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THE CHEMOTHERAPEUTIC ACTION OF PHENANTHRIDINE COMPOUNDS

PART III

THE PHARMACOLOGICAL PROPERTIES OF 3-AMINO-9-*p*-CARBETHOXY-AMINOPHENYL-10-METHYLPHENANTHRIDINIUM SALTS

BY

L. G. GOODWIN, M. D. GOSS, AND J. A. LOCK

From the Wellcome Laboratories of Tropical Medicine, 183, Euston Road, London, N.W.1

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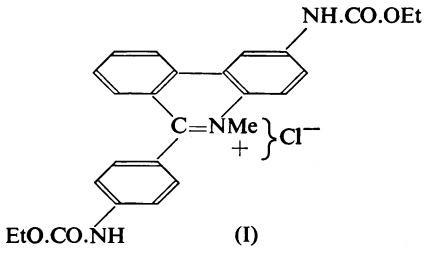
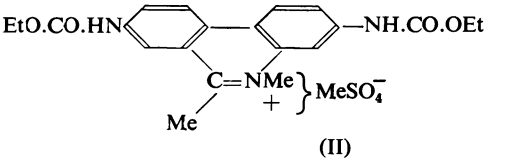
Browning, Calver, Leckie, and Walls (1946) showed that certain phenanthridinium derivatives containing the carbethoxyamino-group (I and II) had appreciable activity against *Trypanosoma cruzi* infections in mice. During the past few years, we have examined a large number of compounds prepared by Dr. Walls and his colleagues, and the general findings have been reported in Part II of this work.

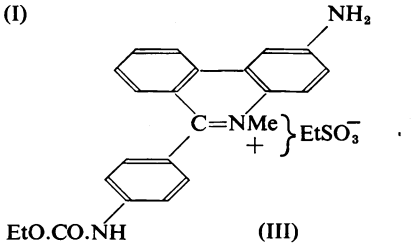
Very few drugs have any value in the treatment of Chagas's disease in man, and therefore any new type of compound which shows promise is worthy of clinical trial if it can be shown to be free from injurious side effects. We have therefore chosen the phenanthridinium compound (III) which was the most active, and have investigated its properties in more detail.



(I)



(II)



(III)

3-Amino-9-*p*-carbethoxyaminophenyl-10-methylphenanthridinium ethanesulphonate or " carbidium ethanesulphonate " (III) is a yellow crystalline solid, readily

soluble in water, especially on heating. It is less soluble in alcohol and other organic solvents. A 2 per cent (w/v) solution may be autoclaved without decomposition. The ethanesulphonate has been used in most of the experiments described below. In a few instances we have used the sulphate, which is almost identical in pharmacological properties, but is more soluble in water.

Our investigations fall into five main groups:

1. A more extensive examination of trypanocidal activity.
2. Acute and chronic toxicity.
3. Effects of injection in man.
4. Absorption and excretion.
5. Actions upon circulation, respiration, and the nervous system.

METHODS

Trypanocidal activity

The methods used for testing drugs against *Trypanosoma cruzi* have been outlined in Part II of this work. In the present extended tests, larger groups of animals were used and the experiments were continued for longer periods. The peripheral blood of treated animals was examined for trypanosomes both by fresh cover-slip preparations, and by stained thick smears. In addition to the strain of *T. cruzi* used in the experiments recorded in Part II we have also used a strain isolated recently in Uruguay and kindly given to us in infected *Triatoma* bugs by Professor R. V. Talice.

Toxicity

Acute toxicity was determined by subcutaneous, intraperitoneal and intravenous injection into mice and rabbits. Chronic toxicity was investigated by giving repeated subcutaneous doses to young mice; the rate of growth was recorded, and the tissues examined histologically when the animals were killed two months later. A more elaborate experiment was made upon rabbits, in which determinations of the haemoglobin concentration, and red and white cell counts were made at intervals during the treatment, and biopsies of liver and kidney were made for histological examination. Blood urea determinations were also made after injection into rabbits, a dog and man. Local irritant activity was estimated by intracutaneous injection into guinea-pigs.

Absorption and excretion

Method of assay.—Carbidium ethanesulphonate has a primary aromatic amino-group in the 3-position and may therefore be diazotized with nitrous acid: subsequent coupling with N-naphthylethylenediamine furnishes an azo-compound which is pinkish-purple in colour and can be estimated colorimetrically. The method used was a modification of that described by Short (1948) for the determination of dimidium bromide.

(i) *Determination in blood.*—1 Ml. oxalated blood was laked in 3 ml. distilled water in a small conical flask; 9 ml. saturated sodium chloride solution and 3 ml. 95% alcohol were added and the mixture was allowed to stand for 5 min.; 4 ml. N-HCl were then added with shaking, and after standing for a further minute the precipitated protein was removed by filtration through paper (Whatman No. 41). 10 Ml. filtrate was mixed with 0.3 ml. aqueous sodium nitrite solution (0.1 g./100 ml.); after 3 min., 0.3 ml. aqueous ammonium sulphamate (0.5 g./100 ml.) was added. After a further 3 min., 0.1 ml. N-naphthylethylenediamine dihydrochloride (0.1 g./100 ml.) in 5 per cent ethyl alcohol was added. After standing 20 min. for full development of colour,

the azo-compound was shaken out with 1 ml. butanol. The butanol layer was separated by centrifuging and the colour estimated in a Spekker absorptiometer with 0.5 ml. cells and a green filter (No. 605 Ilford).

This method gave 80–100 per cent recovery (determined by adding known amounts of drug to blood, extracting, and reading from a standard curve prepared with aqueous solutions of carbidium ethanesulphonate).

(ii) *Determination in tissues.*—For *tissues,* the method used was to grind up the organs thoroughly with sand and dilute to a suitable volume (10 to 30 ml.) with water ; 5 ml. suspension was taken, and 11 ml. of a mixture of three parts of saturated sodium chloride solution and one part of 95 per cent alcohol were added. The mixture was acidified with 4 ml. N-hydrochloric acid and allowed to stand in water at 80° C. for 5 min. in order to get the precipitated protein to flocculate. The liquid was filtered through a Whatman No. 41 paper and 10 ml. of the filtrate were taken for diazotization by the method described above.

(iii) *Determination in bile.*—Samples of *bile* (1 to 2 ml.) were diluted to 5 ml. with water and treated in the same way as tissue suspensions.

