highly specific in causing a reduction in the inactivation of adrenaline by the liver. A number of modifications of the group were investigated, all of which greatly decreased the activity of the parent compound. Thus marfanil (*p*-sulphonamidobenzylamine hydrochloride) and V335 (*p*-sulphomethoxybenzylamine hydrochloride) are the benzylamines ($-C_6H_4.CH_2NH_2$) corresponding to the two benzamidines V147 and V187 ;

the benzylamines have only 1/20 of the activity of the benzamidines. V186 (*p*-sulphonamidobenzamidoxime hydrochloride) is the benzamidoxime ($-C_6H_4.C(:NOH)NH_2$) corresponding to V147, and has 1/100 of the activity of the latter. *p*-Aminobenzoic acid and *p*-aminobenzene sulphonamide (the aniline ($-C_6H_4.NH_2$) compound corresponding to V147) also have only 1/20 of the activity of *p*-aminobenzamidine hydrochloride. Similarly semicarbazide hydrochloride ($NH_2.CO.NH.NH_2$) was much less active than guanidine hydrochloride. Phenylbenzamidine hydrochloride ($C_6H_5.C(:NH)NHC_6H_5$) was also considerably less active than *p*-aminobenzamidine hydrochloride. It is evident that among the various substances which were tested, those containing the amidine group were the most active.

Composition of the Rest of the Molecule.—In compounds containing an amidine group small alterations in the other parts of the molecule influence the activity very greatly. This has already been demonstrated for aliphatic monoamidines, diamidines and diguanidines. The introduction of a carboxyl group, as in creatine and arginine, reduces the activity considerably. Creatine in doses of 15 mg. in a spinal cat occasionally caused a slight potentiation ; 10 mg. arginine was virtually inactive, but 50 mg. caused a slight potentiation. Both were less active than guanidine, molecule for molecule.

Table II gives a list of other substances which also were relatively inactive, and of the doses in which they were injected into spinal cats.

TABLE II

Inactive Substances	*Dose*
Urea	20–30 mg.
Thiourea	80 mg.
Thiouracil (relatively insoluble)	1 mg.
S-methyl *iso*thiourea iodide	1 mg.
Alloxan	10–30 mg.
Sulphaguanidine	2 mg.
Dodecamethylene di-*iso*thiourea dihydrochloride	1 mg.

Discussion

There is a tendency in the literature to over-emphasize the importance of the liver in the physiological inactivation of adrenaline, yet as long ago as 1905 Elliott concluded "that adrenaline disappears in the tissues which it excites." Markowitz and Mann (1929) showed that exclusion of the liver in dogs caused only a slight increase and prolongation of the rise of blood pressure produced by injection of adrenaline, and they suggested that though the liver may play some part in the destruction of adrenaline, this part is no greater than can be accounted for by as much of the vascular tree as is contained therein. Bacq (1937) came to the same conclusion, using the nictitating membrane of the cat as an index of adrenaline activity after temporary occlusion of the circulation to the intestines.

or after total evisceration. This view receives further support from the experiments with guanidine and amidine derivatives. If the liver played any great part in the inactivation of adrenaline in the general circulation, the potentiation by amidines of adrenaline injected into the portal circulation would be matched by a similar potentiation on intrajugular injection, whereas in fact the latter is so small that it was not discovered until particular search was made. The principal physiological problem must therefore be the inactivation of small quantities of adrenaline outside the liver, and the work which has been done on adrenaline inactivation in the liver can only be applied elsewhere by analogy.

Mono-amine Oxidase.—Ephedrine (Blaschko, Richter and Schlossman, 1937), cocaine (Philpot, 1940), and methylene blue are known to inhibit mono-amine oxidase *in vitro*. Philpot and Cantoni (1941) have shown that the pressor effect of adrenaline injected into the portal circulation of spinal cats was greatly augmented by methylene blue, and in this paper the same was found to be true of ephedrine and cocaine. Yet it seems very improbable that amidine and guanidine derivatives reduce the inactivation of adrenaline in the liver by their action on mono-amine oxidase. For the aromatic mono-amidine V147 and guanidine scarcely inhibit mono-amine oxidase at all (Blaschko and Duthie (1945); and personal communication); and pentamidine (which inhibits mono-amine oxidase strongly) potentiates the pressor action of corbasil on intraportal injection, in spite of the fact that corbasil, because of the $-CH_3$ group on the α-carbon atom of its side chain, is not attacked by mono-amine oxidase. There are two possibilities. First, mono-amine oxidase may play an insignificant part in the inactivation of adrenaline in the liver; if this is so we have to seek another explanation for the potentiation of adrenaline by tyramine, ephedrine, cocaine and methylene blue on intraportal injection. It is, for instance, possible that they inhibit the conjugation of adrenaline in the liver to form a sulphuric acid ester (Cf. Richter, 1940). Secondly, mono-amine oxidase may play the main part, but amidine derivatives may prevent adrenaline ever reaching it, by interfering with the uptake of adrenaline by the liver cells.

Bactericidal and Toxic Actions of Amidines and Guanidines.—Other possibilities may be considered. As the experiments with pentamidine show, it seems very likely that amidines and guanidines are rapidly absorbed and metabolized by the liver. It is improbable that they reduce the inactivation of adrenaline by dilating the liver vessels, and so reducing the time available for adrenaline to diffuse into the liver cells. An attempt to show that the reticulo-endothelial system was responsible for the uptake of adrenaline also failed; blocking it with Thorotrast (Maher, 1944) or indian ink did not increase the pressor action of adrenaline injected into the portal vein of a spinal cat.

In 1938 King, Lourie and Yorke examined some of the diamidine and diguanidine derivatives used in this investigation for trypanocidal activity and toxicity. Fuller (1942) studied their action upon a wide range of cocci, anaerobes, dysentery and other Gram-negative organisms in broth and serum. More recently

Blaschko and Duthie (1945) have investigated their action upon mono-amine oxidase *in vitro*. Their toxicity and hypoglycaemic action have also been studied by Bischoff, Sahyun and Long (1929) and by Broom (1936). The activity of these series of drugs increased with the length of the carbon chain up to a maximum, and decreased as the chain was lengthened still further, whether they were tested for toxicity, hypoglycaemic, trypanocidal or antibacterial action, as inhibitors of mono-amine oxidase or for their effect in potentiating the pressor action of adrenaline injected into the portal vein of spinal cats. The chain lengths at which maximum activities were observed, using these four types of measurement, are recorded in Table III. King, Lourie and Yorke (1938) and Fuller (1942)

TABLE III

Series	Chain Length (n) for Maximal Activity					
	King *et al.* (1938): Trypanocidal	Fuller (1942): Bacteriostatic	Blaschko & Duthie: Inhibition of mono-amine oxidase	Hypoglycæmia	Toxicity mice	Potentiation of adrenaline on intraportal injection
$CH_3(CH_2)_nC(:NH)NH_2$			10	3 (Broom)	4 (Broom)	4
$NH_2(HN:)C(CH_2)_n$ $C(:NH)NH_2$	11	12–15	12	8 (Broom)	10 (Broom) 10–18 (King)	14
$NH_2(HN:)C.NH(CH_2)_n$ $NH.C(:NH)NH_2$	10–14	12–18	14	10 (Bischoff *et al*)	12–16 (King)	12

examined only two aliphatic mono-amidines (those in which $n=14$ and 16) and these had little or no activity. The discrepancy between Blaschko and Duthie's figure for the chain length for maximal activity in the mono-amidine series ($n=10$), and that obtained from intraportal injection into spinal cats ($n=4$), provides yet another illustration of the lack of correlation between the inhibition of mono-amine oxidase *in vitro* and the potentiation of adrenaline *in vivo*. With the diamidines and diguanidines, however, the agreement as to the chain length for maximal activity is better. In the diamidine series this is covered by the range $n=8$–18, and in the diguanidine series by $n=10$–18. Fuller (1942) remarked that the increase of activity with chain length in his series suggested that surface active properties might be in part responsible for the activity of these compounds, and that they might act by combining with and denaturing some essential protein constituent. This would provide an explanation for the agreement in the chain length for maximal activity in such diverse biological measurements. But whereas Fuller (1942) found that for bacteriostatic activity the difference caused by interchange of the end groups (with *iso*thioureas, amines, amidines, guanidines and quaternary ammonium salts) was not large, in the present investigation the specificity of the amidine (or guanidine) group was remarkably high.

The pharmacological evidence is not in agreement with Fuller's further suggestion that these drugs may be general protoplasmic poisons. For instance, while V147 in a dose of as little as 0.1 mg. will potentiate the pressor action of adrenaline after intraportal injection into a spinal cat, it is singularly free from any toxic effect upon the heart (Dawes, 1945). On the other hand, there is ample evidence that guanidine derivatives in general cause liver damage in lethal doses, a point which accounts for the close correlation (in homologous series) in the chain length for maximal toxicity and for maximal hypoglycaemic action (Table III). This evidence requires no amplification for guanidine or Synthalin, and the work of Bischoff *et al.* (1929) and of Broom (1936) suggests that many other guanidines, mono-amidines, alkylene diamidines and aromatic mono-amidines also cause liver damage in experimental animals. More recently Daubney and Hudson (1941), Wien, Freeman and Scotcher (1943) and Allen, Burgess and Cameron (1944) have made similar observations with aromatic diamidines in animals, and there has unfortunately been confirmation of their conclusions in man (e.g. Kirk and Henry, 1944). Hawking and Smiles (1941) injected stilbamidine subcutaneously into mice and observed the viscera under ultra-violet light. The fluorescence of stilbamidine gave a rough idea of its distribution, and "judging by the appearances it would seem that the diamidinostilbene collects especially in the liver and kidney." There is therefore evidence for a more general hypothesis. It has not proved possible from the available data to decide upon the specific means by which amidines and guanidines reduce the inactivation of adrenaline during its passage through the liver, but it is possible that this reduction is one easily measurable manifestation of the toxic action of these drugs upon the liver. Indeed one might suggest that all new drugs containing the amidine group which are likely to be used clinically should be examined to see whether they increase the pressor action of adrenaline on injection into the portal vein of a spinal cat. This test is far more rapidly performed than conventional liver function tests, which, of course, it could never replace. But if a new drug did possess a high degree of activity in reducing the inactivation of adrenaline in the liver, then the greatest caution would be required in assessing its action on liver function, and in its preliminary trials on man.

SUMMARY

1. Adrenaline injected into the portal vein of a cat, rabbit or dog has less action on the circulation, spleen or nictitating membrane than when injected into the jugular vein. This difference between intraportal and intrajugular injection is decreased by the simultaneous administration of aromatic and aliphatic diamidine and mono-amidine derivatives, of diguanidines and guanidine itself. These substances are therefore believed to reduce the inactivation of adrenaline during its passage through the liver.

2. The inactivation of adrenaline during its passage through the liver is also reduced by ephedrine, tyramine and cocaine. But whereas the latter also

potentiate the action of adrenaline in the peripheral circulation, amidine and guanidine derivatives either decrease it or have no effect.

3. The amidine group $-C(:NH)NH_2$ was found to be highly specific in producing a reduction of adrenaline inactivation in the liver. Closely related benzylamine, aniline and benzamidoxime derivatives did not possess this property in a comparable degree.

4. The basis for the reduction of the inactivation of adrenaline in the liver by amidine and guanidine derivatives is not known. It is not due to inhibition of mono-amine oxidase, but the facts could be explained by these drugs interfering with the penetration of adrenaline into the liver cells.

5. The variation in activity with change in chemical structure in a series of amidine and guanidine derivatives was examined. In two homologous series of aliphatic diamidines and diguanidines the chain length for maximal activity in reducing adrenaline inactivation in the liver was found to be very similar to that recorded by other observers for maximal toxicity, hypoglycaemic, trypanocidal and bactericidal activity, and activity in inhibiting mono-amine oxidase *in vitro*.

This work was done during the tenure of a grant from the Medical Research Council. I wish to thank Professor J. H. Burn for the guidance and advice he has given throughout.

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OBSERVATIONS ON THE ISOLATED PHRENIC NERVE DIAPHRAGM PREPARATION OF THE RAT

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(Received January 11, 1946)

The work which led up to the investigation described in this paper was concerned with the influence of adrenaline on the action of acetylcholine or on tissue functions normally elicited by acetylcholine. Previously experiments were carried out on animals with a normal circulation or on perfused organs, and any adrenaline effects observed were accompanied by vasoconstriction, so that it was conceivable that its action was due to vascular changes.

The improvement in the transmission of impulses along the motor nerve (Bülbring and Burn, 1939) or the lowering of threshold to submaximal stimuli applied to the sciatic nerve (Bülbring and Whitteridge, 1941) by injecting adrenaline into the circulation might have been due to changes in the distribution of blood in the vascular bed of the nerve trunk leading to an alteration of electrical resistance. However, there was a discrepancy in time relations, the increased nervous excitability lagging behind and long outlasting the vascular effect of adrenaline.

In fatigued skeletal muscle adrenaline—and other vasoconstrictor substances —augment muscle contractions (Bülbring and Burn, 1940), but Maibach (1928) and Corkill and Tiegs (1933) obtained the same result on frog muscle suspended in a bath, showing thereby that the action of adrenaline on the fatigued nerve muscle preparation can be observed without its vascular action. Presumably, in fatigue, neuromuscular transmission gradually fails and is restored by adrenaline. When, for instance, Bülbring and Whitteridge (1941) recorded muscle tension and nerve action potentials, a striking absence of parallelism between the effect of adrenaline on muscle and nerve was observed. At a time when no effect of adrenaline on the nerve response to maximal shocks at 1 per sec. could be seen, 100 per cent increase was seen in the fatigued muscle.

In non-fatigued skeletal muscle stimulated indirectly with maximal shocks, it was found that adrenaline augmented the effect of prostigmine. The results were taken to indicate that adrenaline facilitates neuromuscular transmission and increases the action of acetylcholine on the muscle (Bülbring and Burn, 1942). Though the dose of adrenaline used was always the same, producing a similar degree of vasoconstriction each time, its effect on the muscle was dependent not only on the presence of prostigmine and on the amount of prostigmine given, but

also and chiefly on the rate of stimulation, i.e. on the rate of accumulation of acetylcholine produced at the motor ending. Nevertheless, vascular changes along the nerve might have occurred and it seemed desirable to repeat the experiments on an isolated mammalian nerve-muscle preparation in which the vascular action of adrenaline is without effect.

Dale and Gaddum (1930) described experiments on the denervated kitten's diaphragm suspended in a bath. Therefore an attempt was made to dissect a slip of kitten's diaphragm with the phrenic nerve attached, to suspend it in an isolated organ bath and record the contractions to nerve stimulation. But even with single shocks at a slow rate (5 per min.) the muscle twitches slowly declined, the oxygenation presumably being insufficient. The thinner muscle of a rat's diaphragm was, however, found to work satisfactorily and was used for all the experiments to be described.

