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THE INHIBITION OF SOME PHARMACOLOGICAL ACTIONS OF PENTAMIDINE BY SURAMIN

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When pentamidine and the other aromatic diamidines were first tested in man it was noted that, unlike earlier (and chemically quite unrelated) compounds used in the treatment of sleeping sickness, such as suramin and tryparsamide, they caused an immediate and profound, but transient, reaction on intravenous injection of a dose of therapeutic size. This consisted of a steep and sometimes alarming fall of blood pressure, often accompanied by intense itching, nausea, and colic, and occasionally by vomiting and defaecation. There was often also "a curious puffy suffusion of the face and eyelids" (Lourie, 1942). In laboratory animals Wien (1943) described a number of pharmacological effects, including fall of blood pressure (shown to be caused by peripheral vasodilatation) and stimulation of the isolated intestine of the rabbit and uterus of the guinea-pig and cat. The reactions in man and in animals were later, on sound evidence, attributed by MacIntosh and Paton (1949) to the liberation of histamine by the injected diamidine.

In the course of a "mass treatment" campaign against an epidemic outbreak of sleeping sickness in Sierra Leone in 1939 to 1941, several hundred patients were treated with pentamidine and other diamidines, and many more with suramin and tryparsamide (Lourie, 1942). In the early part of the work it was noticed on occasion that, when a syringe was filled with a 1 per cent aqueous solution of pentamidine dihydrochloride or one of the other diamidines, a fine flocculent precipitate would form in the solution welling into the barrel of the syringe. This was discovered to occur only when the syringe was one that had previously been used for an injection of suramin and that had then been imperfectly washed and sterilized, so that traces of suramin remained present. (The work was done in a remote part of the country, with primitive technical assistance or other facilities.) The precipitate is no doubt attributable to the formation of an insoluble salt complex between the diamidine, which is a strong base, and suramin, which is a polysulphonic acid derivative. Such an interpretation accords well with what is known about the physicochemical reactivity of unsubstituted amidines with sulphonic and other acids (Walker, 1949). Salt- and precipitate-formation similarly occur when mixtures are made of solutions of curare and congo red or related polysulphonated substances (Petroff, 1931). This has recently been re-examined in respect of pure *d*-tubocurarine chloride by Kensler (1949).

It occurred to us that the formation of such a pentamidine-suramin salt complex might impair the pharmacological properties of one or other of the two compounds concerned, and the experiments below were designed to test this possibility. It has, in fact, been shown by Petroff (1924), Kensler (1949) and others that congo red and related substances do inhibit some of the characteristic pharmacological properties of curare. This inhibition was ascribed by Kensler to the reaction which takes place between the two compounds concerned, rather than to an intrinsic effect of congo red or other sulphonated compounds on the tissue sites to which *d*-tubocurarine is adapted. It is in this way also that we would explain the inhibition of some of the pharmacological actions of pentamidine by suramin in the experiments described below.

The actual formation of a precipitate is, of course, not a necessary accompaniment of salt formation (and hence of the possibility of inhibitory properties) between compounds of the two types concerned. This is true not only for concentrations below the thresholds at which precipitation occurs but also at concentrations considerably above those thresholds. As Kensler pointed out, the precipitate formed by congo red and tubocurarine can be dissolved in excess congo red. In the same way we have found that the precipitate formed by suramin and pentamidine can be dissolved in excess suramin. Thus in a series of suramin-pentamidine isethionate mixtures in distilled water where the pentamidine-concentration is constant at 1/100 (the concentration that was usually used for the injections in man) and suramin is present in increasing concentrations, a faint opalescent precipitate appears at about 1/100,000 suramin and becomes dense and flocculent from 1/2,000 to 1/25, but clears up completely at 1/10. If the pentamidine concentration be kept constant at 1/1,000 the dense precipitate present at the lower concentrations of suramin clears up when this approaches 1/50. If the pentamidine be kept at 1/10,000 no concentration of suramin causes a heavy precipitate, and the light precipitate found at lower concentrations of suramin clears up when this exceeds about 1/1,000.

Fall of blood pressure

Table I and Figs. 1 and 2 show the effects of an intravenous injection of pentamidine isethionate on the systolic carotid blood pressure in 16 cats (under chloralose), 8 of which served as controls (i.e., not previously treated with suramin), and the other 8 had previously received an intravenous injection of suramin. The pentamidine dose was usually 1 mg./kg. but ranged up to 2.0 mg. in the few instances shown on the Table, and the suramin ranged from 50 to 250 mg./kg. The blood pressures recorded on the Table are, on the one hand, those immediately before the pentamidine injection and, on the other, the lowest reached within the next five minutes. The initial blood pressures in some of the cats were rather low; this was because those particular cats were used after they had already served for various other experiments, which were, however, of a nature unlikely to affect the present points at issue. The prior dose of suramin sometimes produced a transient fall of blood pressure, or a slow fall returning within 15 minutes to about or near the original level, but the pentamidine was not given until a steady level had been regained. The interval between the injections of suramin and pentamidine varied between five and eighty minutes. Two of the cats that received suramin followed by pentamidine (Exps. 11 and 14) had already received a dose of pentamidine before

TABLE I

FALL OF CAROTID BLOOD-PRESSURE IN CHLORALOSED CATS AFTER INTRAVENOUS PENTAMIDINE, AND ITS INHIBITION BY INTRAVENOUS SURAMIN. PRESSURES RECORDED ARE THOSE IMMEDIATELY BEFORE PENTAMIDINE AND THE LOWEST REACHED WITHIN THE NEXT FIVE MINUTES

Pentamidine alone				Suramin followed by pentamidine					
Exp.	Pentam. dose (mg./kg.)	Blood pressure: Before pentam. (mm.Hg)	Blood pressure: After pentam. (mm.Hg)	Exp.	Suramin dose (mg./kg.)	Interval between suramin and pentam. (min.)	Pentam. dose (mg./kg.)	Blood pressure: Before pentam. (mm.Hg)	Blood pressure: After pentam. (mm.Hg)
5	2.0	33	10	1	200	80	1.2	93	93
6	1.0	56	34	2	100	15	1.0	35	29
7	1.0	33	20	3	100	45	1.7	40	40
10	1.0	145	71	4	50	20	2.0	65	57
12	1.0	58	20	8	250	40	1.0	96	103
13	1.0	128	60	9	250	35	1.0	91	83
15	1.0	87	51	11*	100	5	1.0	107	107
16	1.0	125	79	14*	100	8	1.0	110	110
	Averages	83	43				Averages	80	78

* One injection of pentamidine already given before the suramin in this experiment.