No appreciable " blank " values were given by any tissues except the alimentary tract, by faeces and by the urine of rabbits. The variable nature of the blanks given by the excreta made it difficult to be sure how much drug was leaving the body by these routes.

Blood concentration.—Samples of blood were taken from the ear veins of rabbits at intervals after intravenous doses of carbidium ethanesulphonate. In mice, concentrations were determined in heart-blood removed from groups of animals killed at intervals after injection.

Tissue concentration.—Young rats were injected with doses of 7.5 mg./kg. of carbidium ethanesulphonate and groups of two or four killed at intervals. The tissues were assayed for the drug by the method described above.

Excretion.—Urine and faeces were collected from rabbits and from mice injected with the drug. Excretion in bile was investigated in rats and rabbits. The animals were anaesthetized with ether, the abdomen was opened in the midline and the common bile duct identified. It was dissected free from the portal vein and a cannula of glass capillary or of fine polythene tubing was tied into the duct and the bile collected. One or two initial samples were collected at intervals of 5 or 10 min. and then a dose of drug was given into the jugular vein or intramuscularly. The bile was collected in fractions at intervals of 5 to 30 min. and assayed for carbidium ethanesulphonate.

Excretion was also investigated by ultraviolet light. The drug fluoresces a bright orange colour, and this property was used to follow visibly the fate of the drug after injection.

Pharmacological effects

Rabbits or cats were anaesthetized with pentobarbitone or chloralose, or were decerebrated under ether. Blood pressure was recorded from the carotid or femoral artery. Respiration was recorded by Gaddum's method (1941). Kidney volume was measured by putting the organ in a plethysmograph and recording with a tambour ; an estimate of the renal blood flow was made from time to time by occlusion of the renal vein for a period of five seconds and measuring the dilation of the kidney which ensued. Spleen volume was measured by plethysmograph and tambour. Cardiac volume was recorded with a cardiometer and piston recorder. Duodenal movements were recorded in the living animal by tying in a cannula attached to a closed vessel of saline, and measuring the pressure in the vessel with a tambour. The action upon isolated strips

of gut was determined by adding solutions of the drug to the fluid bathing the tissue in an isolated organ bath. The effect upon the isolated heart was measured by injecting drug into the cannula by which the heart was perfused through the coronary circulation. The effect on the isolated frog's heart was recorded by Straub's method. The action upon blood vessels was demonstrated upon the isolated perfused hind-limbs of a rat, the outflow being recorded with a Stephenson drop-recorder (1948). The action upon the knee jerk was recorded by the method of Schweitzer and Samson Wright (1937).

In mammalian experiments, doses of drug were usually given by injection into a cannula tied in the jugular vein.

RESULTS

1. *Activity against* T. cruzi

The difficulties of using infections of *T. cruzi* for quantitative assays have already been described in Part II of this work. The tendency of the infection to become chronic made it essential to compare treated animals with untreated infected controls in every experiment. Also, in the course of our experiments, we have found that the effect of a drug depends upon the intensity and the stage of development of the infection. A heavy inoculum generally gave a heavy infection, which was rather more resistant to treatment than an infection produced with a lighter inoculum. The greatest effect of a drug was obtained when the drug was given before trypanosomes appeared in the peripheral blood ; the later in the course of the infection that treatment was begun, the less effective the treatment became. *T. cruzi* undergoes multiplication only in the tissues, and not in the blood stream. As the infection progresses, oedema of the tissues is produced by blocking of lymphatics, although Collier, Fulton, and Innes (1942) were unable to demonstrate histologically that the trypanosomes were directly responsible for this lymphatic obstruction. In such circumstances, it may be that a drug introduced into the circulation does not penetrate into the tissues so efficiently, and some of the intracellular parasites may escape an effective concentration of drug. Nevertheless, a drug of high potency should cause all trypanosomes to disappear from treated animals. Unfortunately such a substance has yet to be discovered. " Chagavlon " (the British equivalent of Bayer 7602 Ac), which has been tried in the field for the treatment of Chagas's disease, causes disappearance of trypanosomes from mice for several weeks, but in our experience, even repeated courses of treatment fail to prevent relapse of some of the animals. The action of carbidium ethanesulphonate is similar. Some mice are cured of the infection so far as we can judge from examination of fresh and stained thick smears, and subinoculation into uninfected mice. Others, although they may remain alive long after the control animals have died, continue to show an occasional parasite in the peripheral blood, which may mean that tissue forms are entrenched somewhere in the body where the drug cannot penetrate. So far, however, we have not been able to produce a strain which is demonstrably more resistant to treatment than the original one.

Table I shows the effect of the drug when given at the least favourable time in the course of the disease in mice. There is no doubt of the activity of carbidium ethanesulphonate, and it may prove to be of use in the treatment of Chagas's disease in man, especially at the acute stage. The drug is not effective when given by mouth.

TABLE 1

THE ACTION OF CARBIDIUM ETHANESULPHONATE AGAINST *T. cruzi* INFECTIONS IN MICE

Inoculum (0.1 ml. given s.c.)	Treatment (no. of daily subcutaneous doses)	No. of mice		Weeks after inoculation																	
				2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
3,000 per μl. Reichenow strain	12.5 mg./kg. × 5 from 8th day	5	Tryp. free	0	1	4	4	4	4	4		1 killed (negative) at 2 months									
			Dead	0	0	0	1	1	1	1		2 died (1 positive and 1 negative) at 6 months 1 killed (negative) at 1 year									
850 per μl. Reichenow strain	20 mg./kg. × 5 from 8th day	5	Tryp. free	0	2	5	4	1	5	5	5										
			Dead	0	0	0	0	0	0	0	0										
	Controls	5	Tryp. free	0	0	—															
			Dead	0	1	5															
100 per μl. Talice strain	20 mg./kg. × 5 from 12th day	10	Tryp. free	1	1	10	8	5	8												
			Dead	0	0	0	0	0	0												
	Controls	10	Tryp. free	0	1	0	0	2	2												
			Dead	0	0	2	3	3	3												
375 per μl. Reichenow strain	40 mg./kg. × 10 from 8th day	90	Tryp. free	9	78	78	72	68	62												
			Dead	3	6	8	8	15	21												
	Controls	10	Tryp. free	0	0	3	3	4	5	(Infection in surviving controls became chronic)											
			Dead	0	4	5	5	5	5												
500 per μl. Reichenow strain	Courses of 20 mg./kg. × 5 during weeks 1, 5, 8, and 11 and then 40 mg./kg. × 5 during weeks 13 and 17	89	Tryp. free	6	30	74	66	42	74	71	69	70	75	78	74	72	70	69	66	62	59
			Dead	0	0	2	3	7	7	7	7	7	8	9	10	12	15	18	21	25	25
	Controls	10	Tryp. free	1	0	1	1	0	—												
			Dead	0	1	7	9	9	10												
200 per μl. Reichenow strain	20 mg./kg. (beginning on 12th day) twice weekly until the 8th week, and then 40 mg./kg. twice weekly	89	Tryp. free	0	54	72	77	78	74	55	65	75	78	69	69	67	64	68	68		
			Dead	0	0	3	5	5	7	8	9	9	9	10	12	14	16	17	17		
	Controls	9	Tryp. free	0	0	0	0	0	0	3	3	4	4	4	4	4	4	4	3		
			Dead	0	1	4	5	5	5	5	5	5	5	5	5	5	5	5	5		