METHOD

Adult rats were used. The rat was killed and bled out. After removing the skin over the chest, the thorax was opened along the right side of the sternum and the frontal part of the right thoracic wall was removed. The mediastinum behind the sternum was severed and a cut was made just above the frontal insertion of the diaphragm, taking care not to damage the phrenic nerve, which sometimes attaches itself to the ribs. The frontal part of the left thoracic wall was then removed and the phrenic nerve was seen quite distinctly. Both left lobes of the lung were removed. The left abdominal muscles were cut along the costal margin and the last rib was held with a pair of forceps. A strip of diaphragm was now cut out. Two converging cuts were made through the ribs towards the tendinous part of the diaphragm parallel to its muscle fibres, 3 mm. to the right and 3 mm. to the left of the point where the phrenic nerve enters the diaphragm. The strip was cut out beyond the tendinous part with about 2.5 cm. of phrenic nerve attached to it. The preparation had a fan-like shape, being 3 mm. wide at the tendinous end, while at the costal margin it was about 12 mm. wide. The preparation was fixed by a glass rod with a pin pushed through an intercostal space holding it at the bottom of the organ bath. A thread tied around the tendinous end was attached to a light isotonic heart lever writing on a smoked drum. The nerve was stimulated by a pair of electrodes of the following design (see Fig. 1). Two platinum plates were enclosed in a small block of perspex with wires running up in a perspex handle 6 cm. long. The platinum plates were 1 mm. thick and 3 mm. square, they were lying parallel in the perspex block, 5 mm. square and 6 mm. thick, in such a way that they were separated by 2 mm. perspex. A hole about 1 mm. wide was drilled centrally through this block containing the plates to take the phrenic nerve which thus passed through a tube of perspex with two platinum rings. This arrangement ensured a good contact for the electrical stimulation and also provided a moist chamber for the nerve, preventing it from drying up. The electrodes were fixed vertically just touching the surface of the solution inside the bath. After the phrenic nerve had been pulled up through the hole a moist piece of cotton wool was placed on top. Single shocks were applied

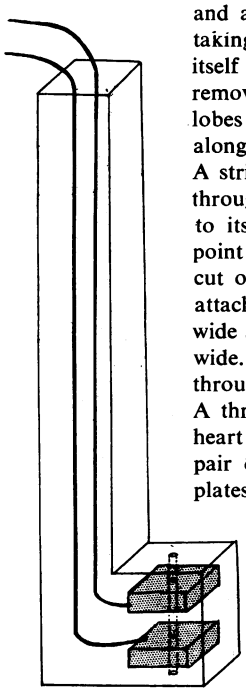

FIG. 1.—Perspex electrodes.

to the nerve from a neon lamp circuit at rates varying from 5 to 50 per min. Constant muscle contractions could be obtained for many hours in response to single shocks at rates not exceeding 12 per min. With faster rates fatigue set in if they were applied for prolonged periods. A tetanus was not maintained by this isolated preparation.

Some experiments were performed using fluid electrodes (Collison, 1933) in which the nerve was stimulated in a chamber sealed off with wax at the bottom so that the fluid surrounding the nerve at the point of stimulation was not in contact with the fluid surrounding the muscle.

Tyrode's solution containing double the usual amount of dextrose was used. A sintered glass gas-distribution-tube was fixed at the bottom of the bath providing vigorous oxygenation with a mixture of 95 per cent O_2 + 5 per cent CO_2. The capacity of the bath was 100 c.c. and the temperature was kept between 36° and 37° C.

RESULTS

1. *The Action of Adrenaline.*

When the phrenic nerve was stimulated with single *maximal* shocks a series of muscle contractions of equal height was obtained. The tension developed in different preparations varied from 10–25 g. It remained constant for several

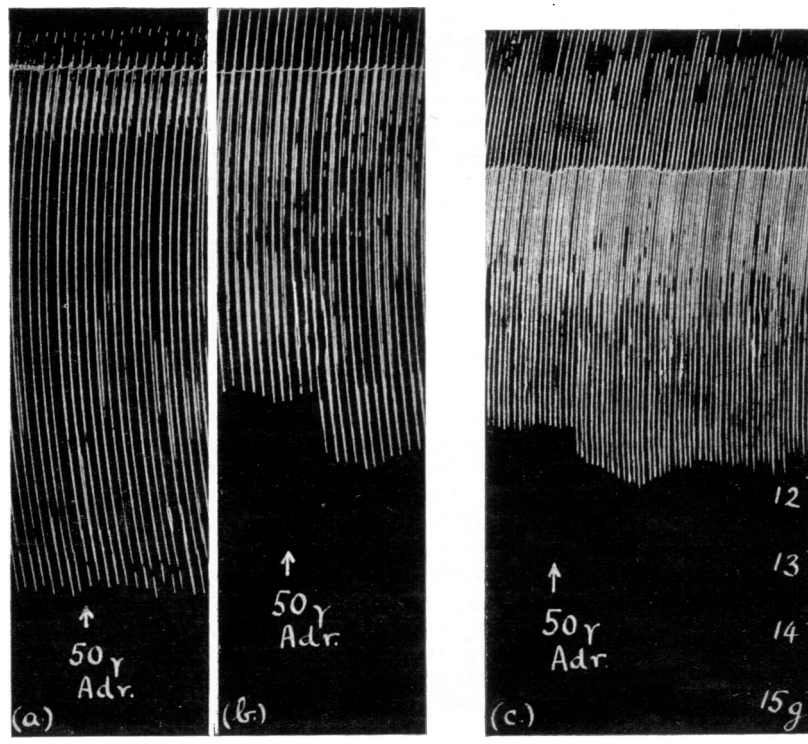

FIG. 2—Contractions of rat's diaphragm. *(a)* and *(b)* stimulation of phrenic nerve with open electrodes, *(c)* with fluid electrodes. The effect of 50 μg. adrenaline on the muscle response to maximal *(a)* and submaximal single shocks *(b)* and *(c)*.

hours if the rate of stimulation did not exceed 12 per min. With faster rates the muscle slowly became fatigued. While the muscle contracted maximally and showed no sign of fatigue, the addition of adrenaline to the bath had no effect on the size of contractions. However, when the phrenic nerve was stimulated with *submaximal* shocks, the muscle contractions were slightly increased by the addition of 10 μg.–50 μg. adrenaline to the bath. This increase never exceeded 20 per cent. In Fig. 2a is shown a record of maximal contractions of the diaphragm ; their size was not affected by adrenaline. In Fig. 2b the same muscle was stimulated with submaximal shocks ; 50 μg. adrenaline now caused a bigger muscle response. When this experiment was repeated on another preparation using fluid electrodes (Collison, 1933) the same result was obtained (Fig. 2c). The chamber in which the nerve was enclosed for stimulation was sealed off with wax at the bottom. Thus the adrenaline which was added to the bath had no direct access to the nerve at the site of stimulation and therefore presumably exerted its action nearer to the muscle. In Fig. 3 is shown another experiment in which fluid electrodes were used. The action of adrenaline on maximal stimuli at a slow rate of 6 per min. (a), was compared with that on submaximal stimuli at the same rate (b). A similar adrenaline effect was observed on submaximal stimuli at a faster rate of 18 per min. in (c). Maximal stimuli were then applied (d) and after prolonged stimulation fatigue reduced the muscle contractions to the same size as they had been initially in response to the submaximal stimulation in (c). The adrenaline effect was very similar both in (c) and (d), suggesting an improvement of transmission in both, which was maintained in (c) but not in the fatigued muscle (d).

2. *The Action of Prostigmine and Eserine.*

As adrenaline had no effect by itself upon maximal muscle twitches the modification of the action of prostigmine and eserine by additional adrenaline was studied with maximal stimuli only. The effect of prostigmine was found to be dependent on the dose and on the rate of stimulation. With slow stimulation

TABLE I

THE EFFECT OF PROSTIGMINE ON THE SIZE OF MUSCLE CONTRACTIONS ELICITED BY MAXIMAL SINGLE SHOCKS

Dose of Prostigmine	Rate of stimulation per min.			
	5–8	9–12	14–16	18–24
0·1 μg.–0·2 μg.	no effect or increase	increase	increase	increase
0·25 μg.–0·3 μg.	increase	increase	uncertain	—
0·4 μg.–0·5 μg.	increase	uncertain	increase followed by depression	—
1–2 μg.	uncertain	increase followed by depression	depression	depression
10 μg.	depression	—	—	—

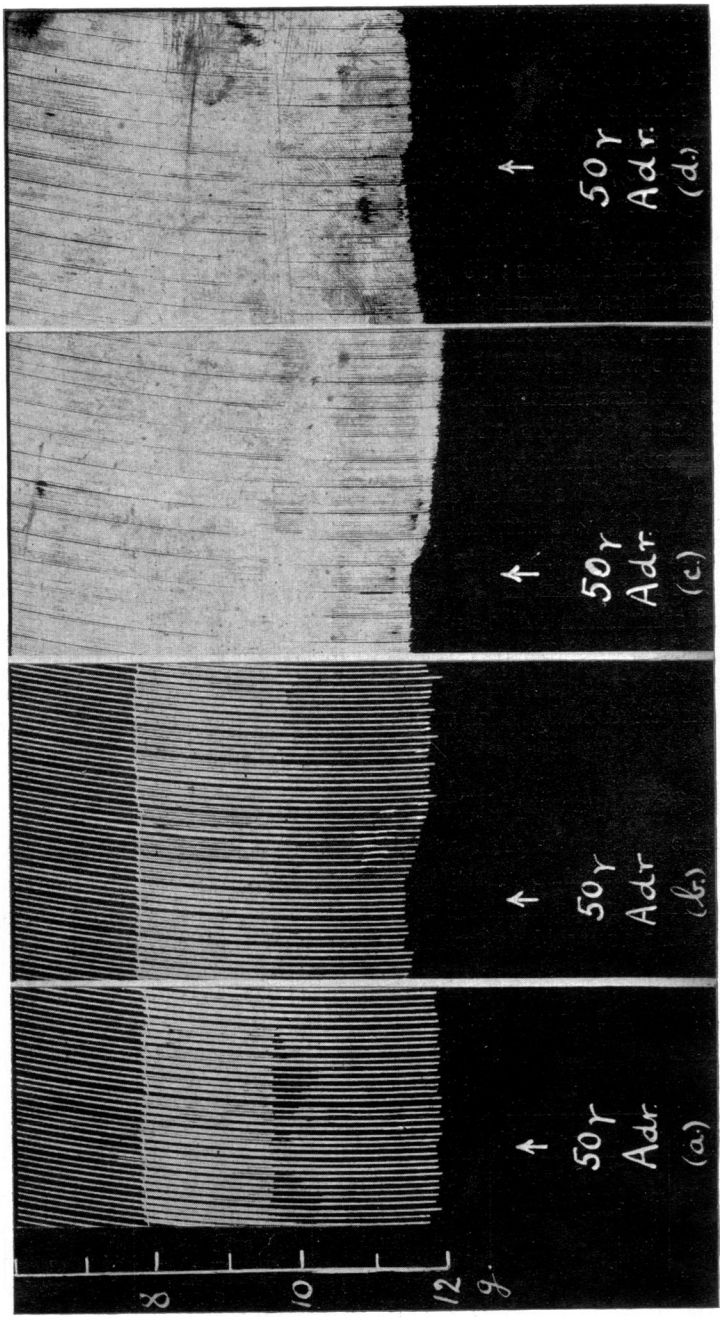

FIG. 3.—Rat's phrenic nerve-diaphragm preparation. Comparison of the adrenaline effect at different rates of stimulation: *(a)* maximal, 6 per min.; *(b)* submaximal, 6 per min.; *(c)* submaximal 18 per min.; *(d)* muscle fatigued by maximal stimulation 18 per min.

(5 per min.) the threshold dose producing increased tension was about 0.1 μg. prostigmine or a concentration of 1 in 1,000 million. The accompanying table was compiled from observations in 20 different experiments.

It can be seen from Table I that small doses of 0.1 μg.–0.2 μg. prostigmine caused an increase of muscle contractions at slow and at fast stimulation rates up to 24 per min. The effect of ten times that amount of prostigmine was uncertain, sometimes producing a temporary augmentation followed by depression.

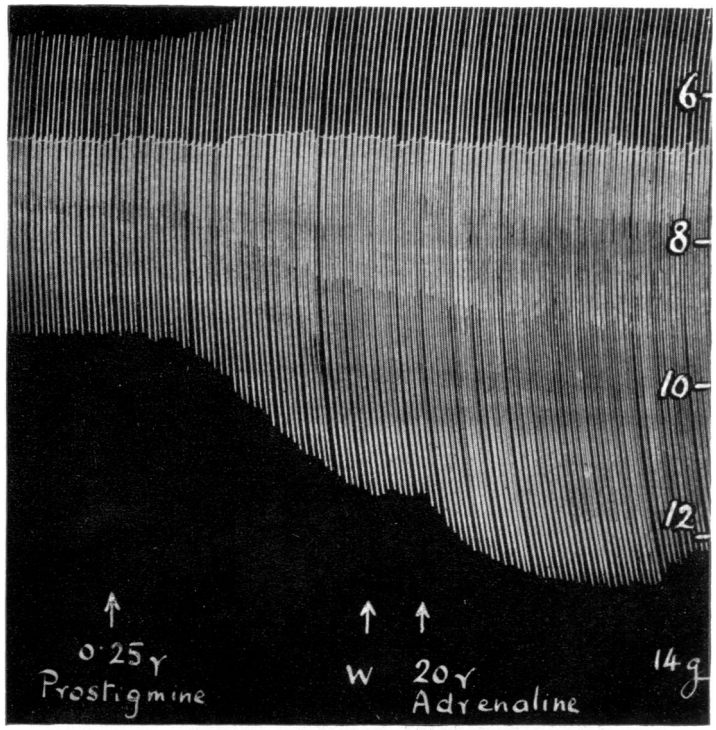

FIG. 4.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 9 per min. Prostigmine causes an increase of muscle contractions, and after a wash out (W) adrenaline causes a further augmentation.

At a slow rate of stimulation, as is seen from Table I, a small dose of prostigmine might either have no effect or cause a rise in muscle contractions. If now adrenaline was added to the bath a further augmentation was sometimes observed. But mostly the adrenaline had no apparent effect unless the prostigmine was washed out before the adrenaline was added. After doses of prostigmine exceeding 0.3 μg. or with faster stimulation, adrenaline usually caused depression of the muscle contractions, but again an augmentation was almost always seen if the prostigmine was first washed out.

Fig. 4 shows the contractions of the diaphragm in response to maximal shocks at 9 per min. A dose of 0.25 μg. prostigmine added to the bath increased the size of contractions. After washing out, 20 μg. adrenaline caused a further increase. If the prostigmine was not washed out, the opposite effect was often observed. In Fig. 5 the rate of stimulation was faster, 18 per min. After 0.1 μg. prostigmine, which by itself scarcely affected the size of contractions, had been washed out, 10 μg. adrenaline caused an augmentation (Fig. 5a); but when the same small dose of 0.1 μg. prostigmine had been repeated, again producing no

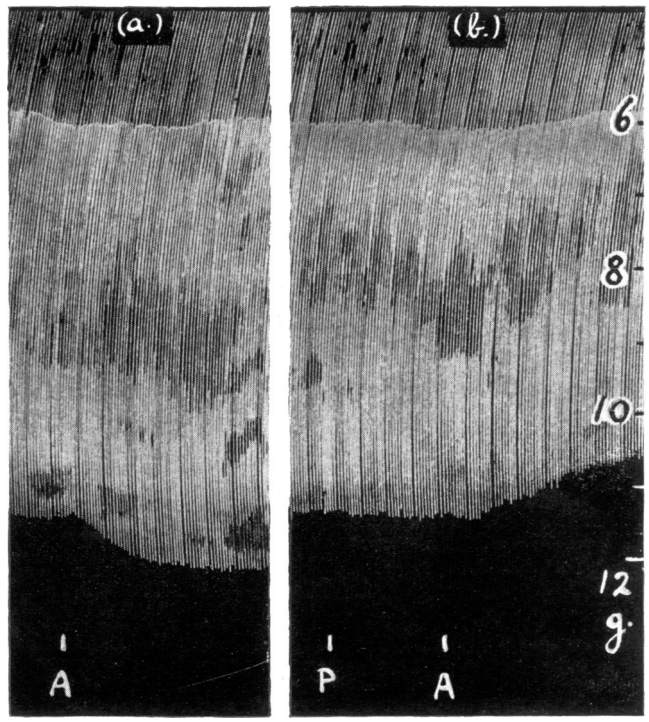

FIG. 5.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 18 per min. 0.1 μg. prostigmine had been given beforehand and was washed out; in *(a)* 10 μg. adrenaline (A) caused a rise in muscle contractions. In *(b)* when 0.1μg. prostigmine (P) was *not* washed out 10 μg. adrenaline (A) caused a fall in muscle contractions.

immediate effect of its own, and was followed 2 min. later by 10 μg. adrenaline, a depression resulted as shown in Fig. 5b.