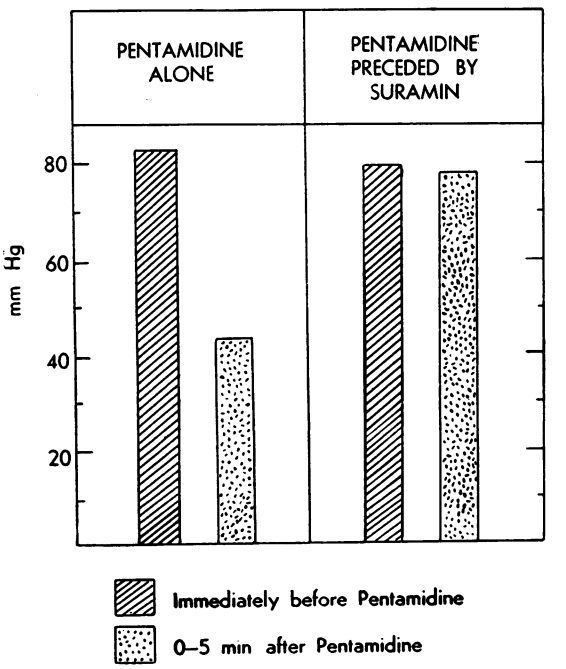

FIG. 1.—Inhibitory effect of suramin on the fall of carotid blood pressure caused by pentamidine. Average pressures in cats (chloralose) immediately before and within 5 minutes after an intravenous injection of pentamidine; 8 cats received pentamidine alone, and 8 received pentamidine preceded by suramin (see Table I).

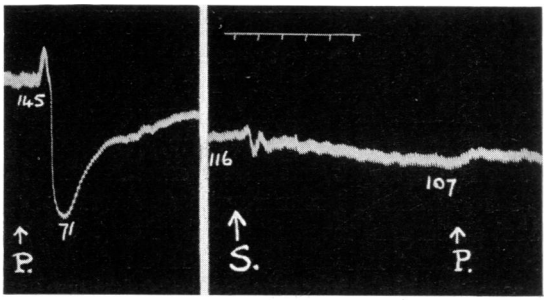

FIG. 2.—Inhibitory effect of suramin on the fall of carotid blood pressure caused by pentamidine. Cats of exps. 10 and 11, Table I. P. = Pentamidine, 1.0 mg./kg. i.v. S. = Suramin, 100 mg./kg. i.v. 30-second time-intervals.

the suramin. In other experiments it was noted that repeated injections of pentamidine usually produced progressively smaller depressor responses (see also Wien, 1943). In the two cats of Exps. 11 and 14, therefore, the inhibitory effect of suramin on the pentamidine-response might have been reinforced by the earlier injections of pentamidine. However, an inhibitor effect of suramin in these two instances remains beyond doubt, since a second injection of pentamidine normally produces merely a smaller response and not the complete abolition observed in the two cases in question. The Table and Fig. 1 show that in the cats that had previously received no suramin the blood pressure was reduced by an injection of pentamidine from an average of 83 to 43 mm. Hg, while in the suramin-treated cats the fall caused by pentamidine was negligible: Fig. 2 shows tracings from two representative experiments (Nos. 10 and 11) of the series.

Broncho-constriction

In view of the known pharmacological actions of pentamidine, to be regarded as the effects of histamine release, some evidence of broncho-constriction is to be expected. This can readily be shown in guinea-pigs by the technique of Konsett and Rössler (1940). Fig. 3 (guinea-pig *a*) represents a typical broncho-constrictor

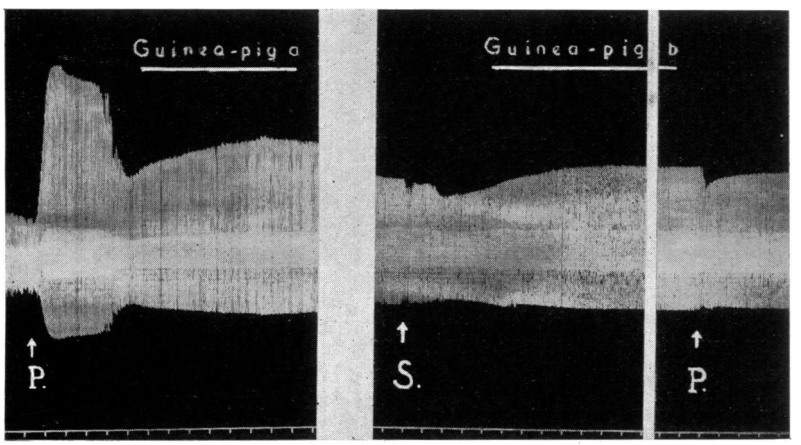

FIG. 3.—Inhibitory effect of suramin on the broncho-constrictor action of pentamidine in guinea-pigs. Urethane anaesthesia. P. = Pentamidine, 25 mg./kg. i.v. S. = Suramin, 250 mg./kg. i.v. 60-second time-intervals. Break in the tracing for guinea-pig *b* represents 20 minutes.