2. *Toxicity*

Acute.—After intravenous injection of lethal doses in mice, rats, and rabbits the animals ceased to breathe, became cyanosed, gave a few convulsive movements and gasps, and died. The hind limbs were often extended. The LD50 by intravenous injection for mice was 10 mg./kg. and it appeared to be about the same for rabbits. Young animals were more tolerant than older ones ; larger doses could be given by slow administration ; there were no delayed deaths. It was found that a dose of 7.5 mg./kg. could be given intravenously without ill effects, and could be repeated daily for at least 10 days in both mice and rabbits. By subcutaneous injection in mice the LD50 was 130 mg./kg., and by intraperitoneal injection 40 mg./kg.

Chronic. (i) *Mice.*—Five daily intraperitoneal doses of 25 mg./kg. given to a group of ten mice caused six of them to die within a week. One was killed by accident. The remaining three were killed and showed fibrous adhesions in the peritoneal cavity ; one mouse showed evidence of intestinal obstruction by a band of these adhesions, which were evidence of the local irritant activity of the drug. The livers, spleens, and kidneys of the mice were apparently normal.

Daily subcutaneous doses of 50 mg./kg. into ten mice for ten days caused irritation at the point of injection, and at the end of treatment the area was beginning to break down. During treatment the mice ceased to grow, but their weights began to increase immediately the doses were stopped. Five of the mice died during treatment ; one was ill and was killed on the 13th day. The remaining four lived apparently in good health until the 68th day, when they were killed. Mice which died during treatment showed no yellow staining of any organs except the upper part of the small intestine. The mice which were killed after 68 days had patches upon their backs where the fur had not grown after the irritation produced by the drug had healed, and there was some induration of the tissues. All had enlarged, dark spleens. The livers of two were rather pale ; the other two were normal in appearance. Two animals had hydronephrotic kidneys on the left side, but this is a not uncommon finding in old mice. All the other organs were normal. Microscopically there were no significant changes in liver or kidneys which could be attributed to the effects of the drug.

(ii) *Rabbits.*—A rabbit weighing 2.6 kg. was given ten doses of 7.5 mg./kg. of carbidium ethanesulphonate intravenously during a period of 14 days. The drug was given as a 1 per cent (w/v) solution in water, into the ear vein. Transient respiratory depression and slight cyanosis occurred after each dose ; the effects were maximal when the dose was given quickly. Transient muscular weakness also occurred. A week after the last dose, the animal was anaesthetized with ether and a piece of the liver was removed for the preparation of sections. The macroscopic appearance and histology were normal. Eight weeks later, the animal was killed and the liver and kidneys examined. The liver showed a few microscopic foci of infection, but there were no changes which could be attributed to the action of the drug. The kidneys were normal.

For a more critical examination of the toxic effects of the drug, six young littermate rabbits were divided into two groups of three. Each animal was kept in a separate cage and was weighed twice a week. At the beginning of the experiment, a fragment of liver was removed from each animal under ether anaesthesia and sections were

prepared. One of the groups was then given a course of 10 daily doses of 7.5 mg./kg. of carbidium ethanesulphonate intravenously (0.75 per cent (w/v) solution in water). The other group was kept as a control and injections of sterile saline were given instead of the drug. Determinations of the haemoglobin concentration, red and white cell counts were made at intervals during treatment, and are recorded in Table II and

TABLE II

THE BLOOD-PICTURE OF RABBITS TREATED WITH CARBIDIUM ETHANESULPHONATE

	Rabbit No.		Days from beginning of experiment										
			1	4	8	11	15	18	22	32	39	49	56
Doses given: (i.v.)					7.5 mg./kg. daily				7.5 mg./kg. daily				
Biopsies at arrows:			↓					↓					↓
Haemoglobin (% Sahli)	1	Controls	85	85	80	80	71	80	74	77	79	74	80
	2		90	92	83	82	81	88	80	82	80	73	74
	3		77	87	80	82	80	78	90	80	77	75	87
	4	Drug	85	84	92	90	90	85	82	98	90	98	95
	5		80	83	85	90	85	81	81	94	85	75	94
	6		86	82	85	80	85	86	76	81	82	83	83
Red cells (millions) per μl.	1	Controls	4.4	5.5	5.3	5.0	4.9	5.2	4.5	5.5	4.8	—	4.6
	2		5.3	5.7	5.4	5.7	4.2	5.7	5.4	6.0	5.5	—	4.4
	3		5.7	5.1	5.1	5.3	4.6	5.4	5.3	5.4	5.8	—	5.7
	4	Drug	5.3	5.2	5.5	5.4	6.1	5.0	4.5	6.0	5.4	—	5.1
	5		5.5	5.0	5.1	6.0	5.1	5.3	6.1	6.1	5.4	—	5.3
	6		4.8	4.6	5.3	5.0	5.1	5.1	5.1	5.8	5.9	—	4.2
White cells (thousands) per μl.	1	Controls	8.0	5.2	9.4	7.4	5.8	9.8	5.8	6.0	7.6	17.4	10.0
	2		9.2	6.4	9.4	14.0	16.4	16.0	8.0	8.0	9.0	7.5	6.8
	3		9.0	5.2	9.8	5.4	5.4	8.2	8.8	8.0	12.2	4.2	14.8
	4	Drug	7.4	4.8	5.6	13.4	9.0	3.8	7.0	7.8	7.8	9.0	7.2
	5		6.0	5.6	5.8	12.8	8.0	8.8	4.6	8.6	17.4	10.6	8.2
	6		8.4	4.2	4.7	8.2	7.8	6.2	10.4	7.4	4.6	8.0	9.6