It may be assumed that the isolated muscle, without a circulation to remove added prostigmine, is progressively affected by the drug, however small the dose, as long as it is left in the bath. Thus more and more acetylcholine is prevented from destruction by cholinesterase. If adrenaline is now added as well, it can only increase muscle contractions before the stage of acetylcholine excess is

reached. The augmentation should therefore be shown best after the prostigmine has been removed from the bath. This is demonstrated in the experiment shown in Fig. 6. The rate of stimulation was 7 per min. ; 0.1 μg. prostigmine was added to the bath. It increased muscle contractions and was allowed to act for 3 min., when it was washed out. Two minutes later adrenaline was added causing a further increase. After the washing out, the muscle contractions slowly returned to their initial level. The dose of 0.1 μg. prostigmine was now repeated and allowed to act for 5 min. causing an augmentation which was, however, less than that produced by the addition of adrenaline before. When now adrenaline was

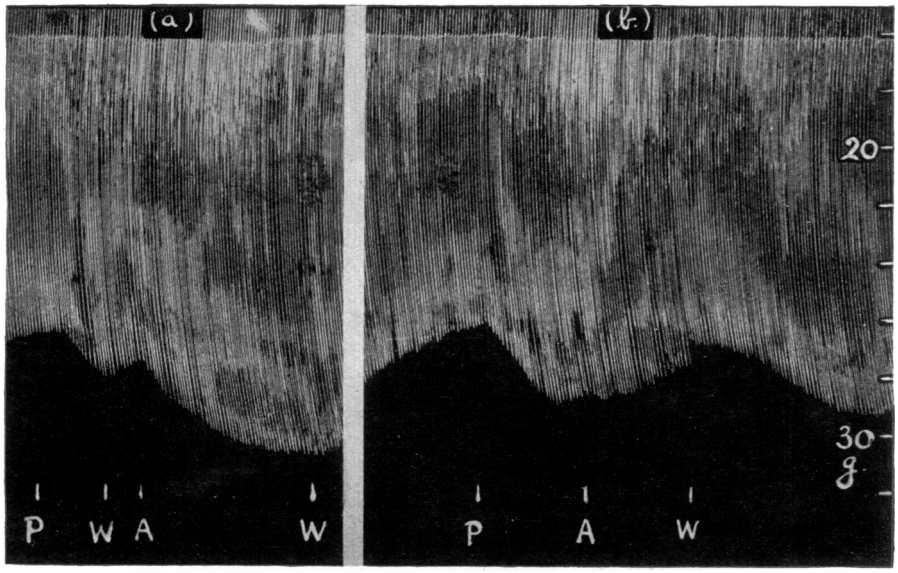

FIG. 6.—Rat's phrenic nerve-diaphragm preparation. Effect of adrenaline *(a)* after and *(b)* before washing out a previous dose of prostigmine. Maximal single shocks 7 per min. P = 0.1 μg. prostigmine ; W = wash, A = 10 μg. adrenaline

added no effect was seen, the size of muscle twitches slowly declining until the solution was changed. The muscle contractions, instead of returning to their initial size, now gradually increased as though the full adrenaline effect only developed at this stage. Similar observations were made repeatedly and an example is shown in Fig. 7. The rate of stimulation was 14 per min. in this experiment. A dose of 0.1 μg. prostigmine produced three times an almost identical augmentation (P). The first dose was washed out, whereupon adrenaline caused its typical further augmentation (A_1). After the solution had been changed, the muscle contractions returned to normal. The second dose of prostigmine was not washed out, whereupon adrenaline (A_2) caused a slight depression. After another wash out, the muscle contractions increased and a

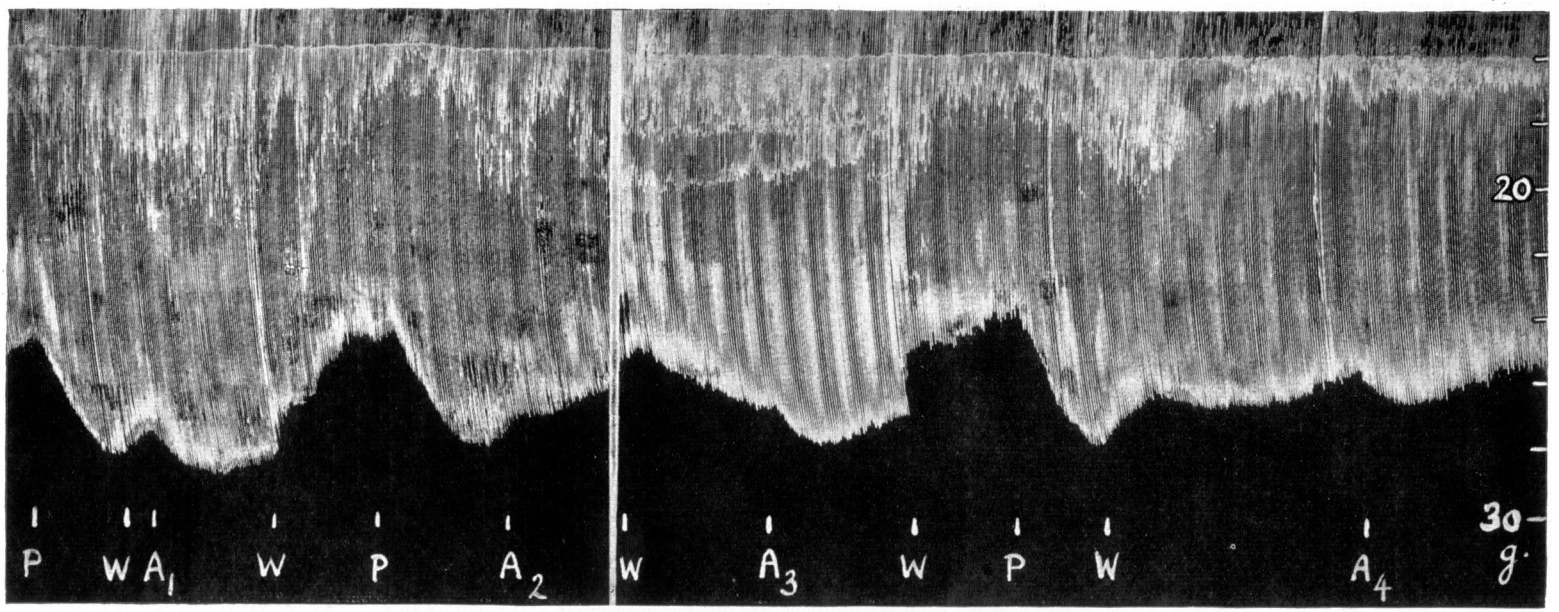

FIG. 7.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 14 per min. P = 0.1 μg. prostigmine, W = wash, A = 20 μg. adrenaline. For description see text.

further rise was obtained with adrenaline (A_3). When this was washed out, contractions once more returned to normal. The third dose of prostigmine was washed out but no adrenaline was added until 10 min. later, when it produced its typical rise (A_4).

There is no qualitative difference between the action of prostigmine and that of eserine, but they differ quantitatively. The threshold dose of eserine was found to be as low as 0.01 μg. or 1 in 10,000 million, which produced a rise in muscle contractions during slow stimulation at 5 per min. In a fresh preparation

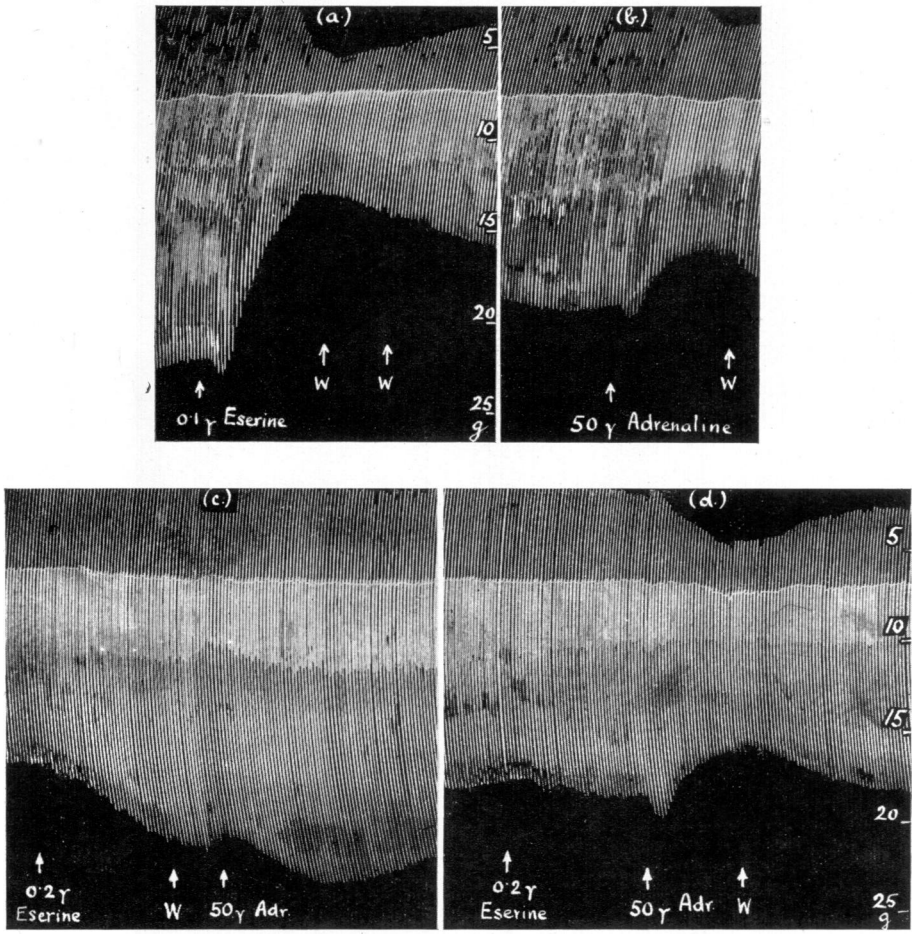

FIG. 8.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 5 per min. W. = wash. *(a)* shows the depression caused by 0.1 μg. eserine on a fresh preparation, *(b)* the effect of 50 μg. adrenaline 35 min. later, *(c)* shows the augmentation caused by 0.2 μg. eserine in the same preparation 3 hours later, and the further increase due to 50 μg. adrenaline after eserine had been washed out. In *(d)* 50μg. adrenaline was added without removing the eserine ; note the similarity between *(d)* and *(b)*.

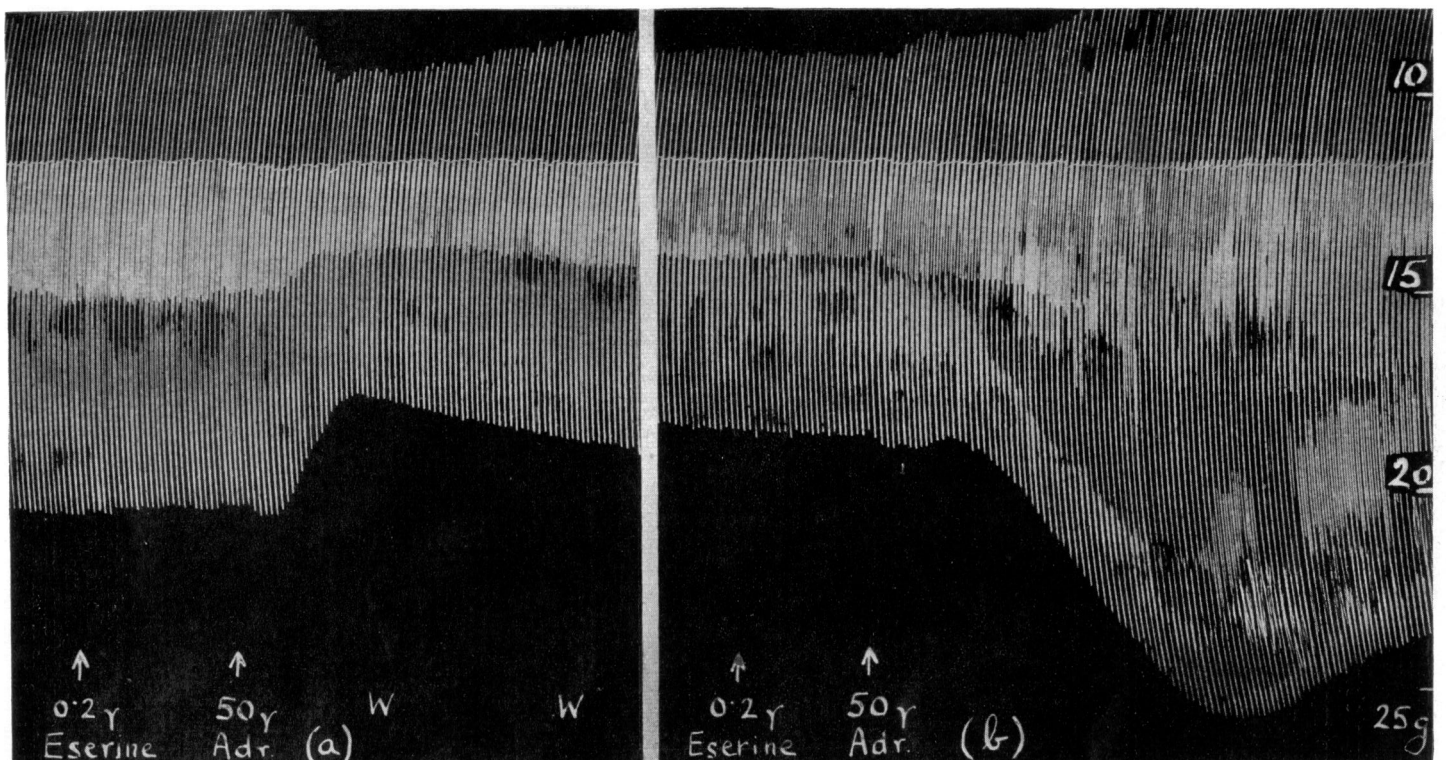

FIG. 9.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 6 per min. In a fresh preparation *(a)* adrenaline caused a depression in the presence of eserine; 3 hours later *(b)* it caused augmentation.

as little as 0.1 μg. eserine was seen to cause depression (Fig. 8a). This depression had not passed off 35 min. later though the solution had been changed twice. When adrenaline was added a further depression was observed (Fig. 8b). The preparation was now left for 4 hours, but in spite of washing, the height of contractions did not return to normal. At this stage, however, 0.2 μg. eserine produced a big rise in tension and, after it had been washed out, adrenaline caused a further rise (Fig. 8c). After the solution had been changed, the contractions were allowed to return to their former level and 0.2 μg. eserine was once more added (Fig. 8d) followed—(without wash)—by adrenaline which caused a depression similar to that of 4 hours previously in Fig. 8b. The two opposite effects of adrenaline shown in Fig. 8c and d in the presence of eserine correspond precisely to those shown in Fig. 5a and b, in the presence of prostigmine.

There was no doubt that if the rat's diaphragm had been prepared for several hours and was stimulated continuously at a slow rate, its sensitiveness to the action of prostigmine and eserine gradually declined. With stimulation at 8 per min. in a fresh preparation, 2 μg. prostigmine caused a short increase of muscle contractions followed by a depression. In another preparation, which had been working for several hours, 20 μg. prostigmine still produced a big increase which then gave way to depression. Similarly adrenaline is more likely to produce a muscular depression in the beginning of an experiment than at a later stage. In Fig. 9a adrenaline following 0.2 μg. eserine caused a big depression, but 3 hours later as shown in Fig. 9b, the same dose produced a big increase.

3. *The Action of Atropine, Curarine and Procaine.*

The action of atropine has been studied both before and after prostigmine or eserine. In big doses atropine shows its well-known "curare-like" action. The contractions of the isolated rat's diaphragm were diminished by doses of 4–5 mg. atropine, a concentration of 1 in 20,000 to 1 in 25,000. Sometimes, however, a very slight transitory increase was seen before the gradual decline. With smaller doses of atropine up to 0.5 mg. no effect on normal muscle contractions could be seen; but after the addition of 1–2 mg. to the bath, making a concentration of 1 in 50,000 to 1 in 100,000, the curious observation was made that the muscle responses to maximal single shocks were increased as is shown in Fig. 10a and 11a. There was never a sudden increase, but the effect was always gradual, at the most 20 per cent and more often less. This increase of normal muscle contractions was only obtained in fresh preparations.