effect, as shown by this method, after the intravenous injection of 25 mg./kg. pentamidine isethionate in a guinea-pig anaesthetized with urethane. A smaller dose does not regularly produce the effect. The reaction begins about a minute after injection but is sometimes delayed for two or even three minutes (a latent period which, in respect of the fall of blood pressure, was stressed by MacIntosh and Paton as probably significant of the underlying mechanism of histamine release). The constrictor response reaches its maximum a few minutes after its onset, and there is then a gradual relaxation, though there is often (as in guinea-pig *a*, Fig. 3) a secondary low-grade constriction lasting for perhaps a quarter of an hour before a stable condition is reached. In numerous experiments we have found that the constrictor action of 25 mg./kg. pentamidine may be aborted by a prior intravenous injection of 250 mg./kg. suramin, and this is shown in the second portion of Fig. 3 (guinea-pig *b*), in which the pentamidine was given 20 minutes after the suramin. In order that the inhibition of the pentamidine-effect by suramin may be attributed with assurance to the latter drug, it is advisable to use a guinea-pig that has had no dose of pentamidine previous to the suramin, since it has been found that a pig that has had one injection of 25 mg./kg. pentamidine may fail completely to respond to a second such injection. It is for this reason that Fig. 3, illustrating inhibition of the broncho-constrictor effect of pentamidine, is derived from observations on two animals, while Fig. 1, dealing with inhibition of the fall of blood pressure, is derived from observations on a single animal. In other experiments we have found, as would be expected, that a suitable dose of an antihistamine compound (e.g., 2.5 mg./kg. mepyramine) is also capable of completely aborting the broncho-constrictor effect of an injection of 25 mg./kg. pentamidine, a result which supports the view that histamine release accounts for at least some of the pharmacological effects of pentamidine with which we are here concerned.

We were interested in comparing the picture produced by the Konsett and Rössler apparatus after an injection of pentamidine with the picture produced by acetylcholine. In the course of these observations an unexpected finding emerged. This was that the tracing for a dose of acetylcholine given before a guinea-pig had received any injection of pentamidine was very different from the tracing produced by the same dose of acetylcholine after an injection of pentamidine (and after time had been allowed for the broncho-constrictor effect of the pentamidine to subside). This is illustrated by Fig. 4, guinea-pig *a*, where the break in the tracing represents a period of 30 minutes, during which a dose of 25 mg./kg. pentamidine was allowed to exercise its effect. Clearly the action of acetylcholine was greatly potentiated by the interposed dose of pentamidine. An explanation of this potentiation was sought in the possibility that there might be a slow release of histamine continuing for some time after the pentamidine injection, this slow release being itself insufficient to cause broncho-constriction but having the effect of exaggerating the response to a dose of acetylcholine injected during the period of release. The explanation was tested indirectly in guinea-pig *b* of Fig. 4 by slowly infusing histamine intravenously at the rate of 1μg. per minute, which was insufficient to cause any constrictor action, and comparing the effect produced by a dose of acetylcholine injected during this infusion with the effect produced by the same dose of acetylcholine before the infusion had begun. The slow histamine infusion did indeed have the effect of potentiating the guinea-pig's response to acetylcholine,

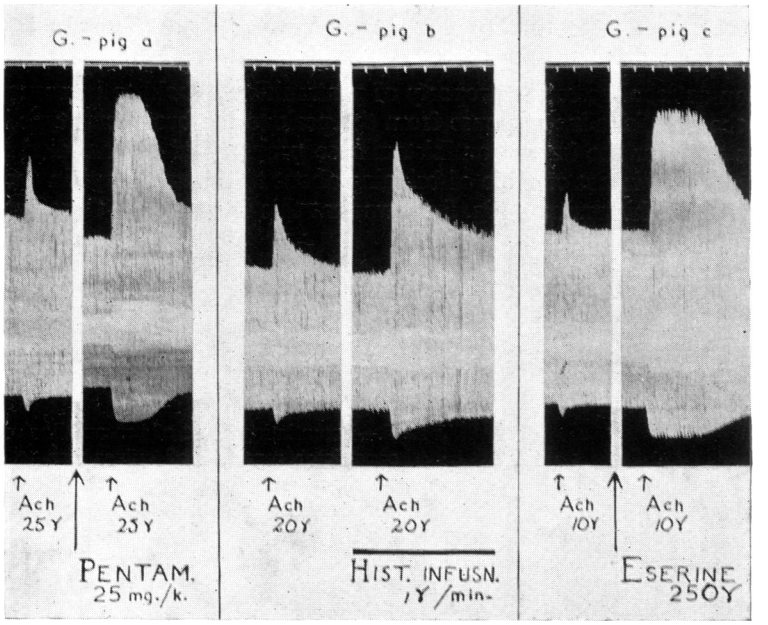

FIG. 4.—Broncho-constrictor response to acetylcholine in guinea-pigs. Potentiation caused by pentamidine (guinea-pig *a*), histamine infusion (guinea-pig *b*), and eserine (guinea-pig *c*). Urethane anaesthesia. Ach. = Acetylcholine. Pentam. = Pentamidine. Hist. infusn. = Histamine infusion. All injections and the infusion i.v. 60-second time-intervals. Break in the tracing for each guinea-pig represents 30 minutes.

but there is an important difference between the tracings for acetylcholine potentiation attributable to the histamine infusion (guinea-pig *b*) and the potentiation provoked by a prior dose of pentamidine (guinea-pig *a*). This is that during the histamine infusion the characteristic shape of the tracing normally produced by acetylcholine is unimpaired; the band immediately widens and forthwith begins to return again to its original state, giving the appearance which, for convenience, we call a "peak" effect (guinea-pig *b*). After a dose of pentamidine, on the other hand, the band again immediately widens in response to an injection of acetylcholine, but it then remains at the maximum width for a relatively long time, which may be as much as two or three minutes, before the effect begins gradually to subside. For convenience, we call this a "broad-end" effect (second part of tracing for guinea-pig *a*). It is clear, therefore, that the exaggerated response to acetylcholine in an animal that has previously received a dose of pentamidine is unlikely to be explained merely as the effect of acetylcholine injected during a period of slow histamine release. An alternative explanation is strongly suggested by the shape of the broad-end tracing, since this indicates not only a greater degree of bronchoconstriction but also a significant persistence of the effect. The alternative explanation is that the potentiation is due to an anticholinesterase effect of the prior dose of pentamidine. Anticholinesterase activity has been demonstrated in the closely related diamidine, stilbamidine, by Bergmann, Wilson, and Nachmansohn (1950). That this is the likely explanation is supported by showing that if a dose of eserine is interposed between two injections of acetylcholine, the response is converted

from the peak to the broad-end type in the same way as when a dose of pentamidine has been interposed (guinea-pig *c*, compared with guinea-pig *a*, Fig. 4).

