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

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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

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 = NH₂, R' = R'' = H, X =), and 1553, 2:7-diamino-9-phenyl-10-methylphenanthridinium bromide (R = R' = NH₂, 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) <i>Diamidines</i> . (i) <i>Stilbene Series</i>	
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) <i>Diamidines.</i> (i) <i>Stilbene Series</i> (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- $\alpha\beta$ -dimethylstilbene
(ii) <i>Diphenoxy Series</i>	
782	4:4'-diamidino- $\alpha\gamma$ -diphenoxypropane
1146	2-bromo-4:4'-diamidino- $\alpha\gamma$ -diphenoxypropane
1272	2-bromo-4:4'-diamidino- $\alpha\epsilon$ -diphenoxypentane
993	4:4'-diamidino- $\alpha\eta$ -diphenoxyhexane
(iii) <i>Miscellaneous diamidines</i>	
1150	Phenanthrene-3:6-diamidine
1346	3:6-diamidinocarbazole
938	4:4'-diamidinodiphenylamine
(b) <i>Phenanthridinium Compounds</i>	
897	7-amino-9- <i>p</i> -aminophenyl-10-methylphenanthridinium chloride
1553	2:7-diamino-9-phenyl-10-methylphenanthridinium bromide
1355	7-amidino-9 - <i>p</i> - amidinophenyl - 10 - methylphenanthridinium chloride
1325	3-amino-9-(3'-pyridyl)-phenanthridine monomethochloride
1324	7-amino-9-(3'-pyridyl)-phenanthridine monomethochloride
193	9-(<i>p</i> -dimethylaminostyryl)-phenanthridine methochloride
196	7-acetamido-9-(<i>p</i> -dimethylaminostyryl)-phenanthridine methochloride
197	7-amino - 9 - (<i>p</i> - dimethylaminostyryl) - phenanthridine methochloride
198	2-methyl - 9 - (<i>p</i> - dimethylaminostyryl) - phenanthridine methochloride

The toxicity data of the three compounds examined in detail,

991	4:4'-diamidino- $\alpha\beta$ -dimethylstilbene dihydrochloride
897	7-amino-9- <i>p</i> -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) <i>Strain I</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) <i>Strain II</i>			
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) <i>Strain III</i>			
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) <i>Strain IV</i>			
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) <i>Strain V</i>			
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	—

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	— NH ₂	NH ₂	H	H	0.094	0.0025	0.009	10.4
1553	—	NH ₂	NH ₂	H	0.061	0.00005	0.0004	152
1355	— Am	Am	H	H	0.046	Inactive	—	—
1325	—	H	H	NH ₂	0.15	0.02	0.06	2.5
1324	—	NH ₂	H	H	0.174	Inactive	—	—
193	— CH : CH 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'diamidino-stilbene 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 (Petrov 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) group-
ing 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.

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