Fig. 1. The treated animals gained weight at the same rate as the controls and there were no significant changes in the blood picture. A second liver biopsy showed no histological differences between the two groups. The animals had by now doubled their initial weights, and a second course of injections was started. The immediate reactions to the doses were more pronounced than with the first series. One animal (no. 5) unfortunately received a dose of the drug into the tissue of the ear instead of into the vein. The ear swelled and there was oedema of the side of the face and of the conjunctiva of the same side. Part of the ear became necrotic and was sloughed off; the face and eye recovered completely. Some weight was lost during this period

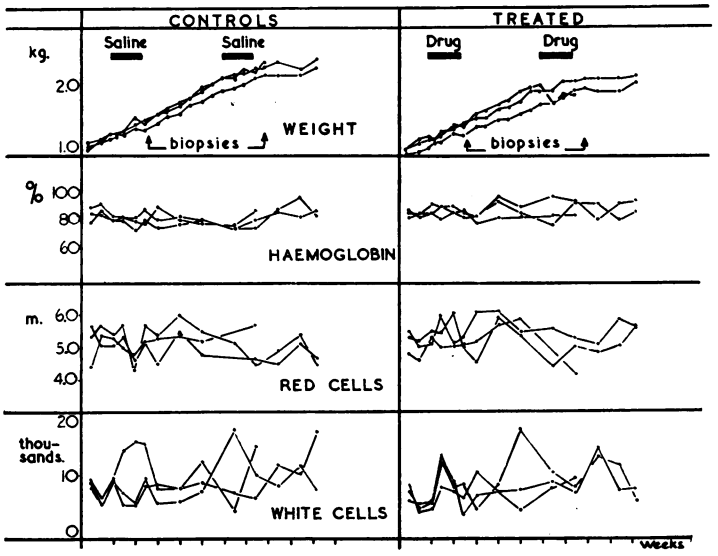


FIG. 1.—The effect of intravenous doses of carbidium ethanesulphonate on the growth and on the blood of rabbits. 6 litter-mate rabbits were used; 3 were given two courses each of 10 daily doses of 7.5 mg./kg. and 3 were given similar treatment with saline. Liver biopsies were taken before treatment and after each course. Kidney biopsies were taken after the second course. The curves show no significant effects upon haemoglobin, red cells, or white cells.

and the animal appeared unwell for about ten days. Doses were stopped after only five had been given to this rabbit in the second course; the rest of the animals received the full course of ten injections. One of them died immediately after the last dose of the series, which was given too rapidly. Autopsy showed a liver normal in appearance and kidneys stained bright yellow with the drug. The histology of these organs was normal. No gross pathological changes were detected in any other organs. The muscles contained 20 μg. carbidium ethanesulphonate per gram. When the second course of injections was completed, biopsies were taken of the livers and kidneys of the surviving animals. One of the controls died from reactionary haemorrhage in the kidney area. All biopsy specimens were normal, except for the kidney of the rabbit which had had necrosis of the ear. In this animal there was considerable damage to the renal cortex with destruction of the glomeruli, and fibrosis. Some of the nephrons showed evidence of obstruction lower down. The blood urea level of this animal was 91 mg./100 ml.; that of the other treated rabbit was 50 mg./100 ml. and of the two surviving controls, 51 and 55 mg./100 ml. respectively. These lesions might well have resulted from the effects of the necrosis of the ear, because this was the only animal in which we had been able to demonstrate such an effect. Nevertheless, the possibility of damage to the kidney must be borne in mind in any study in which phenanthridinium compounds are administered to man.

(iii) *Observations upon the blood urea.*—Wien (1946) showed that subcutaneous injection of 10 mg./kg. of dimidium bromide caused a rise in the blood urea of rabbits. In view of the fact that carbidium ethanesulphonate belongs to the same class of compound, and of the findings recorded above, rabbits were given daily intravenous injections of 5 mg./kg. of carbidium ethanesulphonate, and the blood urea was determined. The results of a typical experiment are shown in Table III. There was a significant rise in blood urea, which fell again when the treatment was discontinued. A transient, slight albuminuria was observed in one animal after the first dose. A liberal water supply prevented any albuminuria with subsequent doses.

TABLE III

BLOOD UREA OF A RABBIT DURING TREATMENT WITH CARBIDIUM ETHANESULPHONATE (5 MG./KG. INTRAVENOUSLY)

Days:	0	1	2	3	4	5	6	7	8
Doses of drug:		5 mg./kg. daily							
Blood urea, mg. per 100 ml. ..	50	50	104	100	120	110	96	—	42

A dog with initial blood urea of 20 mg. per 100 ml. which received four daily intravenous doses of 2 mg./kg. of the drug showed an increase of blood urea to 64 mg./100 ml. after the last dose. There was no albuminuria.

Local toxicity.—From the above experiment on rabbits, it was evident that carbidium ethanesulphonate had an irritant action when injected into the tissues. This was most marked with concentrated solutions, and any strength above 0.1 per cent (w/v) caused redness and oedema after subcutaneous injection. The limiting necrosing concentration after intracutaneous injections in the guinea-pig was about 0.0001 per cent; higher concentrations than this when injected in quantities of 0.05 ml. caused an easily detectable area of redness. No ill effects were observed after careful intravenous injection and it was possible to give at least 10 daily injections of 7.5 mg./kg. into the tail-veins of mice without causing thrombosis of the vessels or necrotic effects. Similar irritant activity was observed for the sulphate, chloride, and methylsulphate.