Since an anticholinesterase property of pentamidine is held to be responsible for changing the acetylcholine-response from the peak to the broad-end type, the question arises whether the known antihistaminase property of pentamidine (Blaschko and Duthie, 1944) is not capable of bringing about a similar change in the response of the bronchi to histamine. Fig. 5 shows that this is so, and it therefore provides

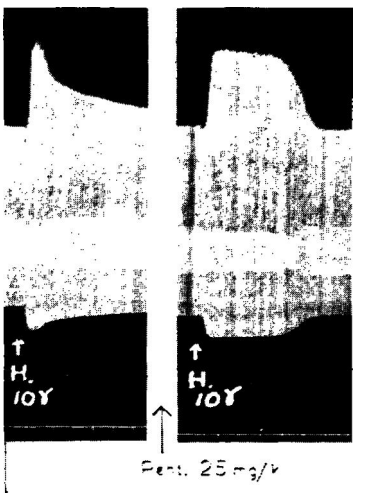

FIG. 5.—Potentiating effect of pentamidine on broncho-constrictor response of guinea-pigs to histamine. Urethane anaesthesia. H. = Histamine. Pent. = Pentamidine. 60-second time-intervals. Break in the tracing represents 30 minutes.

circumstantial evidence of antihistaminase activity on the part of pentamidine. Can similar circumstantial evidence be obtained in favour of an antihistaminase property on the part of suramin as well? This compound is already known to be capable of inhibiting a wide range of other enzyme systems (Town, Wills, Wilson, and Wormall, 1950; Wills and Wormall, 1950). If an injection of suramin were to exercise any antihistaminase activity, then the ability of such an injection to inhibit the broncho-constrictor effect of a subsequent dose of pentamidine must represent a high inhibitory power indeed; for the antihistaminase property of the suramin would tend to potentiate rather than to inhibit the pentamidine response (i.e., the response to liberated histamine). If, on the other hand, suramin has the property of inhibiting and not of potentiating the effects of a dose of histamine, then this antihistamine action might alone be sufficient to account for suramin's inhibitory effect on pentamidine, without the formation of a salt complex playing any essential part in the inhibitory mechanism. It is therefore important to determine the influence which suramin exercises on the response of guinea-pig bronchi to an injection of histamine.

Fig. 6 is a tracing from one of many experiments which showed that suramin has, in fact, some inhibitory action on histamine. The broncho-constrictor effect of 4 μg. histamine was abolished by an injection of 250 mg./kg. suramin, and it was necessary to give 16 μg. histamine after the suramin in order to produce about the same degree of constriction as was caused by one quarter of that dose before the

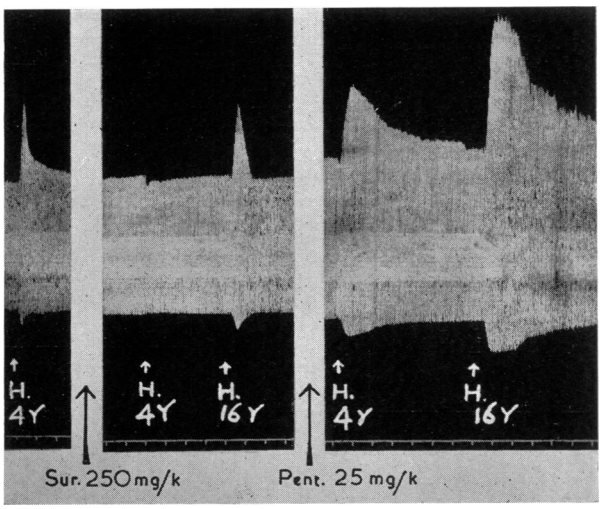

FIG. 6.—Antihistamine effect of suramin on guinea-pig bronchioles, and counteraction of this effect by the potentiating influence of pentamidine. Urethane anaesthesia. H. = Histamine. Sur. = Suramin. Pent. = Pentamidine. 60-second time-interval. Breaks in the tracing represent intervals of 20 and 10 minutes.

suramin. The question therefore becomes acute whether this antihistamine action of suramin can indeed account for its inhibitory effect on pentamidine. This seems unlikely, because Fig. 6 also shows that the antihistaminase action of the pentamidine dose more than counteracted the antihistamine action of the suramin, so that the response to 4 μg. histamine, which had been abolished by the suramin, was now greater than before. The anti-pentamidine action of suramin is therefore unlikely to be fully explained by suramin's antihistamine property, and inactivation of the pentamidine by formation of a suramin-pentamidine salt complex remains a tenable explanation.

It was found, incidentally, that the antihistamine action of suramin is non-specific. An inhibitory action of the same general order is exercised also against acetylcholine. For example, in one typical experiment the broncho-constrictor effect of 5 μg. acetylcholine was abolished by 250 μg. suramin, and it was necessary to give five times that amount of acetylcholine in order to produce about the same effect as before the suramin.

Contraction of isolated gut

As a preliminary to investigating the inhibitory effect of suramin on the power of pentamidine to cause contraction of the isolated gut, we set out to confirm that suramin has an antihistamine effect on this preparation.