The observation that eserine, 1 in 1,000 million, on a fresh preparation might cause a depression of muscle tension produced by maximal shocks (see Fig. 8a) raised the question whether a fresh preparation was in a condition of mild acetylcholine paralysis? Dale and Gaddum (1930) observed that atropine antagonized the action of acetylcholine on the isolated denervated kitten's diaphragm. Brown (1937) showed that in the frog's gastrocnemius the injection

of 0.1 c.c. atropine sulphate, 1 in 1,000, abolished the muscle response to motor nerve stimulation and depressed, but did not abolish, the muscle response to a close intra-arterial injection of acetylcholine. On the other hand, Abdon (1940) found that those doses of atropine which abolished the muscle twitch caused by acetylcholine injected intra-arterially, did not have any influence on the muscle contractions provoked by motor nerve stimulation. In his experiments on frogs the muscle was immersed in atropine sulphate, 1 in 10,000, while in rabbits

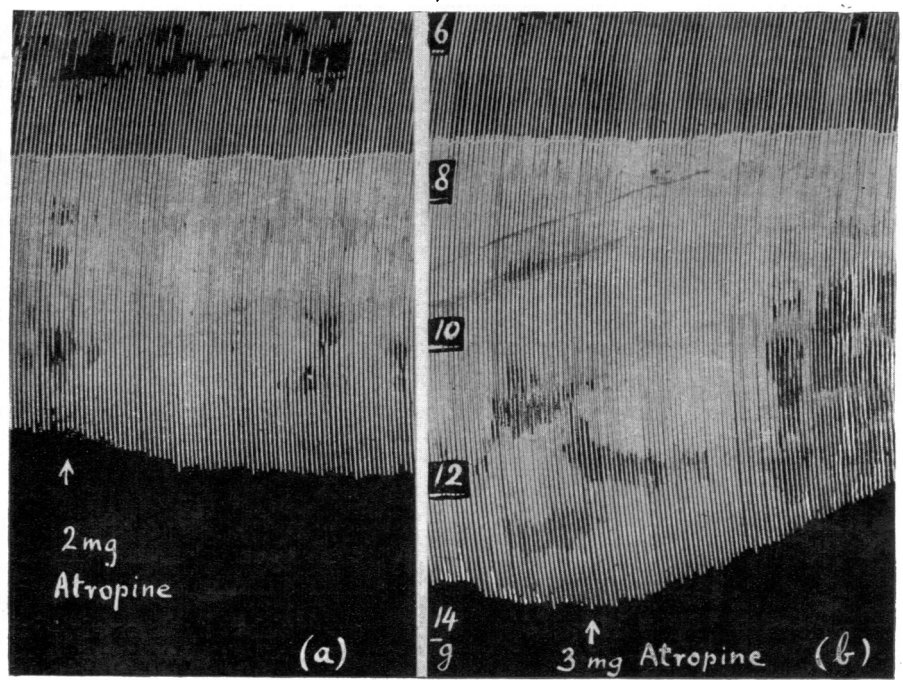

FIG. 10.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 8 per min. The effect of atropine on *(a)* the contractions of a fresh muscle, *(b)* on the contractions increased by 0.2 μg. eserine.

10 mg./kg. atropine sulphate was injected intravenously. Abdon suggested that authors who failed to abolish the effect of intra-arterially injected acetylcholine seemed to have used too small amounts of atropine. But Brown used 10 times as much as Abdon and the discrepancy of their findings cannot therefore be explained in this way.

In the isolated rat's diaphragm atropine caused a depression of muscle contractions in response to maximal nerve stimuli when they were increased by eserine or prostigmine. This is shown in Fig. 10b. While the size of muscle contractions was still increasing, owing to the addition of 0.2 μg. eserine to the bath, 3 mg. atropine reduced them to their original size.

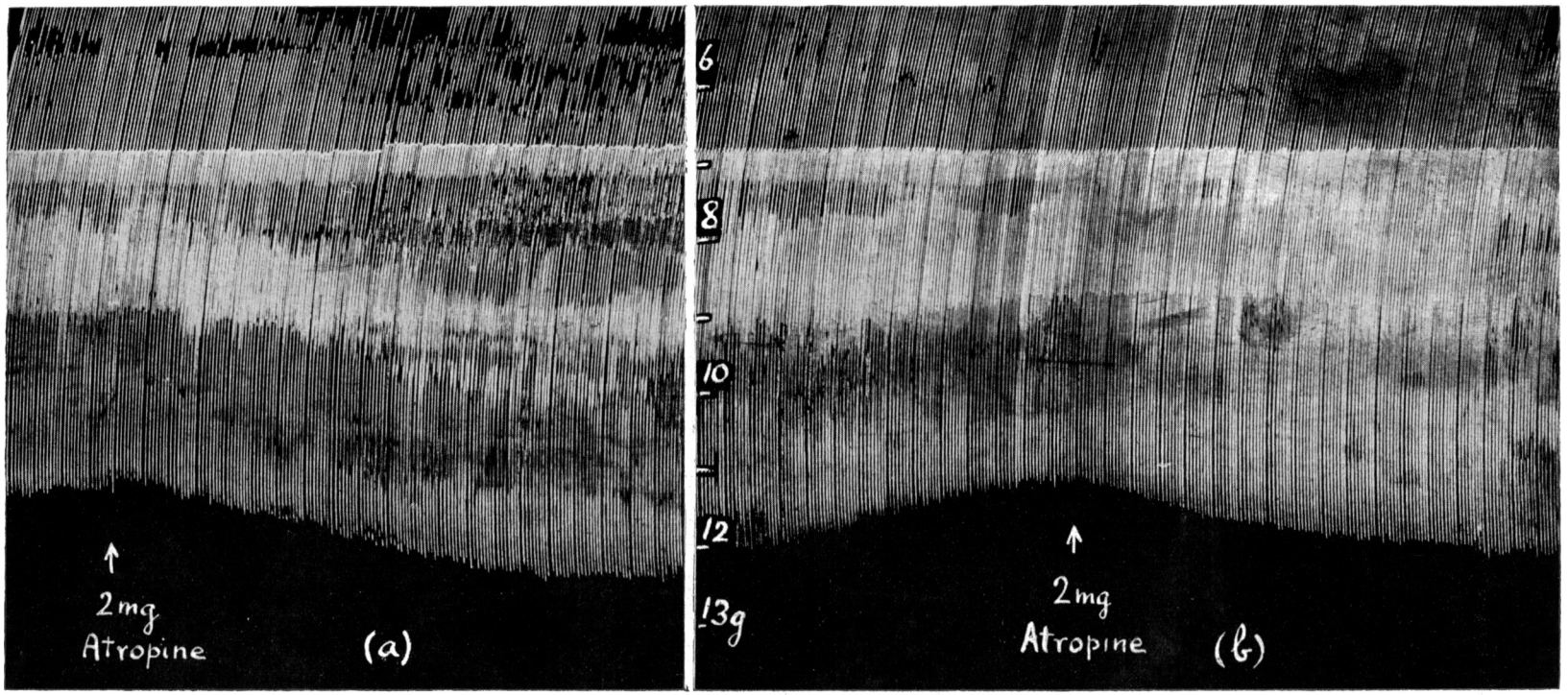

FIG. 11.—Similar to Fig. 10. Maximal single shocks 9 per min. The effect of atropine on *(a)* the contractions of a fresh muscle, *(b)* on the contractions depressed by 20 μg. eserine.

On the other hand, when the muscle contractions were depressed by an overdose of eserine, atropine augmented them. In Fig. 11 is shown an experiment in which atropine, at the beginning of the experiment (Fig. 11a), caused some increase of muscle contractions. The solution was changed several times and the preparation was stimulated continuously for 3 hours. After this period 20 μg. eserine was given and first increased the size of contractions, then depressed them below their original size. When now 2 mg. atropine was added an augmentation was seen (Fig. 11b). There is a striking similarity between the effects in Fig. 10a and b, which suggests that in both instances atropine removes a depression which is due to an excess of acetylcholine.

Briscoe (1936) has shown that subparalytic doses of curarine restore muscle contractions previously depressed by prostigmine, and it seemed interesting to compare the action of atropine with that of curarine. The dependence of the action of prostigmine and of eserine on the frequency of stimulation was studied by Briscoe (1936) and by Bacq and Brown (1937). In Fig. 12a is shown the effect of periods of fast stimulation on the normal muscle before and after 0.5 μg. eserine. Though at a rate of 5 per min. the size of contractions was increased in the presence of eserine, faster stimulation (18 and 50 per min.) caused a rapid decline, the steepness of which was directly proportional to the rate of stimulation. After the administration of larger doses of eserine (2 μg. in Fig. 12b) while the nerve was stimulated at 17 per min. muscle contractions declined rapidly below normal. The addition of 1 mg. atropine stopped this decline and started a slow increase. Further 2 mg. atropine had no more effect than to increase the overthrow of the lever ; (this is due to the muscle relaxing more quickly). When 50 μg. curarine was added to the bath a sudden increase of contractions took place and now, in the presence of curarine, it was found that the relation between the size of muscle contractions and the rate of stimulation was reversed. At a slow rate the height of contractions declined, at a fast rate it increased. It is generally accepted that curarine raises the threshold of the muscle for acetylcholine. Therefore, when the rate of stimulation is slow and, in the presence of eserine, only small amounts of acetylcholine accumulate, the muscle contractions may decline below their maximal size, but with fast stimulation, when more acetylcholine accumulates, a normal height of contractions may be attained.

If one assumes that atropine has only a very slight threshold-raising, curare-like action, this would explain why it was found to be more effective in antagonizing an eserine depression at slow rates of stimulation than at faster rates (compare Figs. 11b and 12b). Fig. 13 shows the muscle contractions of a preparation fully eserinized (100 μg.) and atropinized (4 mg.). At a rate of 5 per min. contractions were bigger than at 18 or 50 per min. But even at this fastest rate no severe depression was seen. If atropine exerted its action only by raising the threshold of the muscle, like curarine but to a lesser degree, then 50 stimuli per min. in the presence of an enormous dose of eserine should have depressed muscle contractions much further. One may therefore attempt to explain the

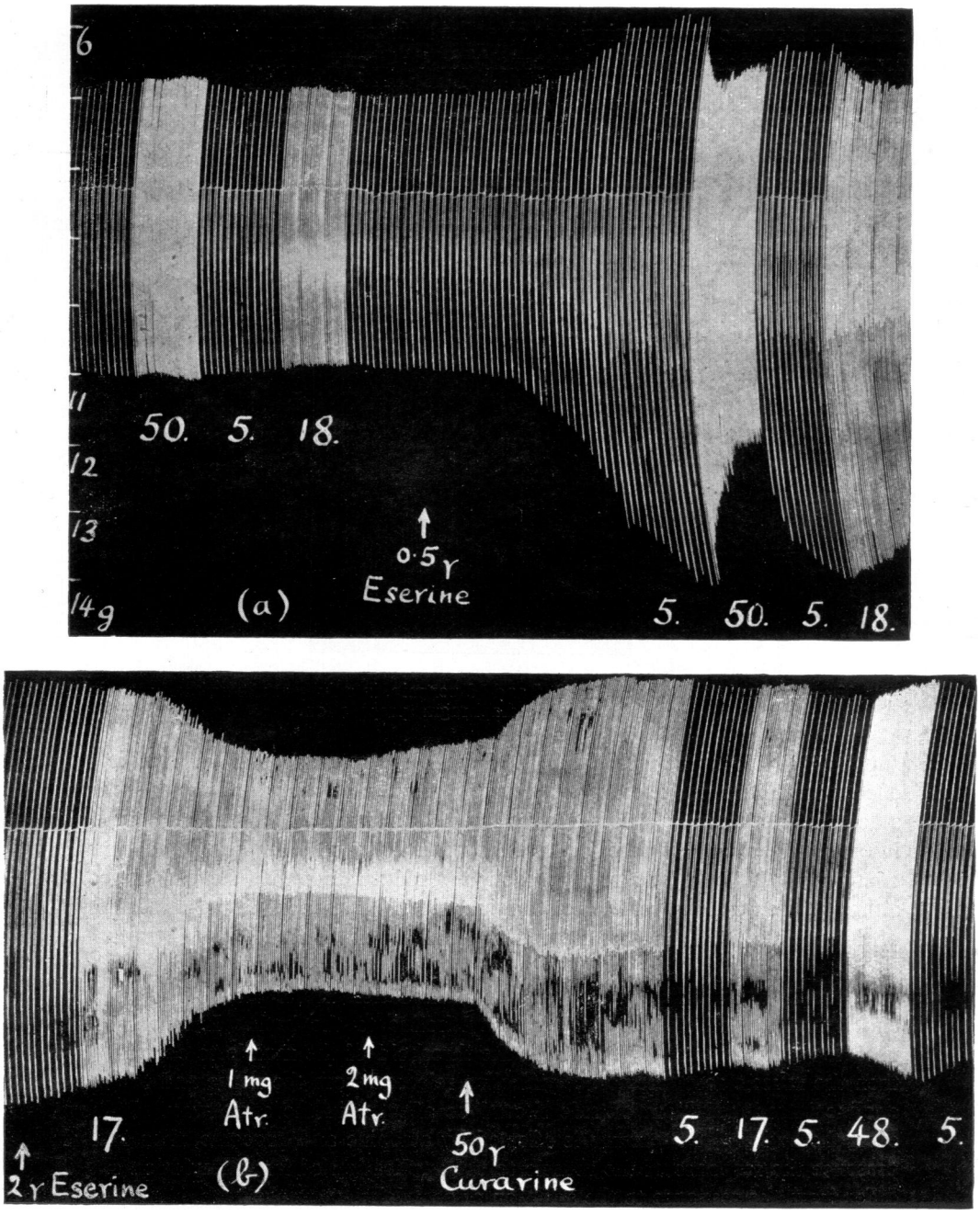

FIG. 12.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks at rates per min. indicated by figures. Note that after eserine *(a)* contractions decline during fast and recover during slow rates, while after curarine *(b)* contractions decline during slow and increase during rapid rates.

action of atropine by assuming that atropine reduces the amount of acetylcholine formed. This possibility was first considered by Brown (1937).

Harvey (1939) showed that procaine suppressed the output of acetylcholine from the superior cervical ganglion during preganglionic stimulation. He suggested that in skeletal muscle procaine, besides acting like curare, also diminished the liberation of acetylcholine from the motor nerve endings. This view was

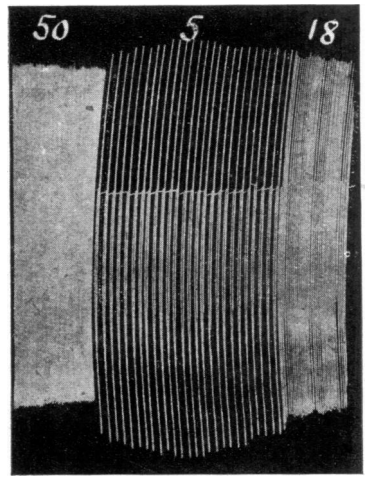

FIG. 13.—Maximal single shocks at 50, 5 and 18 per min. in a fully eserinized and atropinized phrenic nerve-diaphragm preparation.

supported by Jaco and Wood (1944). Fig. 14 shows an experiment in which the effect of procaine was observed (a) during a prostigmine-augmentation, (b) during a depression after an overdose of prostigmine. The increased muscle contractions in (a) were reduced by procaine to their normal height. The diminished muscle contractions in (b) were also at first slightly reduced but then steadily increased although they did not reach their original size. Nevertheless, the gradual onset of this effect is similar to the records obtained with atropine.

It is possible to observe the effect of excess acetylcholine on the muscle contractions by increasing the rate of nerve stimulation in the presence of an anticholinesterase and also by adding acetylcholine to the bath. If atropine and procaine act like curarine by raising the threshold of the muscle to acetylcholine then the muscle response to acetylcholine released from the nerve and to acetylcholine introduced from outside should be affected in the same way. If, however, the activity of the nerve is affected by atropine and procaine, then in their presence the response of the muscle to the application of fast stimulation should differ from the response to the addition of acetylcholine to the bath.