3. *Effects of injection in man*

Intramuscular injection.—2 Ml. of 0.5 per cent (w/v) solution of carbidium sulphate were injected into the right deltoid muscle. The sensation upon injection was only that of the tightness which follows any intramuscular injection; there was no pain or stinging. On the following morning the muscle was slightly swollen and tender, and warmer than the normal side. In the evening the swelling was definite and tender to pressure. The arm felt stiff as after heavy labour, and there was some swelling also of the lateral and upper parts of the pectoralis major and triceps. There was a tired feeling in the forearm, and very slight tingling over the sensory area supplied by the ulnar nerve. On the second day the swelling was much reduced and gradually disappeared, leaving only a slight tenderness to pressure over the site of injection, which persisted for two or three weeks.

Intravenous injection.—8 Ml. of 0.5 per cent (w/v) solution were injected intravenously in 20–25 seconds. The blood pressure fell very slightly and the pulse rate increased slightly. The pulse was back to the initial value five minutes after the injection. A bitter taste was perceptible before the injection was complete, and lasted for two hours. The only other effect was a flushing and feeling of warmth in the face, especially in the malar region and the sides of the neck, which persisted for three and a half hours. This is probably evidence of the peripheral dilatation also observed in other animal experiments. There were no subjective sensations in the abdomen, and a large meal was eaten three hours after the dose. Sleep was

TABLE IV

BLOOD LEVELS OF CARBIDIUM ETHANESULPHONATE AFTER REPEATED INTRAVENOUS INJECTIONS OF 7.5 MG./KG. INTO A 2.6 KG. RABBIT

Day	No. of dose	Blood concentration, mg. per 100 ml.						
		Before dose	Immed. after dose	15 min.	30 min.	1 hr.	2 hr.	3 hr.
1	1	0	1.22	0.48	0.43	0.39	0.34	0.32*
2	2	0.005	3.90	0.36	0.26	0.14	0.22	0
4	3	0.10	1.80	0.22	—	0.14	—	—
5	4	0.05	1.24	—	0.10	—	—	—
8	5	0.09	1.08	0.12	—	0.15	—	—
9	6	—	3.68	0.49	—	0.31	—	—
10	7	0.17	4.24	1.17	—	0.29	—	—
11	8	0.24	1.96	0.29	—	0.10	—	—
12	9	0.18	8.40	0.74	—	0.36	—	—
15	10	—	1.60	0.42	—	0.22	—	—

* Levels at 4, 6, and 9 hr.: 0.24, 0.16, and 0.11 respectively.

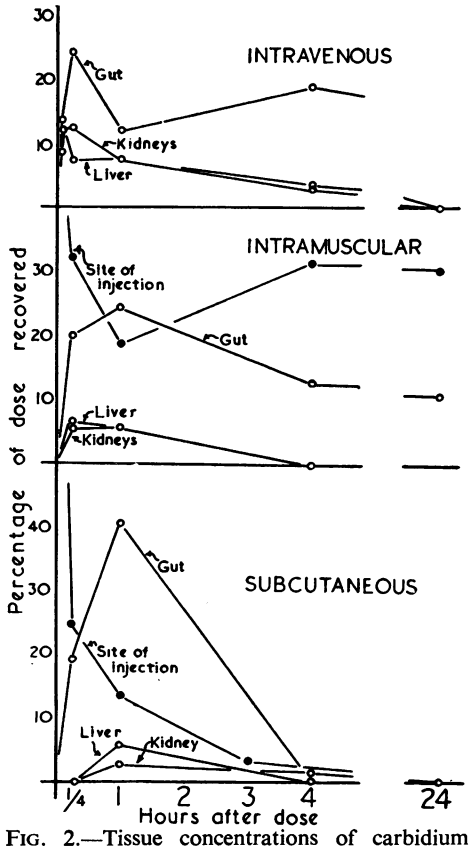


FIG. 2.—Tissue concentrations of carbidium ethanesulphonate after injection of doses of 7.5 mg./kg. into young rats.

unaffected, and no effects were observed after the flushing of the face had passed off. The blood urea, which was 35 mg./100 ml. four and a half hours before the dose, fell to 26 mg. one hour after the injection, then rose to 51 mg. two and a half hours later, but it had fallen to 32 mg. by the next morning. There was a very faint cloud of albumin in the urine collected overnight, but no red cells or casts could be found in the deposit. No symptoms or signs of late toxicity developed during the following four months.

4. *Absorption and excretion in laboratory animals.*

Blood concentrations. — In rabbits which received 7.5 mg./kg. of carbidium ethanesulphonate intravenously, the highest concentration in the blood occurred immediately after injection; the height observed depended upon the rate at which the blood sample was withdrawn. The greatest value obtained was 8.4 mg./100 ml. The concentration then fell rapidly and very little could be detected twenty-four hours after the dose. However, a slight rise in the blood level was found twenty-four hours after a dose

towards the end of a series of 10 doses, as shown in Table IV. When injections were given intraperitoneally or subcutaneously no drug could be detected in the peripheral blood by the method used, although low concentrations were undoubtedly present.

In an experiment with mice the pooled blood of animals injected with 7.5 mg./kg. intravenously contained 0.54 mg./100 ml. immediately after the injection ; a quarter of an hour later 0.20 mg./100 ml. was present but none was detectable half an hour, three quarters of an hour, two or four hours later.

Tissue concentrations.—The percentages of the dose recovered from rat tissues after intravenous, intramuscular, and subcutaneous injections are recorded in

TABLE V

DISTRIBUTION OF CARBIDIUM ETHANESULPHONATE AFTER INTRAVENOUS, INTRAMUSCULAR, AND SUBCUTANEOUS INJECTION INTO RATS (GUT FIGURES ARE CORRECTED FOR " BLANK ")