With a piece of guinea-pig ileum suspended in oxygenated Locke solution at 32° C., it was found that suramin in a concentration of 1/1,000 caused tonic and spasmodic contractions ; concentrations of 1/1,000 to 1/5,000 caused in some experiments occasional spasmodic contractions during only about the first 10 minutes of exposure, and concentrations below 1/5,000 had little or no visible effect. Observations were then made on the response of guinea-pig ileum to a uniform dose of

histamine introduced at 5- or 10-minute intervals (*a*) before the addition of suramin to the surrounding Locke solution; (*b*) in the presence of varying concentrations of suramin; and (*c*) in the absence of suramin but after the gut had been exposed to that substance for varying lengths of time. The method used and results obtained are sufficiently shown on Table II and Figs. 7 and 8, from which it will be seen

TABLE II

SUMMARY OF EXPERIMENTS TO SHOW INFLUENCE OF SURAMIN ON THE RESPONSE OF ISOLATED GUINEA-PIG ILEUM TO HISTAMINE

Suramin concentration	No. of exps.	Frequency of histamine test*	Duration of contact with suramin (in min. in respective experiments)	Histamine response, in relation to response before contact with suramin	
				During contact with suramin	After contact with suramin
>1/10,000 ..	3	5 min.	10, 30, 30	Potentiation	Inhibition
	1†	10 min.	60	Potentiation	Inhibition
1/10,000–1/25,000	5	5 min.	30, 30, 35, 40, 60	Potentiation	Inhibition
	2‡	10 min.	50, 80	Inhibition§	Inhibition
1/40,000–1/100,000	7	5 min.	30, 30, 30, 30, 60, 75, 90	No change‖	No change¶
	2	10 min.	50, 120	No change	No change

* Histamine dosage (chosen to give sub-maximal response at the beginning of the experiment) kept constant throughout each experiment; usually such that concentration in the bath was 10^{-8}. Replaced by Locke or suramin-Locke solution as soon as full response had been produced.

† See Fig. 7.

‡ See Fig. 8.

§ Appeared in 10–20 and 20–30 minutes, respectively.

‖ Except in one experiment with 1/40,000 suramin, in which there was potentiation.

¶ Except in one experiment with 1/80,000, in which there was inhibition.

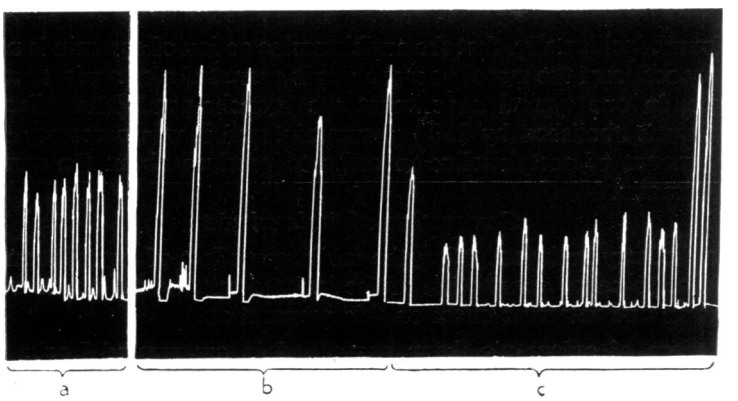

FIG. 7.—Potentiation of the response of isolated guinea-pig ileum to histamine in the presence of suramin and inhibition after its removal. Histamine 10^{-8} tested every 10 minutes in oxygenated Locke solution at 32° C. (*a*) before adding suramin to the Locke solution, (*b*) in the presence of 1/4,000 suramin, and (*c*) after replacing the suramin-Locke by normal Locke solution; last 2 contractions are in response to 5×10^{-8} histamine.

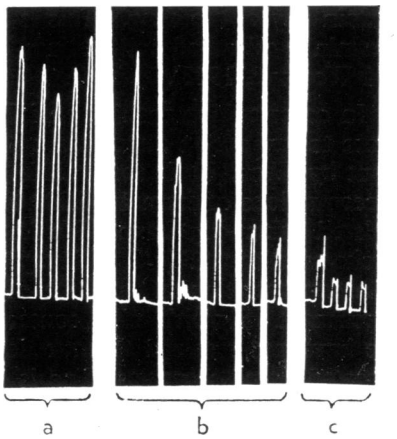

FIG. 8.—Inhibition of the response of isolated guinea-pig ileum to histamine both in the presence of suramin and after its removal. Histamine 10^{-8} tested every 10 minutes in oxygenated Locke solution at 32° C. (*a*) before adding suramin to the Locke solution, (*b*) in the presence of 1/10,000 suramin, and (*c*) after replacing the suramin-Locke by normal Locke solution. Section *b* is interrupted because the drum was not stopped between tests in this part of the experiment.

that suramin at concentrations above 1/10,000 in the fluid surrounding the gut serves to potentiate its response to histamine, but as soon as suramin ceases to be present in the surrounding fluid the histamine-response becomes inhibited (in relation to the effect observed before suramin was present). This inhibition persists for periods of from 20 minutes to at least 70 minutes (the limit of the observation-period), depending on the concentration of suramin that had been used and the duration of exposure. When the surrounding Locke solution contains suramin in concentrations of 1/25,000 to 1/10,000 there may again be potentiation of the histamine-response, followed by inhibition in suramin-free Locke solution. But the potentiation in the presence of suramin at these concentrations was observed only in those experiments (five in number) in which the response to histamine was tested every five minutes. In the two experiments in which the histamine-response was tested every 10 minutes the effect observed in the presence of suramin was inhibition instead of potentiation. Whether any significance attaches to the fact that the one effect was observed when the histamine-response was tested every five minutes and the other when it was tested every 10 minutes can be decided only by further experiment. When the suramin concentrations used were below 1/25,000 there was usually no observed change in the histamine-response either in the presence of suramin or after its removal.