In Fig. 15 (a) is shown the effect of fast stimulation on a muscle treated with prostigmine. The size of muscle contractions declined and a further depression

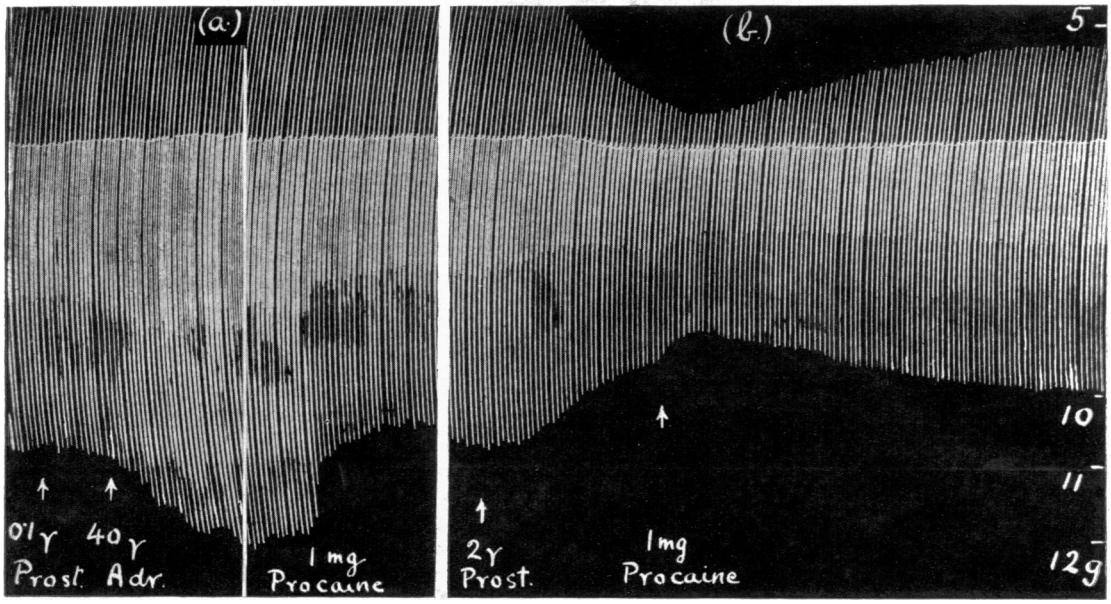

FIG. 14.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 8 per min. The effect of 1 mg. procaine on the size of muscle contractions *(a)* when increased, *(b)* when depressed by prostigmine.

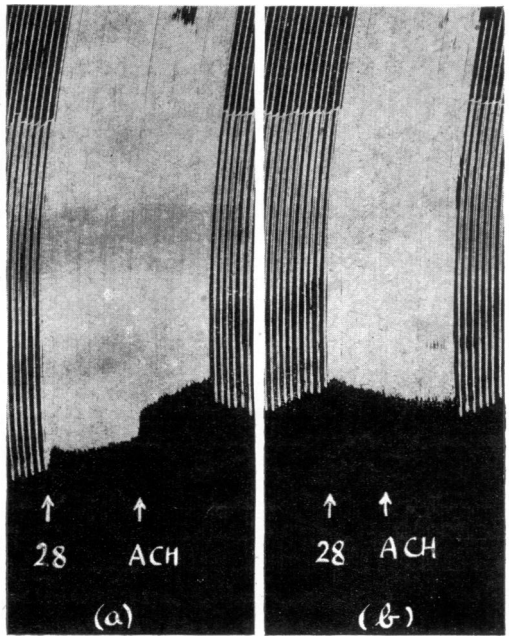

FIG. 15.—Rat's phrenic nerve-diaphragm preparation. Maximal single shocks 5 per min; 2 μg. prostigmine present throughout. The effect of increasing the rate of stimulation to 28 per min. and of 0.5 mg. acetylcholine *(a)* before and *(b)* after 35 μg. curarine.

was produced by the addition of 0.5 mg. acetylcholine to the bath. In (b) after 35 μg. curarine the muscle contractions slowly increased during the period of faster stimulation and the addition of 0.5 mg. acetylcholine did not interrupt this slow augmentation. As curarine raises the threshold of the muscle to acetylcholine neither the rapid stimulation nor the addition of 0.5 mg. acetylcholine to the bath caused a depression. We know that in the presence of curarine the nerve liberates acetylcholine as before (Dale, Feldberg and Vogt, 1936) and the amount introduced into the bath remained the same. But the muscle, being less sensitive, was not paralysed.

In Fig. 16 is shown a comparison between the action of atropine and curarine. Throughout 2 μg. prostigmine was added to the bath to ensure the accumulation

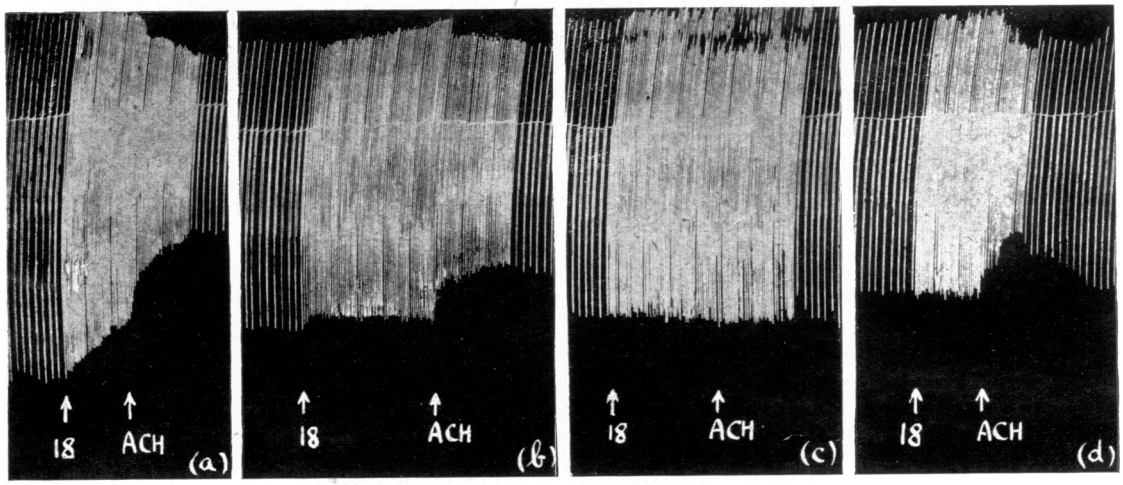

FIG. 16.—Similar to Fig. 15. Maximal single shocks 6 per min.; 2 μg. prostigmine present throughout. The effect of increasing the rate of stimulation to 18 per min., and of 0.5 mg. acetylcholine *(a)* before and *(b)* after 2 mg. atropine; *(c)* after 35 mg. curarine; *(d)* after 2 mg. atropine.

of acetylcholine. In (a) a depression was produced by fast stimulation and a further depression by additional acetylcholine. In (b) 2 mg. atropine was added. The muscle contractions were at first slightly depressed during fast stimulation but steadily regained their original height; the addition of 0.5 mg. acetylcholine caused a marked depression. After the preparation had been washed out several times during an interval of 20 min., 35 μg. curarine was added to the bath (c); this abolished both the depression due to rapid nerve stimulation and that due to added acetylcholine. After another interval in which the curarine was washed out the sequence was once more repeated in the presence of atropine (d).

The action of procaine was indistinguishable from that of atropine. This is shown in Fig. 17. Throughout 2 μg. prostigmine was present in the bath and a

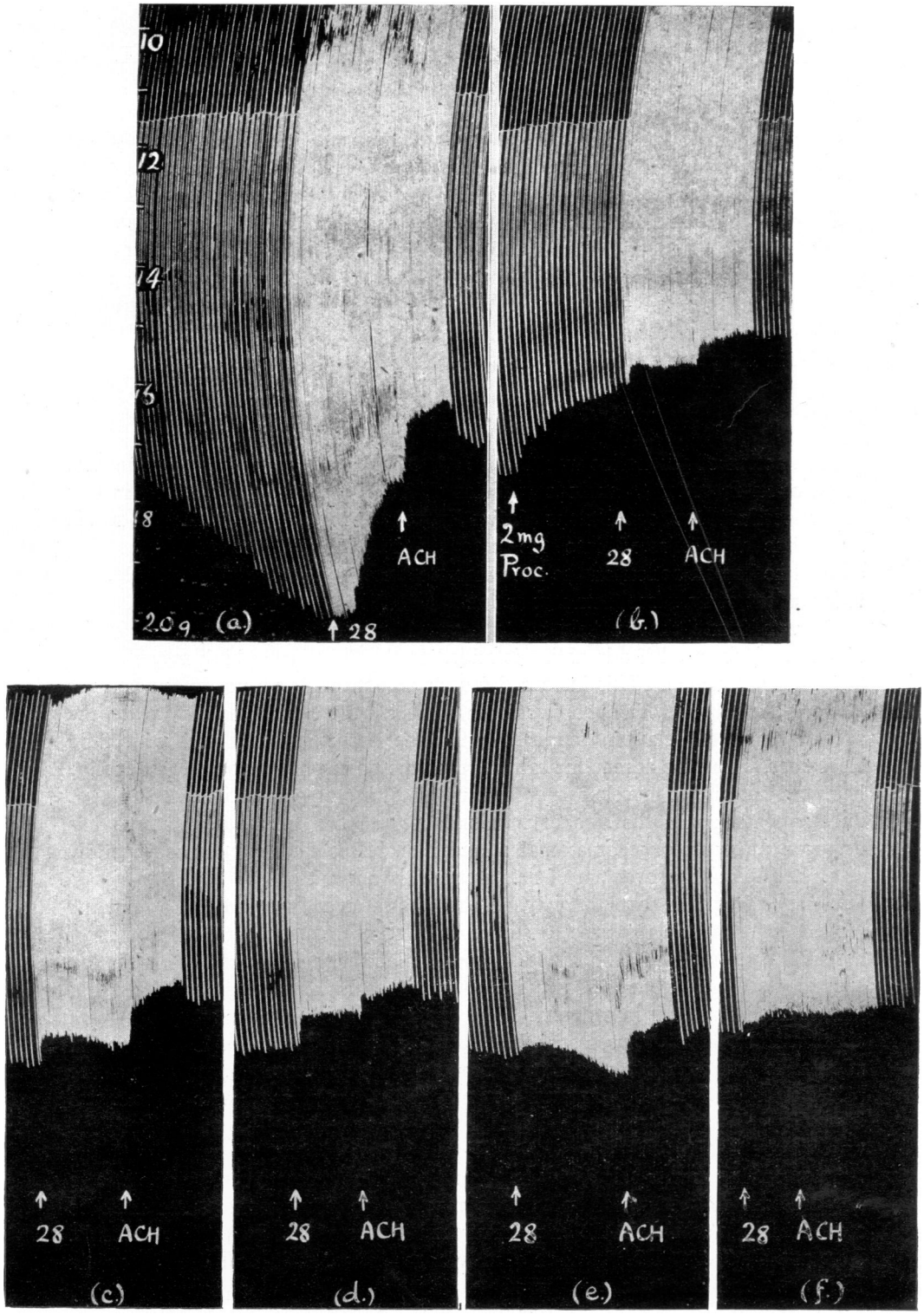

FIG. 17.—Similar to Fig. 15. Maximal single shocks 6 per min.; 2 μg. prostigmine present throughout. The effect of increasing the rate of stimulation to 28 per min., and of 0.5 mg. acetylcholine *(a)* before and *(b)* after 2 mg. procaine; *(c)* after washing out; *(d)* after 2 mg. atropine, and *(e)* after washing out; *(f)* after 35μg. curarine.

depression was first produced by fast stimulation and secondly by additional acetylcholine. In the presence of 2 μg. prostigmine only (a) both effects were marked. After 2 mg. procaine (b) and also after 2 mg. atropine (d), muscle contractions at first declined with increased rate of stimulation but soon remained steady, whereas the addition of 0.5 mg. acetylcholine caused a progressive depression. When the bath was changed, both after procaine (c) and after atropine (e), rapid stimulation caused an initial depression followed by a gradual increase of contractions, but added acetylcholine caused a profound decline. Finally, in the presence of 35 μg. curarine (f), both effects were abolished.

Discussion

The action of adrenaline was studied on an isolated mammalian nerve muscle preparation in order to exclude vascular effects. The results described in this paper confirm the conclusions drawn from previous experiments in which adrenaline was injected into the bloodstream. They leave no doubt that adrenaline has an action on the muscular response to nerve stimulation which is independent of its vasoconstrictor action.

It was found that muscle contractions in response to submaximal nerve stimuli are increased if adrenaline is added to the fluid in the bath in which the muscle is suspended. This increased muscle response is seen not only when the nerve is stimulated with open electrodes, in which the adrenaline may reach the site of nerve stimulation, but also when the nerve is stimulated with fluid electrodes, where the adrenaline cannot reach the point of stimulation. Adrenaline has no effect on the muscle response to maximal motor nerve stimuli. It causes an augmentation in the size of maximal contractions only when fatigue has occurred after prolonged maximal stimulation. This phenomenon is generally attributed to an improvement of neuromuscular transmission.

It is difficult to explain why in the non-fatigued preparation adrenaline increases the muscle response to submaximal stimuli whereas it does not affect maximal contractions. The results so far obtained with submaximal stimulation suggest that adrenaline acts by improving neuromuscular transmission; but further investigation is in progress. This explanation would be in agreement with observations on the perfused superior cervical ganglion (Bülbring, 1944) in which adrenaline improved synaptic transmission and thus increased the response of the nictitating membrane to submaximal stimulation of the preganglionic nerve fibres. The effective concentration of adrenaline in the perfusion fluid of the ganglion was, however, 1 in 100 to 1 in 200 million, whereas in the fluid surrounding the isolated muscle it was 1 in 2 to 1 in 10 million. The discrepancy may be due to the different way in which adrenaline reaches the tissue; much less may be required if it is carried in the circulation than if it acts by diffusion from outside. Dale and Gaddum (1930) used an even stronger concentration of adrenaline, 1 in 75,000, which increased the action of acetylcholine on denervated muscle.

Another action on skeletal muscle was described by Bülbring and Burn (1942), who found that adrenaline augmented the action of eserine or prostigmine. It was possible that this was due to a vascular effect. But the results were interpreted as indicating that adrenaline lowered the threshold of the muscle, thereby potentiating the action of acetylcholine which accumulated in the presence of the anticholinesterase. This view is supported by the results obtained on the isolated preparation. If the phrenic nerve is stimulated with maximal shocks at slow rates the contractions of the diaphragm are increased by prostigmine or eserine and a further increase is produced by the addition of adrenaline to the bath. If the nerve is stimulated at more rapid rates or if the anticholinesterase is allowed to exert a more prolonged action, then adrenaline causes a depression of muscle contractions. Both observations indicate that adrenaline in the absence of vascular changes intensifies the effect on the muscle of an accumulation of acetylcholine.

In the isolated muscle, especially at the beginning of an experiment, the effects of excess acetylcholine are more readily observed than in a muscle with its normal circulation. This fact is reminiscent of conditions in the perfused superior cervical ganglion described by Brown and Feldberg (1936). They showed that in the perfused ganglion during continued maximal preganglionic stimulation the output of acetylcholine started at a high level and fell rapidly during the first 20–60 min. after which it continued at a steady low level. Consequently, when the perfusion fluid contained eserine, the effect of excess acetylcholine on the contraction maintained by the nictitating membrane during continued preganglionic stimulation differed in the initial stage from the effect later on. An increase in strength of stimulation, as well as the injection of acetylcholine into the perfusion fluid, at first caused a depression, but 44 min. later both produced an augmentation. The results obtained on the isolated striated muscle preparation suggest that similar conditions prevail. In the beginning of an experiment motor nerve stimulation seems to produce such a large amount of acetylcholine that the addition even of a small dose of an anticholinesterase may cause an accumulation sufficiently high to produce paralysis. The same dose of eserine, given later, may cause an increase of muscle contractions. Similarly, in a fresh preparation, after a dose of eserine which by itself may exert no action, adrenaline causes a depression, whereas several hours later the same dose of adrenaline causes augmentation.