Route	Tissue	Percentage of dose recovered at intervals after injection. Means in parentheses				
		5 min.	½ hr.	1 hr.	4 hr.	24 hr.
Intravenous	Liver	16, 11 (13.5)	9.8, 4.4 (7.1)	7.7, 7.2 (7.45)	3.3, 4.1 (3.7)	0
	Kidneys	13, 11 (12.0)	17, 8 (12.5)	6.8, 7.6 (7.2)	2.0, 3.5 (2.75)	0
	Gut	14, 3 (8.5)	29, 20 (24.5)	3, 21 (12.0)	28, 10 (19.0)	0
	Spleen	Trace	Trace	Trace	Trace	0
	Brain	0	Trace	Trace	Trace	Trace
	Lung	Trace	Trace	Trace	1.2, 1.2 (1.2)	0
	Muscle (0.5 g.)	—	Trace	Trace	Trace	0
	Heart	1.4	3.5, trace (1.75)	Trace	0	0
Intramuscular	Liver	—	6.5, 6.7 (6.6)	8.6, 5.0, 4.0, 5.3 (5.73)	0	0
	Kidneys	—	4.3, 6.0 (5.15)	3.7, 5.0, 6.4, 5.7 (5.20)	0	0
	Gut	—	22, 18 (20.0)	55.0, 30.0, 6.0, 7.0 (24.5)	11, 14 (12.5)	11, 10 (10.5)
	Spleen	—	3.7, 3.7 (3.7)	2.8, 1.8 (2.3)	0	0
	Brain	—	0	0	0	0
	Lung	—	0	0	0	0
	Muscle (0.5 g.)	—	0	Trace	0	0
	Heart	—	Trace	2.3, 1.3 (1.8)	0	0
	Site of injection	—	31, 33 (32)	15, 13, 20, 26 (18.5)	32, 31 (31.5)	33, 28 (30.5)
Subcutaneous	Liver	—	0	5.1, 3.2, 7.5, 8.1 (6.0)	0	0
	Kidney	—	0	2.8, 2.2, 3.0, 3.5 (2.9)	1.8, 1.0 (1.4)	0
	Gut	—	38, 0 (19.0)	28, 53 (40.5)	0	0
	Spleen	—	0	0	0	0
	Brain	—	0	0	0	0
	Lung	—	Trace	0	0	0
	Muscle (0.5 g.)	—	—	0	0	0
	Heart	—	Trace	Trace	0	0
	Site of injection	—	17, 40, 21, 21 (24.8)	12, 8, 20, 15 (13.8)	3.5, 3.4 (3.45)	0

Table V and in Fig. 2. There were considerable differences between individual animals, but it is clear that the drug appeared in the liver and kidneys quite soon after injection; it also appeared in considerable quantities in the alimentary canal. Traces were found in the spleen, heart, and lungs. The drug disappeared more rapidly from the site of injection after subcutaneous than after intramuscular administration. The figures given for the amount of drug in the alimentary canal are only approximate, because of the variable "blank" value obtained with the guts of untreated animals, but the main trend of the observations is clear. The average "blank" amounted to the equivalent of about 1.1 mg. of carbidium ethanesulphonate per kg. of rat, that is, about 15 per cent of the dose given. All the figures in Table V have been corrected accordingly.

Excretion.—Collection of the urine of rabbits showed that if any of the drug is excreted by this route, it is only a very small amount. None at all could be detected in the urine collected from a group of 10 mice in an all-glass metabolism cage, after doses of 7.5 mg./kg. given either intravenously or subcutaneously. Heating the urine with alkali or acid, in order to hydrolyse any metabolic derivative, also failed to reveal any of the drug.

It was noted in the toxicity tests that animals which died during treatment showed a yellow staining of the upper part of the alimentary tract. This suggested that the drug was excreted by the liver into the bile, and further experiments confirmed that this was so.

Table VI shows the results of typical experiments in which bile was collected under anaesthesia. It will be seen that after intravenous injection the drug began to appear in the bile within a few minutes. The rate of excretion increased to a maximum at about half to one hour after injection, and then gradually declined. At the end of four or five hours, about a quarter of the dose given had been recovered from the bile. After intramuscular injection, a steady and prolonged excretion occurred, showing that the drug was being passed out by the liver as soon as it was absorbed from the site of injection.

Carbidium ethanesulphonate also has the useful property of fluorescing a bright orange colour in ultraviolet light. This property was used to follow visually the fate of the drug after injection. Anaesthetized, or freshly killed rats, rabbits, and cats were observed under the beam of a mercury vapour lamp screened with Wood's glass. Within two minutes of intravenous injection into a rat, the bile was seen to fluoresce with the characteristic orange colour of the drug and it continued to do so for several hours (until the end of the experiment) thus confirming the results of chemical assay. The upper part of the small intestine also fluoresced because of the bile which it contained, and segments of the fluorescent material could be seen passing down the gut. When the orange fluorescence reached the caecum it became more difficult to trace because of dilution with the caecal contents, and also because the lower ileum often contained material which fluoresced a deep red and masked the orange of the phenanthridinium compound mixed with it; this red fluorescence was present in normal animals which had not received the drug. The liver always fluoresced a warm orange colour after injection of the drug, and in rabbits which had received a course of injections, the muscles also had a definite yellow sheen. Single doses were insufficient to give a recognizable fluorescence in the muscles.

TABLE VI

EXCRETION OF CARBIDIUM ETHANESULPHONATE IN THE BILE OF RATS AND RABBITS

Rat		Rabbits		
Time after injection (min.)	(i.v.) μg. in bile	Time after injection (min.)	(i.v.) μg. in bile	(i m.) μg. in bile
−10 to −5	0.4	−20 to −10	0	—
−5 to 0	0.4	−10 to 0	0	0
0–5	6.8	0–10	160	0
5–10	12.2	10–20	500	76
10–15	11.2	20–30	260+	58
15–20	9.1	30–40	460	84
20–25	7.7	40–50	360	102
25–35	7.6	50–60	360	94
35–45	8.6	60–70	360	86
45–55	5.4	70–80	230	84
55–65	3.0	80–90	250	96
65–75	2.1	90–100	200	110
		100–110	180	83
	Total = 73.7 μg. Injected = 700 μg. Recovered = 10% in 75 min.	110–120	180	86
		120–130	160	76
		130–140	170	90
		140–150	130	76
		150–160	140	89
		160–170		91
		170–180	Total = 4,100 μg. Injected = 20,000 μg. Recovered = 20% in 160 min.	62
		180–190		100
		190–200		84
		200–210		73
		210–220		78
		220–230		74
		230–240		60
		240–250		72
		250–260		60
		260–270		59
		270–280		70
		280–290		56
		290–300		46
		300–310		52
		310–320		90
		320–330		70
		330–340		46
				Total = 2,533 μg Injected = 24,000 μg. Recovered = 10% in 340 min.