A reasonable interpretation of these findings is that within a narrow concentration-range suramin does exercise an inhibitory action on the response of the isolated gut to histamine. While this is clear from some of the above experiments, the conditions necessary for its consistent demonstration remain to be accurately determined. In the presence of higher concentrations the tissue becomes abnormally excitable and its reactivity to histamine is enhanced. (It is possible that this hyper-excitability may be due to impurities rather than to the suramin itself; "Antrypol" brand, as commercially supplied by I.C.I., was used throughout.) During exposure of the gut to suramin, especially at the higher concentrations, some of the substance is adsorbed by the tissue cells and, depending on the actual concentration used and the duration of exposure, sufficient is retained for the cells to remain in the inhibitory range of suramin-concentration for a long time after the surrounding fluid has ceased to

contain any of the drug. This is in accordance with the well-established fact that suramin is adsorbed and retained by body tissues for long periods of time (Dewey and Wormall, 1946).

In order to demonstrate an inhibitory effect of suramin on the isolated gut we chose to use rabbit duodenum rather than guinea-pig ileum, because of the statement by Wien (1943) that rabbit gut is consistently stimulated by pentamidine at concentrations of from 1/25,000 to 1/10,000, while no marked effect is exerted on isolated guinea-pig ileum. We found that isolated rabbit duodenum in oxygenated Locke solution at 35° C. will respond repeatedly to successive doses of pentamidine at a concentration in the bath of 1/25,000. Fig. 9 shows the tracing from one experiment in which nine consecutive contractions were produced in this way. We have not resolved the question how far this ability of the gut to respond to repeated doses of pentamidine is consistent with histamine release as an explana-

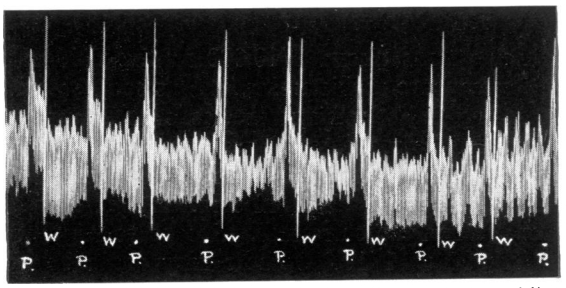

FIG. 9.—Response of rabbit duodenum to repeated contact with pentamidine isethionate in oxygenated Locke solution at 35° C. P. = Pentamidine 1/25,000. W. = Wash.

tion of the underlying mechanism of the stimulant action of pentamidine on the gut. In this connexion, though, an antihistamine (mepyramine) at a concentration of 1/100,000 can be shown to prevent the response normally caused by 1/25,000 pentamidine.

Fig. 10 also shows consecutive pentamidine responses, of rather greater degree than those of Fig. 9, and, on another piece of gut, abolition of the response to this

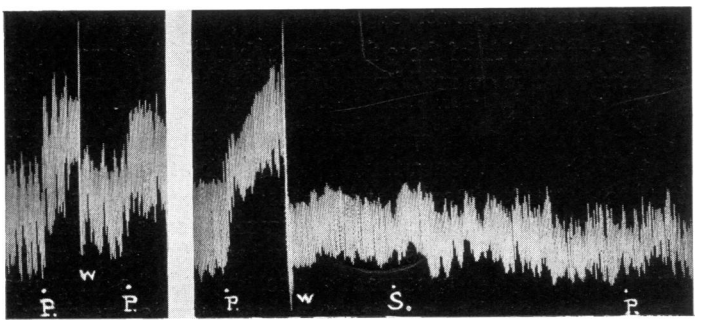

FIG. 10.—Inhibitory effect of suramin on the stimulant action of pentamidine on isolated rabbit duodenum in Locke solution at 35° C. P. = Pentamidine 1/25,000. S. = Suramin 1/5,000. W. = Wash.

concentration of pentamidine (1/25,000) by the presence in the Locke solution of suramin at a concentration of 1/5,000. In view of the possible role of histamine release in the mechanism of pentamidine activity on the gut, and the antihistamine action of suramin demonstrated in the experiments above, we cannot exclude the possibility that suramin's anti-pentamidine action on the gut may be partly due to antihistamine activity as well as to the formation of an inactive salt complex.

Changes in hydrogen ion concentration cannot be held to account for the effects on the histamine- or the pentamidine-response that we have attributed to suramin. Using a Muirhead *p*H-Meter (type D-303-B) we found that changes in the hydrogen ion concentration of Locke solution caused by the presence of up to 1/200 suramin are negligible.

*Curare-like action**

Depression of neuro-muscular transmission.—Bergmann *et al.* (1950) claimed that stilbamidine blocks neuro-muscular transmission in the frog sciatic nerve gastrocnemius preparation. This led us to seek an action that might possibly be interpreted in this way on the rat phrenic nerve diaphragm preparation of Bülbring (1946). Fig. 11 shows the results of a typical experiment in which the preparation was set up in double dextrose Tyrode solution at 37° C. and single shocks of 0.73 mv. for 0.8 msec. were applied at 10-second intervals by means of a square-wave stimulator. Pentamidine in a concentration of 1/10,000 caused a slight temporary inhibition of the muscle contractions. The response to such a dose is repeatable a number of times in a single preparation, and in the experiment of Fig. 11 it was obtained six times, at intervals of about ten minutes, immediately before making the observations recorded on the Figure. It will be seen that the presence of 10^{-4} suramin in the double dextrose Tyrode solution used completely blocked the pent-