It seems possible that when this isolated muscle is freshly prepared, acetylcholine accumulates in it even in the absence of an anticholinesterase. This hypothesis is supported by the observation that, in a fresh muscle, contractions were slightly augmented by the addition of atropine to the bath. This observation was puzzling until it was shown that atropine antagonized any effects of increased amounts of acetylcholine accumulated by nerve stimulation in the same way as it antagonizes the action of acetylcholine, eserine and prostigmine in the spinal cord (Bülbring and Burn, 1941). Thus, when muscle contractions were

increased by eserine or prostigmine, the addition of atropine reduced them to normal. On the other hand, when muscle contractions were decreased by an overdose of an anticholinesterase or by a faster rate of stimulation atropine counteracted the depression. In both instances atropine acts like curarine. A difference was, however, observed when excess of acetylcholine was not only produced by rapid nerve stimulation in the presence of eserine or prostigmine but also by addition of acetylcholine from outside. Curarine in the presence of an anticholinesterase prevented the muscular depression caused by fast stimulation and that caused by a dose of acetylcholine alike. But atropine did not prevent the depressant effect of a dose of acetylcholine added to the bath. The conclusion was therefore drawn that atropine interfered with the liberation of acetylcholine from the motor nerve ending rather than that atropine affected the muscle in the same way as curarine. Brown (1937) came to the same conclusion when he found that in frog's muscle atropine rendered nerve stimulation ineffective, leaving, however, a large part of the acetylcholine response still present.

In the phrenic nerve-diaphragm preparation the effects caused by atropine were very similar to those obtained with procaine. As procaine depresses the activity of sensory nerves a similar action on motor nerves is not surprising. It is well known that atropine possesses a weak local anaesthetic action. But while Harvey (1939) showed that procaine diminished the output of acetylcholine from the perfused superior cervical ganglion during preganglionic stimulation, Brücke (1937) showed that atropine increased the output of acetylcholine in the frog's heart during vagus stimulation. The amount of acetylcholine escaping into the perfusion fluid may not be a true measure of the amount liberated from the nerve, but may be modified by the amount which is capable of or prevented from combining with the receptive substance. Recently Dawes (1946) has shown that atropine and procaine in large dosage exert a similar action on heart muscle, i.e. both of them prolong the refractory period in the same way as quinidine.

Summary

1. A strip of the rat's diaphragm with the phrenic nerve can be used as an isolated mammalian nerve muscle preparation.

2. If single submaximal shocks are applied to the phrenic nerve the contractions of the diaphragm muscle are increased by the addition of adrenaline to the bath. This effect is observed whether the adrenaline reaches the site of nerve stimulation or not. Unless the muscle is fatigued, adrenaline has no effect on the muscle response to maximal nerve stimuli.

3. The muscle contractions elicited by maximal nerve stimuli are increased by small doses of eserine or prostigmine at slow rates of stimulation. A depression is produced by prolonged action or by an overdose of the anticholinesterase or by increasing the stimulation rate in the presence of the anticholinesterase.

4. Adrenaline augments the action of eserine and prostigmine. This may be

observed as an increase or as a decrease in the size of muscle contractions according to various conditions. Muscle contractions are increased when the amount of the anticholinesterase is small and when the rate of stimulation is slow. The size of contractions is depressed by adrenaline after an overdose of the anticholinesterase or with faster rates of stimulation.

5. The depressant effect of eserine or prostigmine and of subsequent adrenaline is more readily observed in a fresh preparation than in one which has been stimulated for several hours. The possibility that acetylcholine may accumulate during the initial stages of motor nerve stimulation in this isolated preparation is discussed.

6. Atropine increases slightly the size of muscle contractions elicited by maximal nerve stimuli in a fresh preparation.

7. If muscle contractions are increased by eserine or prostigmine, atropine reduces them to their normal size. If muscle contractions are depressed by an overdose of an anticholinesterase or by a faster rate of stimulation then atropine counteracts the depression.

8. In the presence of an anticholinesterase curarine abolishes the depressant effects of excess acetylcholine, whether this be produced by rapid nerve stimulation or by adding acetylcholine to the bath. Atropine differs from curarine. It abolishes the depressant effect of rapid motor nerve stimulation but it does not abolish the depression caused by the addition of acetylcholine to the bath.

9. The action of atropine was found to be very similar to that of procaine. The conclusion was therefore drawn that atropine acts not only by raising the threshold of the muscle, as curarine does, but also by interfering with nervous activity.

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THE EFFECT OF ETHANOL, METHANOL, PARALDEHYDE AND ACETONE ON THE PRESSURE OF THE CEREBROSPINAL FLUID OF THE DOG

BY

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(Received January 16, 1946)

Evidence has been presented in an earlier paper (Bedford, 1941), that ether dilates the blood vessels of the brain and consequently raises the pressure of the C.S.F. In the present series of experiments, a study has been made of the effect of ethanol and certain related compounds on the pressure of the C.S.F.

Experimental Procedure.—Dogs were anaesthetized with sodium *iso*-amyl ethyl barbiturate (Amytal, Lilly), administered intravenously. Details of the technique are given in an earlier paper (Bedford, 1938). After anaesthesia was complete, a tube with side-valve was tied into the trachea. In all experiments, a minimal degree of pulmonary ventilation was maintained by means of a pump. The pressure of the C.S.F. was recorded from the cisterna magna by means of a vertical glass manometer of 1 mm. bore; readings of the pressure were taken at minute intervals. The mean arterial pressure was recorded from a cannula in the femoral artery.

Special attention was paid to the action of ethanol; some experiments were, however, performed with methanol, paraldehyde or acetone.

In one group of experiments, the dogs were made to inhale air containing varying amounts of the vapour of the liquid under investigation. This was effected by placing a reservoir containing the undiluted liquid between the respiratory pump and the animal; the air from the pump bubbled through the liquid. By means of a simple device, it was possible to vary at will the proportion of air from the pump which passed through the liquid. No absolute measurements of concentrations could be made. The reservoir was immersed in a water bath at 40° C.; the tubing from the reservoir to the animal was also heated over the greater part of its course.

In another series of experiments, the effect of intravenous administration was studied. The liquid in 10 per cent (v/v) concentration in isotonic NaCl solution, was introduced at a measured rate into a tributary of the saphenous vein. The duration of an average experiment was 1½ hours.

RESULTS

The Effect of Ethanol on the Pressure of the C.S.F.

(a) *Inhalation.*—The effect of inhalation of ethanol was studied in five dogs. No change was noticed in the pressure of the C.S.F. until a fall occurred in the mean arterial B.P. An immediate fall in the pressure of the C.S.F. was then observed; this fall was directly proportional to the fall in B.P. The pressure of

the C.S.F. and the mean arterial B.P. rose simultaneously to their original levels when the concentration of the ethanol vapour was reduced. Apart from changes resulting from a fall in B.P., the results were the same when ethanol was administered suddenly in high concentration and when dogs were made to breathe a low concentration of ethanol vapour which was gradually increased to maximum tolerance. Abrupt cessation of administration of ethanol was also unaccompanied by any change in the pressure of the C.S.F. The results of a typical experiment are illustrated in Fig. 1.

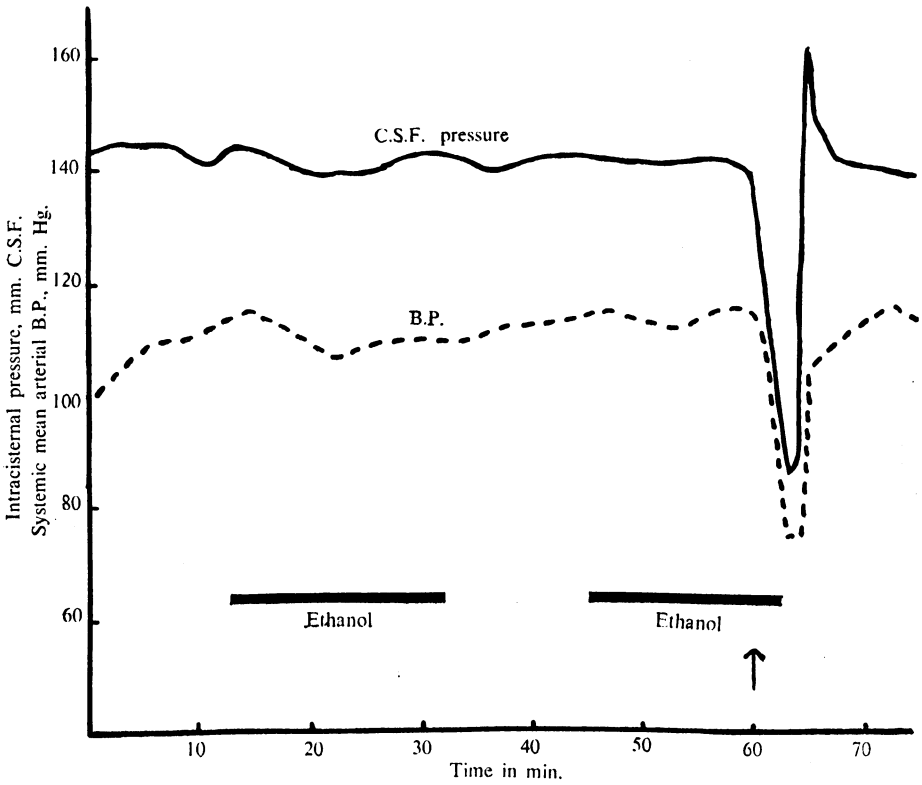

FIG. 1.—The effect of inhalation of ethanol on the pressure of the C.S.F. The concentration of ethanol vapour during the first period of administration failed to cause a fall in B.P. During the second period of administration, the ethanol vapour was increased at the arrow, to a concentration that caused a fall in B.P. The temporary rise in C.S.F. pressure to a level higher than its original level, which followed the replacement of ethanol vapour by air, is not specific for ethanol but is generally found to accompany any sudden rise in systemic B.P.

(b) *Intravenous Administration.*—The intravenous administration of ethanol was studied in six dogs. A 10 per cent (v/v) concentration of ethanol in isotonic NaCl solution was introduced at a rate of 2 ml. per min. ; a greater rate than this usually produced a rapid fall in blood pressure. The maximum

volume of this solution administered during a single experiment was 96 ml.; it was introduced over a period of 48 min. into a dog of 10 kg. The concentration of ethanol in the blood would be well in excess of that needed to cause coma in man. No change was noticed in the pressure of the cerebrospinal fluid except in those instances in which the alcohol produced a fall in B.P. The accompanying fall in the pressure of the C.S.F. was clearly dependent on the fall in blood pressure.

The Effect of Methanol, Acetone and Paraldehyde on the Pressure of the C.S.F.

Inhalation of methanol (4 dogs), paraldehyde (8 dogs) and acetone produced effects on the B.P. and C.S.F. very similar to those described above for ethanol. The effect of intravenous administration of paraldehyde (10 per cent v/v in isotonic NaCl) was more dramatic than that observed with ethanol, a profound fall of arterial B.P. occurring when the solution was introduced at a rate exceeding 1 ml./min. (3 dogs). The effect of intravenous administration of methanol was indistinguishable from that of ethanol (2 dogs).

Summary

It would appear from the above experiments that ethanol, methanol, paraldehyde and acetone have no immediate influence on the pressure of the C.S.F. unless they are administered in amounts sufficient to produce a fall of systemic B.P.; a fall then occurs in the pressure of the C.S.F. which is directly dependent on the fall in B.P. The same results were obtained whether ethanol, methanol and paraldehyde were administered by inhalation or intravenously; acetone was only administered by inhalation. It is concluded that any direct action these compounds may have on the cerebral blood vessels is insignificant in character.

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Brit. J. Pharmacol. (1946), **1**, 65–80.

CHEMOTHERAPEUTIC ACTION OF AMIDINE AND PHENANTHRIDINIUM COMPOUNDS IN *T. CONGOLENSE* INFECTIONS

BY

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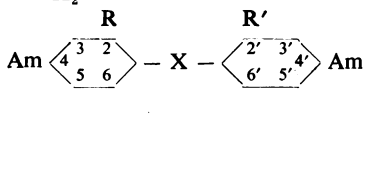
(Received January 17, 1946)

In evaluating the therapeutic activity of a series of diamidine compounds we found that while certain of them (particularly nuclear halogen-substituted derivatives of stilbamidine) were highly active against *Trypanosoma equiperdum* and *T. rhodesiense,* few were curative in *T. congolense* infections. One of the most active was 4:4′-diamidino-$\alpha\beta$-dimethylstilbene (991 or 'dimethyl stilbamidine'). Its structure is shown from the general formula I, where

Am = $-C(=NH)-NH_2$, X = $-C(Me):C(Me)-$, R = R′ = H:



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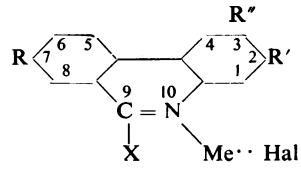
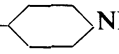


II

The activity of this compound has been described in laboratory animals by Fulton & Warrington Yorke (1943, a and b) and in cattle by Carmichael & Bell (1943, 1944) and Daubney & Hudson (1943). It was found active in field trials but the therapeutic index was small: there is usually a narrower margin of safety in large animals than in small laboratory animals.

Recently certain phenanthridinium compounds have been found highly active against *T. congolense,* with a greater therapeutic index than that of the amidines (Walls, 1945; Browning, Morgan, Robb and Walls, 1938; Hornby, Evans and Wilde, 1943; Carmichael and Bell, 1943; Browning and Calver, 1943, and Bell, 1945). The phenanthridinium compounds have the general formula II

The most active were 897, 7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (R = NH_2, R′ = R″ = H, X = ), and 1553, 2:7-diamino-9-phenyl-10-methylphenanthridinium bromide (R = R′ = NH_2, R″ = H, X = ).

Although dimethyl stilbamidine had been shown to be less promising in field trials than the phenanthridinium compounds, we were interested in carrying the

comparison further in the laboratory, since the early experiments had been conducted with an acute strain in mice, whereas in cattle the disease takes a chronic course. This paper is therefore concerned partly with a comparison of dimethyl stilbamidine and the phenanthridinium compound 897 against different strains of *T. congolense,* and also with the examination of other diamidine and phenanthridinium compounds, including some observations on the pharmacological actions of the compound 1553. The results have clarified to some extent the relationship between chemical structure and activity against *T. congolense,* but no correlation of activity has been found between this infection and *T. equiperdum, rhodesiense* or *brucei* infections: diamidines which were highly active against the latter trypanosomes were ineffective against *T. congolense,* and conversely, phenanthridinium compounds which were highly active against *T. congolense* showed little activity against *T. equiperdum.*

METHODS

Subinoculations of each strain were made from infected mice by withdrawing heart blood, diluting appropriately with serum (normal horse serum 20 per cent)—citrate–saline, and injecting 0.3 cc. of the suspension intraperitoneally into each mouse. This was carried out at the acme stage when trypanosomes were numerous in the peripheral blood (3 or 4+); 1+ = 1 – 10, 2+ = 20 – 30 trypanosomes per microscopic field, and so on. Groups of albino mice from an inbred laboratory strain were used, and only those were selected which after some days showed a moderate degree of parasitaemia (up to 2+). Solutions of the drugs were given by intravenous injection into the tail vein or subcutaneously: the latter method was more frequently used, since by this route the therapeutic index is almost always greater. Wet smear preparations of blood from the tail were examined periodically; a mouse was regarded as negative if no trypanosomes were found in at least 20 microscopic fields. Evaluation was made by determining the E.D.50 (50 per cent of the animals cleared of trypanosomes from the peripheral blood within seven days), C.D.50 (50 per cent of the animals free of trypanosomes for at least 28 days, 42 days for chronic strains), and L.D.50 (average lethal dose). The calculations were made either by Litchfield and Fertig's method (1941) or by Irwin and Cheeseman's (1939) modification of Kärber's method. Where required the standard errors of these results are given. The chemotherapeutic index was determined by dividing the L.D.50 by the C.D.50, which is a more exact ratio than the usual ratio M.L.D./M.C.D. A similar method of assaying trypanocidal activity has recently been employed by Chen, Geiling, and MacHatton (1945). The five different strains of *T. congolense* were utilized only in the comparative experiments with dimethyl stilbamidine and phenanthridinium compound 897; in other experiments only the acute strain III was used.