At about the same time as the drug became detectable in the bile, the kidneys also began to fluoresce. The fluorescence became more and more intense and was most marked in animals which had received a large amount of drug intravenously during the recording of effects upon the blood pressure and respiration. In such experiments total quantities of 200 mg. or more have been given, and this is sufficient to make the kidneys bright yellow under ordinary illumination. The fluorescence was confined to the cortex of the kidney ; none appeared in the medulla, and none

in the urine. In some experiments the urine was seen to fluoresce a faint blue-green colour, but the characteristic orange-yellow colour was never seen to pass beyond the cortex in a living kidney. In two rabbits and a cat, after repeated doses of drug, the aorta was exposed and 5 ml. of a 5 per cent (w/v) solution of methylene

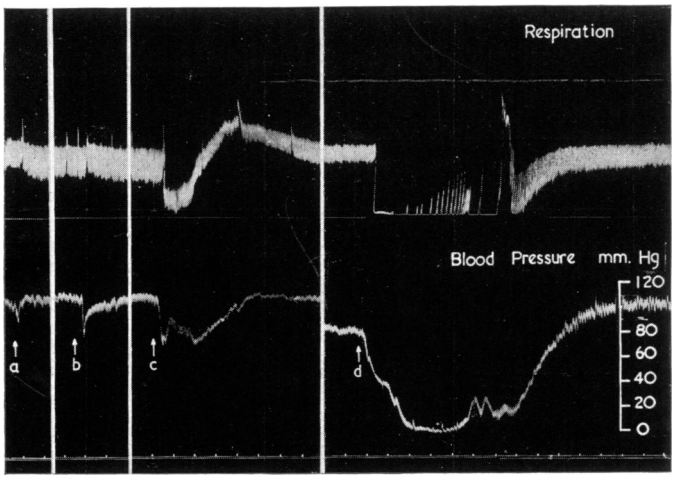


FIG. 3.—The effect of carbidium ethanesulphonate upon respiration and blood pressure. Rabbit, ♀, 3.6 kg. Pentobarbitone. Time signal = 60 sec. At (*a*) 1 mg. carbidium ethanesulphonate, intravenously; at (*b*) 7.5 mg., at (*c*) 15 mg., and at (*d*) 20 mg.

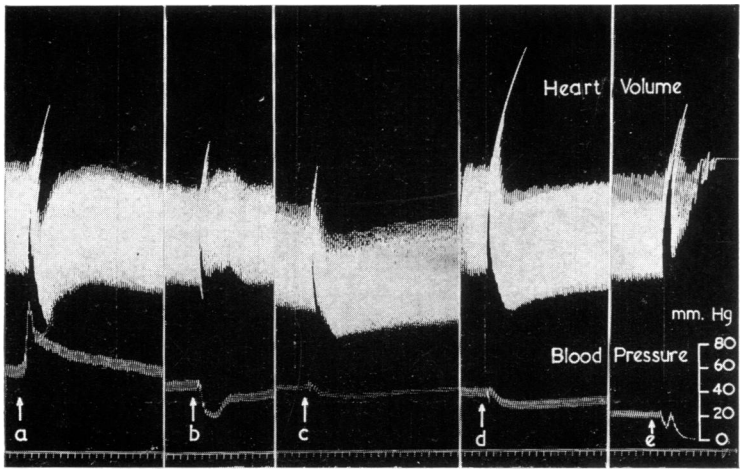


FIG. 4.—The effect of carbidium ethanesulphonate upon heart volume and blood pressure. Rabbit, ♂, 2.6 kg. Pentobarbitone; artificial respiration. Time marker: 10 sec. At (*a*) 10 μg. adrenaline: rise in blood pressure and diminution of heart-volume. At (*b*) 10 μg. acetylcholine: fall in blood pressure and dilation of the heart. At (*c*) 2 mg. carbidium ethanesulphonate: little effect on blood pressure and diminution of heart volume. At (*d*) 4 mg. carbidium ethanesulphonate: small fall in blood pressure and diminution of heart volume. At (*e*) 15 mg. carbidium ethanesulphonate: fall in blood pressure, and arrest of heart in diastole.

blue was injected into it so that it passed into the renal arteries. Examination of the cut surfaces of the kidneys under the ultraviolet lamp then showed the interlobular vessels and glomeruli clearly defined as a dark purple pattern among the fluorescent tubules. Although there was so much drug in the cortex of the kidney, there appeared to be no diversion of the blood supply from the cortex. Frozen sections of these kidneys, observed under the microscope by ultraviolet light, confirmed the fact that the drug was present in the cells of the tubules.

5. *Pharmacological actions*

Effect upon blood pressure and respiration.—Small doses of carbidium ethanesulphonate (0.25 –2 mg./kg.) caused a transient fall in blood pressure. Larger doses (2–4 mg./kg.) usually caused a small rise followed by a more prolonged fall. The blood pressure returned to its initial level within about 5 minutes (Fig. 3). Sometimes the rise in blood pressure was more prominent than the fall; this was most likely to be observed after a considerable amount of drug had already been given

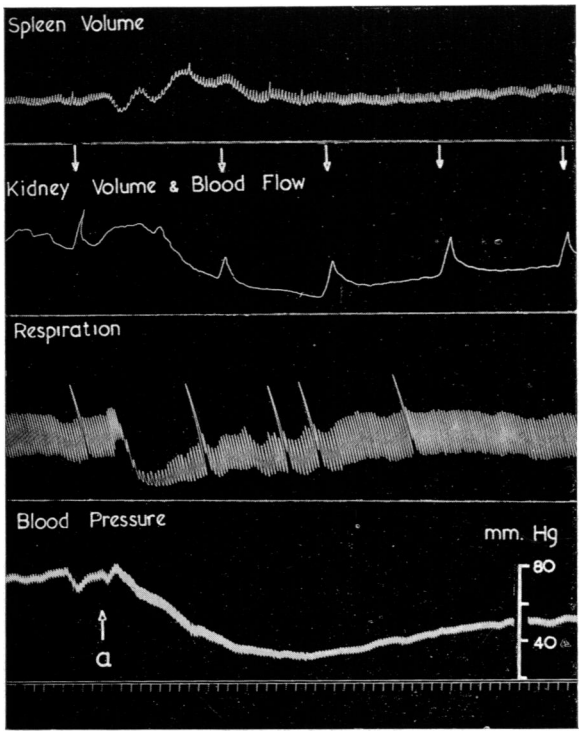


FIG. 5.—The effect of carbidium ethanesulphonate upon the spleen, kidney, respiration, and blood, pressure. Cat, 3 kg. Pentobarbitone. Time marker—10 sec. At (*a*) 7.5 mg. of carbidium ethanesulphonate intravenously. The spleen dilated a little, the kidney constricted after a slight dilation. The constriction was parallel to the drop in blood pressure. The blood flow through the kidney was diminished as shown by the heights of the peaks on the volume recording. These were produced by occlusion of the renal vein for 5 sec. at each arrow. The respiration was inhibited.

and the blood pressure was low. The fall in blood pressure was less marked when the blood was well oxygenated.