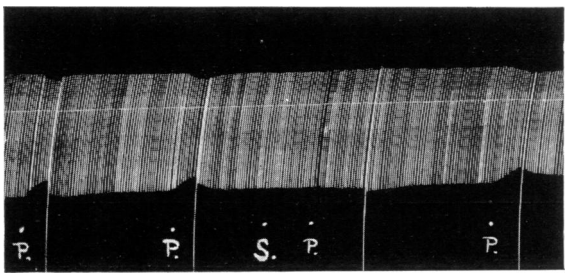

FIG. 11.—Depression of muscle contractions by pentamidine in the rat phrenic nerve diaphragm preparation, and abolition of this effect in the presence of suramin. Oxygenated double dextrose Tyrode solution at 37° C. P. = Pentamidine 1/25,000. S. = Suramin 1/10,000.

amidine effect, which could then be elicited again after washing. A slight precipitate formed in the bath while suramin and pentamidine were both present. In order to eliminate the possibility that the inhibition might be due simply to effective amounts of pentamidine being withdrawn from solution by the precipitate, similar observations were made using an excess of suramin (1/200) so that there should

* The term curare-like is used here very loosely and without implying that the mechanism of action of pentamidine is exactly the same as that of curare.

be no actual precipitate present in the suramin-pentamidine mixture. In this concentration of suramin (before adding pentamidine) the contractions became somewhat greater than before. On the addition of pentamidine to give a concentration of 1/10,000, the suramin excess prevents the formation of any precipitate (or if formed it immediately goes into solution) and the inhibitory action of pentamidine on the contractions is abolished in the same way as when weaker (precipitate-forming) concentrations of suramin are used. At such a high concentration of suramin, however, the interference with pentamidine activity is much longer-lasting. In one experiment it persisted after eight Tyrode washings during a period of about 80 minutes, as tested by the introduction of pentamidine at a concentration of 1/10,000 shortly before each wash. That the ability of the preparation to respond to pentamidine was, however, not completely abolished was shown by inhibition of the muscle contractions when the pentamidine test-dose was increased to give a concentration of 1/2,000 in the bath. Persistence of the suramin-effect long after the surrounding fluid has been replaced by suramin-free Tyrode solution is not surprising in view of what is known about the power of tissue cells to adsorb and retain suramin, to which reference was made in the section above on contraction of isolated gut.

Paralysis in frogs.—The demonstration that pentamidine blocks neuro-muscular transmission on the phrenic nerve diaphragm preparation leads naturally to a consideration of other properties that this compound may have in common with curare. Its ability to paralyse frogs when injected into the ventral lymph sac was therefore investigated, together with the question whether this paralysis could be inhibited by suramin.

TABLE III

Inhibitory effect of suramin on pentamidine-induced paralysis in frogs. Both drugs injected into the ventral lymph sac. Suramin-treated frogs received only one dose of suramin, at the beginning of the experiment, but pentamidine was given daily to all frogs as long as they survived

Exp.	Frog serial numbers	Number of frogs paralysed, in relation to the number treated							
		1st day		2nd day		3rd day		4th day	
		Drug and dose*	Result	Drug and dose*	Result	Drug and dose*	Result	Drug and dose*	Result
1	1–6	P. 0.75	4/6	P. 1.5	6/—	P. 1.5	2/2	P. 1.5	1/1
	7–12	S. 20 + P. 0.75	0/6	P. 1.5	1/5	P. 1.5	0/2	No survivors	
2	13–18	P. 1.5	6/6	P. 1.5	3/3	P. 1.5	1/1	No survivors	
	19–24	S. 40 + P. 1.5	0/6	P. 1.5	0/1	P. 1.5	0/1	No survivors	

* P. = Pentamidine. S. = Suramin. Dose in mg./20 g.

The injections into the ventral lymph sac were made in aqueous solution in 0.5 ml. per 20 g. body weight. Suitable doses of pentamidine were found regularly to cause a generalized flaccid paralysis which, if the frog recovered at all, lasted for two to five hours. Suramin was injected 10 minutes before the pentamidine, and the paralysis associated with the latter drug could thus be aborted; for the demonstration of this inhibition the suramin dose should not exceed 40 mg./kg., since larger doses in the frog cause a generalized spastic or tetanic condition which obscures any possible inhibitory influence that may be exerted on the effects of a subsequent pentamidine injection. Following Kensler (1949), paralysis was defined as inability of the frog to right itself five times consecutively when placed on its back, and Table III records the results of two experiments from which the following conclusions were drawn:

1. Pentamidine in a dose of 0.75 mg./20 g. usually or often paralyses, and a dose of 1.5 mg./20 g. regularly does so. The larger dose produced this effect on each of the nineteen occasions on which it was tested (without prior suramin).

2. The larger dose (1.5 mg./20 g.) may eventually kill the frog (after it has recovered from the paralysis), though some frogs will support repeated injections at 24-hour intervals for two or three days.

3. A prior injection of suramin in a dose of 20 or 40 mg./20 g. can protect a frog against the paralysing action of 1.5 mg./20 g. pentamidine.

4. This protective effect of a single injection of suramin may last for at least 48 hours.

Toxicity for mice

An intraperitoneal injection of 2.75 or 3.25 mg./20 g. pentamidine isethionate is normally lethal to mice within 48 hours of treatment. After the larger dose (3.25 mg.) most of the deaths occur within six hours of the injection, but if

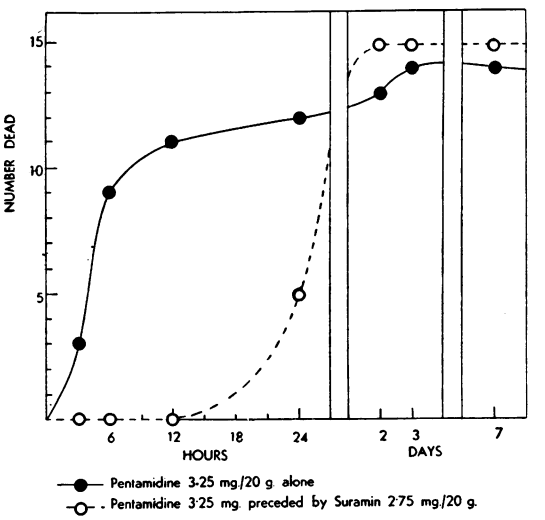

FIG. 12.—Delaying effect of suramin on the lethal action of pentamidine in mice. Intraperitoneal injections. Suramin half-an-hour before the pentamidine. Fifteen mice in each group.