The strains of *T. congolense* employed were essentially of two types, namely those which produced acute and chronic infections in mice. Acute infections in mice may be fatal in about six days, whereas in cattle the chronic infection persists for a considerable time. The virulence in mice is often enhanced by passage, but in cattle the reverse may occur. The size of inoculum influences the onset of the infection, heavy inocula hastening the appearance of trypanosomes in the peripheral blood, although the effect is not very marked. The route of administration has a similar effect, intraperitoneal injection causing a quicker appearance than the subcutaneous route.

Strains of T. Congolense

I and II.—These two strains have been employed by Browning and Calver (1943), and Prof. C. H. Browning kindly supplied both strains. Strain I was described earlier by Brown-

ing, Cappell and Gulbransen (1934), and produces a chronic infection with a relapsing course. Since it was originally obtained from Dr. C. M. Wenyon it was probably derived from the same source as strain V. We confirmed the chronic nature of strain I and found that parasites appeared more quickly in the blood after intraperitoneal (four days) than after subcutaneous injection (five to eight days). After the first appearance of the parasites they increased usually to about 3+ within three to four days, although sometimes a longer period was observed. A negative phase followed, in which trypanosomes were not found in the peripheral blood, a period which might last for four weeks, followed by a relapse when numerous trypanosomes could be found. Subinoculations were made at the first appearance at the acme stage, i.e., at the height of the infection, as described by Browning: we confirmed that subinoculations made at other times altered the character of the infection. The majority of the mice survived this chronic infection, which corresponds to the condition found in cattle, in which the host may harbour trypanosomes for very long periods without succumbing.

Strain II produces an acute infection, the parasites becoming numerous in the blood and persisting until the death of the animal after a period varying from a few days to several weeks. It is not so virulent as strains III or IV. Prof. C. H. Browning has stated that it was received in a guinea pig in 1933 from Hornby in Tanganyika; its previous history is unknown.

III.—This strain, kindly supplied by Dr. J. D. Fulton, runs an acute course in mice, and we found it very constant in its virulence. After subinoculation trypanosomes appeared in the blood within two to four days, and all the mice died within seven to nine days. When the passage was made by subcutaneous injection these times were lengthened somewhat, the blood being full of parasites within nine days, and the mice dying within twelve days.

IV.—This strain was kindly supplied by Dr. F. Hawking, who originally obtained it from the Liverpool School of Tropical Medicine. As would be expected, the course of the infection and its virulence were very similar to those of the parent strain III.

V.—This strain was kindly supplied by Mr. L. G. Goodwin. After subcutaneous injection of peripheral blood no parasites are usually seen until eight to ten days. They increase up to fourteen days, and then decrease until at eighteen days none may be found. Subsequently relapses occur and the process may be repeated, the majority of the mice surviving for a long period. Subinoculations were always made at the first appearance of the parasites. In maintaining this strain for these experiments we used heart blood and subinoculated intraperitoneally. By this method and the use of a heavy inoculum it was found that the trypanosomes appeared within four days, and the acme stage was reached within five to eleven days; the parasites disappeared from the blood three days later, with subsequent relapses. The course of the infection showed considerable variation in different animals, and in this respect was entirely different from the acute strains. This factor also made this strain more difficult to work with when assessing the value of drugs. Only treatment which was effective within, say, seven days, could be attributed to the drug, since spontaneous cures would occur later, with temporary disappearance of trypanosomes.

RESULTS

The compounds* examined are given in the following list:

No.	Name of Compound.
(*a*) *Diamidines.* (i) *Stilbene Series*	
744	4:4′-diamidinostilbene
1311	2-methyl-4:4′-diamidinostilbene

* All the compounds in *(a)* were dihydrochlorides, except 1126, which was the dilactate. Compounds 744 to 1126 of the stilbene series were prepared by Ashley and Harris (1946).

No.	Name of Compound.
(*a*) *Diamidines.*	(i) *Stilbene Series* (continued)
1350	2:2′-dimethyl-4:4′-diamidinostilbene
1032	2-methoxy-4:4′-diamidinostilbene
1011	2-hydroxy-4:4′-diamidinostilbene
1129	2:2′-dihydroxy-5:5′-diamidinostilbene
1118	2-iodo-4:4′-diamidinostilbene
1015	2-amino-4:4′-diamidinostilbene
1025	2-acetamido-4:4′-diamidinostilbene
1126	4:4′-di-N-ethylamidinostilbene
1005	4:4′-diamidino-α-methylstilbene
991	4:4′-diamidino-αβ-dimethylstilbene
	(ii) *Diphenoxy Series*
782	4:4′-diamidino-αγ-diphenoxypropane
1146	2-bromo-4:4′-diamidino-αγ-diphenoxypropane
1272	2-bromo-4:4′-diamidino-αε-diphenoxypentane
993	4:4′-diamidino-αη-diphenoxyhexane
	(iii) *Miscellaneous diamidines*
1150	Phenanthrene-3:6-diamidine
1346	3:6-diamidinocarbazole
938	4:4′diamidinodiphenylamine
(*b*) *Phenanthridinium Compounds*	
897	7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride
1553	2:7-diamino-9-phenyl-10-methylphenanthridinium bromide
1355	7-amidino-9-*p*-amidinophenyl-10-methylphenanthridinium chloride
1325	3-amino-9-(3′-pyridyl)-phenanthridine monomethochloride
1324	7-amino-9-(3′-pyridyl)-phenanthridine monomethochloride
193	9-(*p*-dimethylaminostyryl)-phenanthridine methochloride
196	7-acetamido-9-(*p*-dimethylaminostyryl)-phenanthridine methochloride
197	7-amino-9-(*p*-dimethylaminostyryl)-phenanthridine methochloride
198	2-methyl-9-(*p*-dimethylaminostyryl)-phenanthridine methochloride

The toxicity data of the three compounds examined in detail,

991	4:4′-diamidino-αβ-dimethylstilbene dihydrochloride
897	7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride
1553	2:7-diamino-9-phenyl-10-methylphenanthridinium bromide

are given in Table I.

TABLE I
Toxicity to Mice of 991, 897 and 1553

Compound	Injection	No. of Mice	L.D. 50 (mg./g.)	Limits % (p = ·95)
991	i.v.	40	0·049	85–118
	s.c.	40	0·198	83–121
897	i.v.	60	0·015	89–112
	s.c.	70	0·094	91–110
1553	i.v.	80	0·020	88–114
	s.c.	80	0·061	90–111

In Table II (i–v) the results are given of the comparative activities of 991 and 897 against five different strains (acute and chronic) of *T. congolense.*

TABLE II
Comparison of activity of 897 and 991 against different strains of *T. congolense*
Infection: in mice, 1–20 trypanosomes/microscopic field.
Cure: disappearance of trypanosomes for at least 28 days.
Administration of compounds: subcutaneously.

Dose (mg./g.)	No. of Mice	897	991
		No. of Mice Cured	
	(*i*) *Strain I*		
0·02	10	10	8
0·01	10	6	6
0·005	10	8	6
0·0025	10	6	2
0·00125	10	4	0
C.D. 50 (mg./g.)		0·0027	0·0057
Limits % (p = ·95)		58–173	58–171
Therapeutic index		35	35
	(*ii*) *Strain II*		
0·02	10	10	10
0·01	10	8	10
0·005	10	10	6
0·0025	10	6	2
0·00125	10	4	0
C.D. 50 (mg./g.)		0·0020	0·0041
Limits % (p = ·95)		63–160	63–160
Therapeutic index		46	49
	(*iii*) *Strain III*		
0·04	10	9	8
0·02	10	8	8
0·01	10	5	1
0·005	10	4	0
C.D. 50 (mg./g.)		0·0090	0·0162
Limits % (p = ·95)		60–167	60–167
Therapeutic index		10	12

(*Continued overleaf*)

Dose (mg./g.)	No. of Mice	897	991
		No. of Mice Cured	
	(*iv*) *Strain IV*		
0·04	10	9	10
0·02	10	5	4
0·01	10	4	1
0·005	10	0	0
C.D. 50 (mg./g.)		0·0157	0·020
Limits % (p = ·95)		59–169	61–163
Therapeutic index		6	10
	(*v*) *Strain V*		
0·04	10	8	9
0·02	10	6	10
0·01	10	1	5
0·005	10	2	4
C.D. 50 (mg./g.)		0·0151	0·0078
Limits % (p = ·95)		59–171	61–164
Therapeutic index		6	25

Table III shows the results of the evaluation of the activity of 1553 against an acute strain (III) of *T. congolense.*

TABLE III

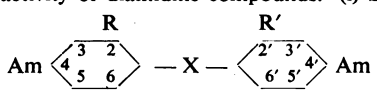
Evaluation of the activity of 2: 7–diamino–9–phenyl–10–methylphenanthridinium bromide (1553) against *T. congolense*, Strain III

Dose (mg./g.)	No. of Mice	No. of Mice cleared of Trypanosomes	
		3–7 Days	28 Days
0·001	10	10	10
0·0005	10	10	6
0·00025	10	9	2
0·000125	10	9	1
0·00008	10	8	—
0·000051	10	5	—
0·000032	10	3	—
0·00002	10	0	—
E.D. 50 (mg./g.)		0·00005	
Limits % (p = ·95)		72–139	
C.D. 50 (mg./g.)		0·0004	
Limits % (p = ·95)		61–165	
Therapeutic index		152	

The results on the diamidines, phenanthrenes and phenanthridinium compounds examined (28 compounds in all) are given in Tables IV, V and VI. The compounds were injected subcutaneously and the mice were infected with *T. congolense*, strain III.

TABLE IV

Toxicity and activity of diamidine compounds. (i) Stilbene series.



No.	Substituent Groups			L.D. 50 (mg./g.)	E.D. 50 (mg./g.)	C.D. 50 (mg./g.)	Index
	X	R	R′				
744	—CH : CH—	H	H	0·18	0·02	0·08	2·3
1311	Do.	Me	H	0·146	0·01	0·03	4·9
1350	Do.	Me	Me	0·140	0·015	Not curative	—
1032	Do.	OCH_3	H	0·100	0·015	Do.	—
1011	Do.	OH	H	0·137	0·025	Do.	—
1129	*Do.	OH	OH	0·095	Inactive	—	—
1118	Do.	I	H	0·332	0·025	Not curative	—
1015	Do.	NH_2	H	0·075	0·015	0·038	2·0
1025	Do.	NH.Ac	H	0·150	0·008	0·038	4·0
1126	**Do.	H	H	0·180	0·04	Not curative	—

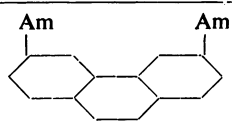
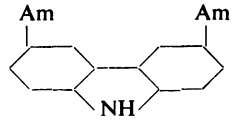
* Am = 5 : 5′. ** Am = Et NH—C(=NH)—

When the activities of 897 and 991 were compared the results showed that against the acute strains both compounds had approximately the same therapeutic indices : 897 was about twice as active and twice as toxic. The level of significance was taken at two to three times the standard error. The same result was obtained with the chronic strain I, but with strain V the compound 897 was only about half as active and consequently had a poorer therapeutic index. The difference, however, was not highly significant and only one comparison was made. The results with the phenanthridinium compound 1553 (Table III) showed quite clearly that it was significantly very much more active than either its analogue 897 or the diamidine 991.

It will be observed from Tables IV, V and VI that although a number of compounds showed activity, only 12 had a curative action, and eight of these—namely, 744, 1311, 1015, 1025, 1005, 1146, 1325 and 197, had therapeutic indices which were too small to be of interest. The activities of the phenanthridinium compounds 897 and 1553 and of the diamidine 991 have already been considered. It is noteworthy that phenanthrene-3:6-diamidine, 1150, displayed activity of a fairly high order.

TABLE V

Toxicity and activity of diamidine compounds. (ii) Stilbene and diphenoxy series. X, R and R′ as in Table IV.

No.	Substituent Groups			L.D. 50 (mg./g.)	E.D. 50 (mg./g.)	C.D. 50 (mg./g.)	Index
	X	R	R′				
1005	— C(Me) : CH —	H	H	0·12	0·01	0·04	3·0
991	— C(Me) : C(Me) —	H	H	0·198	0·004	0·007–0·016	12–28
938	— NH —	H	H	0·050	0·01	Not curative	—
782	— O — $(CH_2)_3$ — O	H	H	0·055	Inactive	—	—
1146	Do.	Br	H	0·107	0·01	0·05	2·1
1272	— O — $(CH_2)_5$ — O	Br	H	0·23	Inactive	—	—
993	— O — $(CH_2)_7$ — O	H	H	0·063	Do.	—	—
1150				0·160		<0·01	>16
1346				0·065	0·01	Not curative	—

Observations on the Pharmacological Properties of the Phenanthridinium Compound 1553

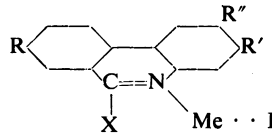
Action on Respiration

Respiration was recorded by Gaddum's method. In rabbits anaesthetized with urethane the intravenous injection of 5 or 10 mg. of 1553 produced a marked fall in the depth of respiration, with little alteration in the rate, accompanied by a fall in blood pressure (Fig. 1): the effects were transitory. The analogue 897 had a greater effect and the initial fall of blood pressure was followed by a large secondary rise. These effects were not reflex in origin since they occurred after vagotomy and denervation of the carotid sinus. There was some anoxaemia during the rise of blood pressure: the arterial blood was noticed to become darker and there was a slight fall in the alkali reserve. When the injections were made into the right carotid artery the depressant effect on the respiration was markedly less, and the subsequent rise of blood pressure did not occur. With a sufficiently large dose death was caused by respiratory failure ; this was noticed particularly with compound 897, the depressant effect of which on the respiration was more pronounced. In cats anaesthetized with either chloralose, pentobarbitone, or

urethane, and in decerebrate preparations, the respiration was much less affected than in rabbits. In some preparations there was little alteration, or even an initial slight increase ; the greatest depression of the respiration was observed in urethanized animals.

TABLE VI

Toxicity and activity of phenanthridinium compounds.



No.	Substituent Groups				L.D. 50 (mg./g.)	E.D. 50 (mg./g.)	C.D. 50 (mg./g.)	Index
	X	R	R′	R″				
897	— C6H4 —NH_2	NH_2	H	H	0·094	0·0025	0·009	10·4
1553	— C6H4 —	NH_2	NH_2	H	0·061	0·00005	0·0004	152
1355	— C6H4 —Am	Am	H	H	0·046	Inactive	—	—
1325	— C5H4N —	H	H	NH_2	0·15	0·02	0.06	2·5
1324	— C5H4N —	NH_2	H	H	0·174	Inactive	—	—
193	—CH : CH C6H4 $N(CH_3)_2$	H	H	H	0·004	Do.	—	—
196	Do.	NHAc	H	H	0·200	Do.	—	—
197	Do.	NH_2	H	H	0·035	0·01	0·02	1·8
198	Do.	H	CH_3	H	0·003	Inactive	—	—

Action on Circulation

Blood Pressure.—The general effect in anaesthetized rabbits and cats was a transitory fall in blood pressure, which was sometimes followed by a secondary

rise, especially with large doses, in urethanized rabbits. The compound 897 had a greater depressant action than 1553 in cats ; the administration of a full dose of atropine reduced the fall in blood pressure but did not abolish it. The secondary rise in blood pressure may have been partly due to the anoxaemia, since it did not occur when the injections were given intra-arterially, and the effect on the respiration was not so marked. In rabbits ergotoxine did not abolish the rise.