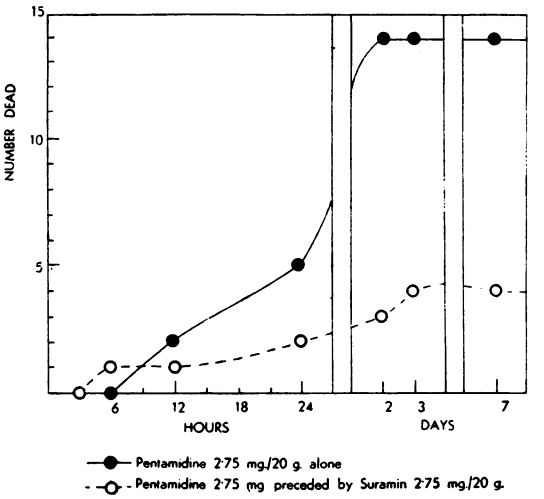

FIG. 13.—Inhibitory effect of suramin on the lethal action of pentamidine in mice. Intraperitoneal injections. Suramin half-an-hour before the pentamidine. Fifteen mice in each group.

2.75 mg./20 g. suramin be injected intraperitoneally half-an-hour before the pentamidine, the toxic action of the latter is delayed, so that most of the mice do not die until more than 24 hours later. This is shown in Fig. 12. Against the smaller dose of pentamidine (2.75 mg.) the protective action of 2.75 mg. suramin is, of course, less severely tested, so that most of the mice recover completely. This is shown in Fig. 13.

The question of suramin's antihistamine action, exercised against histamine released by the pentamidine injected, may be dismissed in explaining the inhibitory effect of suramin on pentamidine in mice and frogs, because of the relative insensitivity of these animals to histamine. Also, in frogs the effects observed were in no way to be associated with the known actions of histamine.

The question of chemotherapeutic interference

Both pentamidine and suramin are extensively used in the treatment of sleeping sickness. It is therefore not only of academic interest but perhaps also of some practical importance to decide whether the inhibition of pharmacological activity exercised by the one drug on the other extends also to an inhibition of therapeutic efficacy. Investigation of this aspect is not yet complete but is sufficiently important to warrant speculation at the present stage.

Chemotherapeutic interference between these compounds is, on the whole, unlikely. The pharmacological responses we have studied have all been of the kind that come into effect almost immediately after injection while the pentamidine is present in the system during the very short period of its maximum concentration. This is obvious enough in our investigations on blood pressure, bronchial and intestinal tone, and neuro-muscular transmission. It is less obvious in regard to toxicity for mice, where many of the deaths occur not immediately but some hours after the injection, though here also the result is probably largely dependent on the

impact of high concentrations of drug on vital centres immediately after the injection. The pharmacological interference we have studied occurs, therefore, during the very short period when the two compounds are present in the system in maximal concentrations and when conditions are accordingly appropriate for formation of an inactive salt complex. Chemotherapeutic activity, on the other hand, comes into play when either compound is present not only at much lower concentrations than are necessary for the pharmacological effects studied, but also during a very much longer period of exposure of the reactive tissue (in this case the trypanosome cell) to drug. Trypanosomes may not disappear from the blood stream for several days after a curative dose of either suramin or pentamidine, and Wormall and his colleagues (Town *et al.*, 1950) have produced strong evidence for the view that, after an injection, suramin is gradually released from drug-protein complexes in the plasma and may then enter the trypanosome in amounts which would be slightly inhibitory over a long period of time. Quite a low grade of inhibitory enzyme action, if maintained sufficiently long, could so disorganize the trypanosome's metabolism as to lead to its death and fully account for the therapeutic effect. Pentamidine has been less studied from these points of view, but it also is known to act relatively slowly, and the antitrypanosomal action of both suramin and pentamidine is known to persist for a very long time after an injection, as testified by a protective period of several months after the injection of a single prophylactic dose of either compound. Under these conditions of relatively slow action over a long period of time in low concentrations, complete dissociation of the suramin-pentamidine salt complex is to be expected, and each compound should exercise its action on the trypanosome unobstructed by the other.

This work therefore provides a basis of evidence, and of theory which yet requires further experimental support, for a unique situation in therapeutics where two compounds tend to annul one another's pharmacological or toxic effects while acting in concert (if not synergistically) as chemotherapeutic agents.

SUMMARY

1. The chance observation that a precipitate is liable to form in mixtures of dilute solutions of suramin and pentamidine raised the question whether the simultaneous presence of these two substances in the body might tend to inhibit the pharmacological effects of either of them. This has been substantiated by showing that a previous injection of suramin (or the presence of suramin) can inhibit the following actions of pentamidine: fall of blood pressure, broncho-constriction, contraction of gut, "curare-like" action on the rat phrenic nerve diaphragm preparation, paralysis in frogs and toxicity for mice.

2. Reasons are given for attributing this inhibitory effect mainly to the formation of an inactive salt complex between the suramin and pentamidine. The salt complex may, of course, be present even when the concentrations of the component substances are outside the range of those necessary for the actual formation of a precipitate.

3. Some of the pharmacological effects of pentamidine are known to be attributable to histamine release, and we have found that suramin can exercise antihistamine activity under certain conditions. It is possible, therefore, that this antihistamine

action contributes towards suramin's inhibitory influence on pentamidine, but reasons are given for believing that it plays a secondary role in some of the examples studied and is negligible in others.

4. Theoretical considerations make it unlikely that this inhibition of the pharmacological effects of the one drug by the other embraces also an inhibition of chemotherapeutic activity.

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