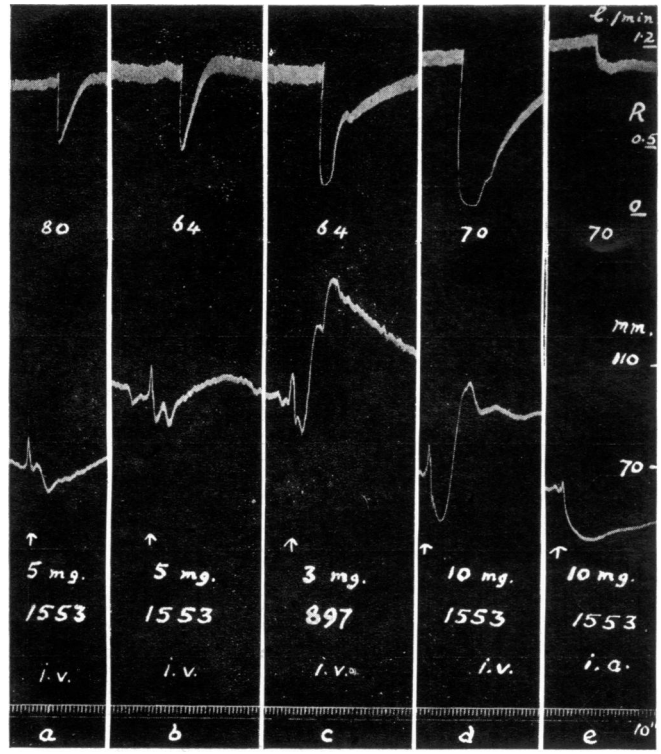


FIG. 1.—Rabbit, urethane anaesthesia. Between *a* and *b* vagotomized and carotid sinus denervated. Upper record respiration ; figures indicate rate per min. Lower record blood pressure.

In decerebrate and spinal cats a fall of blood pressure was produced which was, however, much less in the spinal preparation. In the latter preparation a marked increase in the pressor response to adrenaline was observed immediately following the intravenous injection of 1553 (Fig. 2), in contrast to the decrease caused by the amidines (Wien, 1943 ; Dawes, 1945).

Spleen Volume.—In decerebrate cats 1553 in doses of 1 to 2 mg. injected intravenously or intra-arterially produced a marked increase in spleen volume (Fig. 3) accompanied by a fall in blood pressure. There was little effect in the spinal cat, in which there was also little effect on the blood pressure.

Heart.—On the isolated rabbit's heart (Langendorff's preparation), perfused with Ringer's solution, doses up to 1 mg. caused slight stimulation in both rate and amplitude and some reduction of the effects of adrenaline. The effects were small and would be of little significance in the whole animal.

Action on Smooth Muscle

On the isolated rabbit's intestine suspended in a bath of 50 c.c. of Tyrode's solution, doses up to 5 mg. caused stimulation with increase in tonus, while larger doses produced relaxation and inhibition of the movements. The compound 897 had a much greater stimulant action, being about 25 times more active.

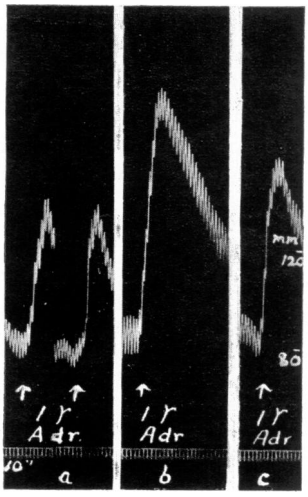


Fig. 2

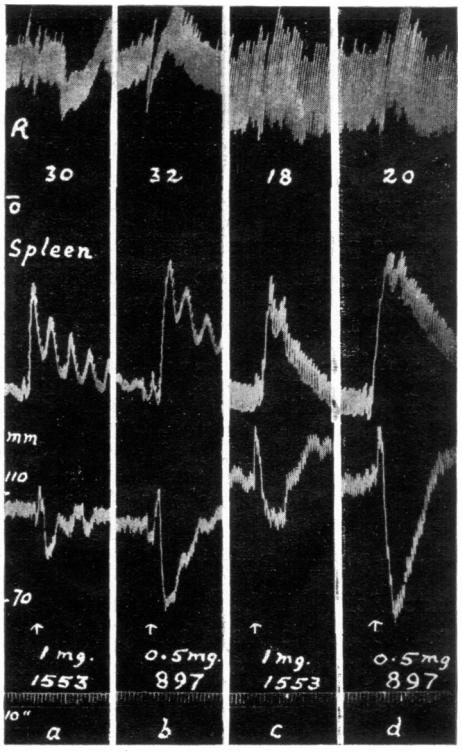


Fig. 3

FIG. 2.—Cat, spinal. Blood pressure record. All injections intravenously. Between *a* and *b*, 10 mg. 1553, which had only a slight depressor effect; but note that 1553 increased the response to adrenaline. At *c* the adrenaline response after 30 minutes was still raised.

FIG. 3.—Cat, decerebrate. All injections intravenously. Between *b* and *c* vagotomized. Upper record respiration; figures indicate rate per min. Middle record spleen volume. Lower record blood pressure.

Action on Blood Sugar and Blood Urea

Six rabbits were used. In doses of 1 to 10 mg./kg. given intravenously there was no significant effect on the blood sugar. In doses of 1 to 5 mg./kg. there was little effect on the blood urea, but with 10 mg./kg. an increase in the blood urea was usually observed. The effect was more pronounced if further injections were given later. The following figures illustrate this:

EFFECT ON BLOOD UREA

Rabbits given (*a*) 2 daily subcutaneous injections of 10 mg./kg. of 1553; (*b*) the same injections repeated after 14 days.

Blood urea	(*a*)	44	68	80	122	84
mg./100 c.c.	(*b*)	63	102	101	248	—
Hours		0	5	24	48	120

The high blood urea levels were accompanied by proteinuria, and at post mortem there was slight fatty degeneration and cloudy swelling in the tubules of the kidney.

Action on the Liver

Guinea pigs were used, since it has been found that they are more sensitive than other animals to the hepato-toxic action of the amidines in demonstrating fatty degeneration. The phenanthridinium compound 1553 was given intramuscularly in daily injections of 0.5, 1.0, 2.0 and 5.0 mg./kg. for 5 days. The animals were killed some days after the end of treatment, and frozen sections of the liver were stained with sudan. On the lowest dose no pathological changes were observed, but with 1.0 mg./kg. there was some fatty degeneration, and with 2 and 5 mg./kg. the fatty degenerative changes were pronounced, resembling in this respect the toxic effects of the amidines.

Action on Urinary Porphyrins

Since Bell (1945) has found photosensitization effects in cattle, it was of interest to see whether this was associated with a porphyrinuria, but no increase in urinary porphyrins was found in rats even after 15 daily subcutaneous injections of 10 mg./kg.

DISCUSSION

A consideration of the results obtained with the phenanthridinium compounds showed certain differences from those obtained by Browning and Calver (1943) and Calver (1945). They found that strain I (chronic infection) responded much better to treatment with 897 than strain II (acute infection), the C.D.50 figures being respectively 0.0005 and 0.005 mg./g.—that is, a tenfold difference. They found also that treatment was much more effective at the acme stage, when parasites were abundant, than when few parasites were present. In the latter

case the above doses produced cures not in 50 per cent of the mice but in 0–6 per cent. With both strains they observed that if the acme stage was passed and a chronic stage ensued the trypanosomes were far more resistant, and the approximate C.D.50 figures were increased to 0.005 and 0.025 mg./g. respectively. We have not, however, observed these differences between the two strains in their response to treatment. The figures found in these experiments for the C.D.50 for strains I and II were 0.0027 (limits 49–205 per cent, $p=.99$) and 0.002 mg./g. (limits 54–185 per cent, $p=.99$). Treatment was given when trypanosomes were present to the extent of up to 20 per microscopic field. This infection may have been lighter than that employed by Browning and Calver, and may account for some difference in sensitivity, but it would not explain the lack of difference in response between the two strains. The passaging of the strains in different mice may also have some effect, although the characteristic virulence of each strain was maintained. Confirmation of these observations was provided by the results obtained with the other chronic strain, V, against which the phenanthridinium compound was even less effective and gave a C.D.50 figure of 0.015 mg./g., (limits 50–203 per cent., $p=.99$). We were unable therefore to find that 897 was more effective in chronic than in acute infections.

Comparison of the results obtained with the phenanthridinium compound 897 and the diamidine 991 showed that they had the same therapeutic indices except for the chronic strain V, against which 897 was less active. Our results are therefore in agreement with those of Fulton and Yorke (1943), who found 991 as active as 897, but differ from those of Calver (1945), who found it considerably less active.

An analysis of the results obtained with the series of substituted compounds tested revealed some interesting relationships between chemical structure and trypanocidal activity. Although *T. congolense* infections only are considered here, the compounds have been examined for activity against other trypanosomes, and it may be stated that nuclear substitution in the 2-position in 4:4′diamidinostilbene by Me, OH, Cl, Br or I led to a marked increase in activity against *T. equiperdum* and *T. rhodesiense* infections. These observations will be published in detail in another paper.

Walls and Browning (1945) have already pointed out the specificity of the action of the phenanthridinium compounds ; greatest activity was obtained with the 2-amino substituents. While they are highly active against *T. congolense* they have only slight activity against *T. brucei* and *T. equiperdum*. A certain type of chemical structure, different from that required for other trypanosomes, is obviously required for *T. congolense*. Conversely, whereas the nuclear substituted stilbamidine derivatives are highly active against *T. equiperdum* and *T. rhodesiense*, they show little activity against *T. congolense*.

The active amidine compounds, 991, 1005, and 1311, in the stilbamidine series have a methyl substituted grouping, but in the phenanthridine series 3-amino-9:10-dimethylphenanthridine and 3-amino-9-methylphenanthridine are inactive

(Morgan and Walls, 1932, 1938). Substitution in the unsaturated linkage of the amidines was more effective than nuclear substitution, and the dimethyl was more active than the monomethyl derivative. One requirement, therefore, appeared to be the presence of a methyl grouping, although amino substituted amidines, 1015 and 1025, had some activity, and a more interesting compound, 1150 (a phenanthrene diamidine) had no methyl grouping. This compound displayed a fairly high order of activity, and had a therapeutic index of at least 16. The C.D.50 has not been evaluated definitely since insufficient material was available. It is only slightly active (therapeutic index=3) against *T. equiperdum*, thus showing specificity of action against *T. congolense*. The preparation of the compound has been described by Barber and Stickings (1945). It is also of interest since it lacks a quaternary nitrogen atom, which is apparently necessary for optimal activity in the salts of the phenanthridinium compounds (Morgan and Walls, 1938). The examination of an analogous compound in which the amidino substituents are replaced by amino substituents would determine the relative importance of the amidine groupings. It was found, however, that an analogous compound, 3:6-diamidinocarbazole, was inactive ; in this compound the amidine groupings were retained but the phenanthrene was replaced by a carbazole nucleus.

The compounds 1325, 1324, 193 to 198 and 1355 are all phenanthridinium compounds and are substituted in the positions shown by Walls to confer optimal activity. The compounds 1325 and 1324 are 9-β-pyridyl-phenanthridines (Petrow and Wragg, 1946), and it was found that the replacement of the 9-aminophenyl grouping by 9-β-pyridyl led to a reduction in activity. The 3-amino derivative (1325) showed slight activity, while the 7-amino derivative (1324) was inactive: both compounds were also inactive against *T. equiperdum*. With compounds 193 to 198 we have studied the effect of the introduction of an unsaturated linkage between phenyl at C 9 and the phenanthridine nucleus. These compounds have a structural resemblance to the trypanocidal styryl-quinoline derivatives of Browning, Cohen, Ellingsworth and Gulbransen (1929). They were found to be completely inactive with the exception of the 7-amino derivative (197), which was slightly active against *T. congolense* but inactive against *T. equiperdum*. The substitution of amidine for amino groups in the phenanthridinium compounds, as in 1355, led to a loss of trypanocidal activity. It is interesting to note that although the introduction of an unsaturated linkage led to a decrease in trypanocidal activity, it produced an increase in antibacterial activity. Similarly the substituted amidines in the diphenoxy series were highly active bactericides although they were of little interest as trypanocidal agents.

The activity of the phenanthridinium compound 1553 (Walls and Browning, 1945) was evaluated for comparative purposes against *T. congolense* strain III. By subcutaneous injection the E.D.50 was found to be 0.00005 mg./g., the C.D.50 0.004 mg./g., and the L.D.50 0.061 mg./g., giving a therapeutic index of 152. This is of course valid only for mice: in cattle Bell (1945) has found recently an index of only 6.

A brief examination of the pharmacological actions of the phenanthridinium compounds 1553 and 897 has shown a similarity in some respects to the properties of the amidines. They have a depressant action on the circulation and are toxic to the liver ; the phenanthridinium compounds also have a depressant action on the respiratory centre. Of some interest was the finding that 1553 increases the pressor effects of adrenaline, whereas the amidines cause a decrease. Although photosensitization symptoms have been reported in cattle we have been unable to find any increased output of porphyrins in the urine of experimental animals. It is quite possible that there may be an accumulation of porphyrin pigments in the liver which is not accompanied by any increased porphyrinuria. The toxic effects in cattle, where the animals may die six weeks after injection, are indeed suggestive of hepatic and renal degenerative changes.

SUMMARY

1. A comparison has been made of the activities of 7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride (897) and 4:4′diamidino-$\alpha\beta$-dimethylstilbene dihydrochloride (991) against several acute and chronic strains of *T. congolense*. The phenanthridinium compound was twice as toxic and active against the acute strains, the therapeutic indices being the same, but against one chronic strain it was less active.

2. An evaluation of 2:7-diamino-9-phenyl-10-methylphenanthridinium bromide (1553) showed that by subcutaneous injection in acute infections the C.D. 50 was 0.0004 mg./g., the L.D. 50 was 0.061 mg./g., and the therapeutic index 152.

3. A study of the correlation between chemical structure and *T. congolense* activity led to the following conclusions:

Diamidine Compounds

(a) The introduction of methyl groupings into the unsaturated stilbene linkage enhanced activity ; nuclear substitution was not so effective.

(b) Nuclear substitution by various groupings in the 2-position did not increase activity, except for a slight effect with methyl, amino and acetamido derivatives, although it was found that the introduction of halogen markedly increased activity against *T. equiperdum* (results to be published). Alkyl substitution in the amidine grouping and substitution of a diphenylamine for the stilbene linkage were without effect.

(c) The comparatively high activity of phenanthrene-3:6-diamidine was of interest. An analogous compound, 3:6-diamidinocarbazole, was devoid of activity.

Phenanthridinium Compounds

(a) The replacement of amino by amidine groupings resulted in loss of activity.

(b) The substitution of the 9-(3′-aminophenyl) by the 9-(3′-pyridyl) grouping led to a reduction in activity.

(c) The introduction of an unsaturated linkage (*p*-dimethylaminostyryl) between phenyl at C 9 and the phenanthridine nucleus also led to a reduction in activity.

4. Some of the pharmacological properties of the phenanthridinium compound 1553 have been described. In general it has a depressant action on the circulation and respiratory centre, and in high doses, a toxic action on the liver and kidneys.

I am indebted to Mr. G. Newbery and Dr. H. J. Barber for the compounds examined, with the exception of Nos. 193 to 198, which were prepared for the Therapeutic Research Corporation, London, and kindly supplied by Dr. V. A. Petrow, Queen Mary College. I wish to acknowledge particularly the very valuable assistance given by Miss U. Pardoe, who considerably facilitated the investigation by her careful and painstaking microscopic examinations. Mr. L. Joyner kindly performed the biochemical estimations. My thanks are due to the directors of May & Baker, Ltd., for permission to publish the results.

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BRITISH JOURNAL OF PHARMACOLOGY AND CHEMOTHERAPY

EDITED FOR

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In co-operation with the EDITOR of the British Medical Journal

VOLUME I, 1946.

LONDON

BRITISH MEDICAL ASSOCIATION

TAVISTOCK SQUARE, W.C.1

Printed in Great Britain by Fisher, Knight and Co., Ltd.,
The Gainsborough Press, St. Albans.

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Printed in Great Britain by FISHER, KNIGHT & Co., LTD., Gainsborough Press, St. Albans.