Small doses caused a transient fall in the rate and amplitude of respiration, followed by a compensatory increase. With large doses (more than 4 mg./kg.) the respiration sometimes ceased altogether, but the animal could usually be kept alive by artificial respiration until normal breathing was re-established. The drug still produced these effects after denervation of the carotid sinuses and section of the vagi.

Effect upon the heart.—In anaesthetized animals, very little direct effect upon the heart was observed, even after doses large enough to paralyse the respiration. In a rabbit in which the heart volume was recorded with a cardiometer, a dose of 2 mg./kg. caused a diminution of heart volume similar to that given after a dose of 4 μg./kg. of adrenaline, although the systemic blood pressure fell, presumably as a result of peripheral vasodilation (Fig. 4). There was no change in heart rate. A large dose (6 mg./kg.) caused arrest of the heart in diastole.

Upon the isolated perfused rabbit heart, small doses had no effect, and the very large dose of 1 mg. injected into the cannula was required to arrest the heart beat. It resumed beating feebly and slowly after a time, and was restored to normal rhythm and amplitude by a dose of 2 μg. adrenaline. A similar action was observed

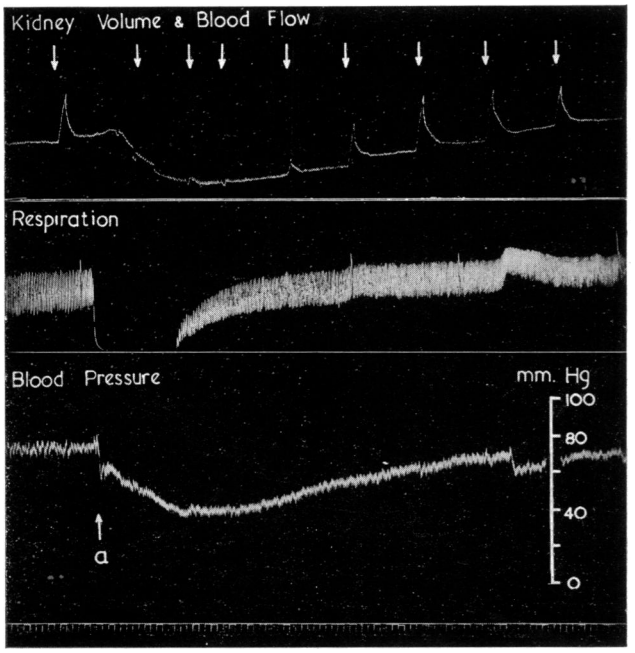


FIG. 6.—The effect of carbidium ethanesulphonate upon kidney volume and blood flow, respiration, and blood pressure. Rabbit, ♂, 3 kg. Pentobarbitone. Time marker—10 sec. At (*a*) 7.5 mg. of carbidium ethanesulphonate intravenously. At the arrows, the renal vein was occluded for periods of 5 sec. and the peaks produced on the record show the rate of blood flow. The flow was abolished at the trough of the fall in blood pressure; during the rise, there was a compensatory increase in flow. The kidney constricted after a slight initial dilation. (See also Fig. 5.)

upon a perfused frog's heart with a concentration of 0.05 g./100 ml. of carbidium ethanesulphonate in Ringer. Frog hearts were stained yellow by the drug. One frog heart showed heart-block after three doses of the drug.

Effect upon the spleen volume.—The usual effect of the drug was to cause dilatation of the spleen of the cat (Fig. 5). The organ probably takes part in a general vasodilatation produced by the drug. In one experiment, where a small rise instead of a fall of blood pressure was observed in an animal under artificial respiration, the spleen diminished in volume. A transient diminution in volume was also observed early in the same experiment with a small dose of drug which was insufficient to produce a fall in blood pressure.

Effect upon perfused vessels.—When injected into the cannula supplying Ringer-Locke solution to the hind limbs of a rat, or a rabbit's ear, the drug produced a slight vasoconstriction. The amount of carbidium sulphate required to give a definite reduction in flow was about 0.5 mg.

Effect upon the kidney.—The effect upon kidney volume was to cause a small dilatation followed by a constriction which corresponded approximately with the fall in blood pressure. The blood flow through the kidney was greatly reduced during the fall in blood pressure; there was a compensatory rise in the rate of flow when the blood pressure began to rise again (Figs. 5, 6). The changes in the kidney are therefore probably secondary to the effect upon the blood pressure. This was confirmed in an experiment in which the fall in blood pressure produced by the drug was mimicked by the slow infusion of acetylcholine; the renal blood flow was correspondingly reduced.

Effect upon the intestine.—Intravenous injection of carbidium ethanesulphonate into a cat caused a strong stimulation of the duodenum. The stimulation was not

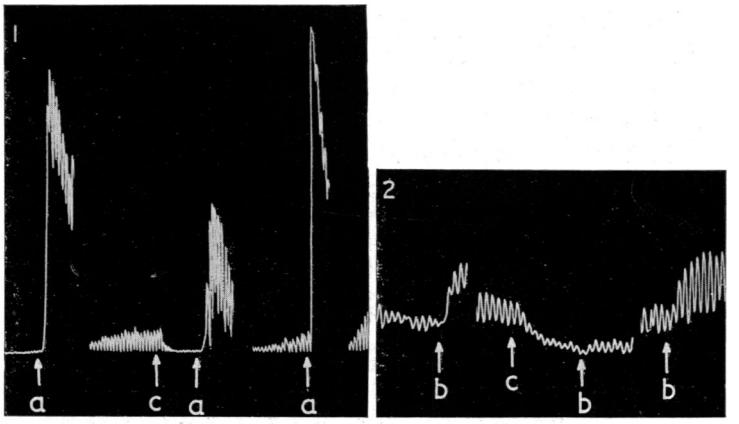


FIG. 7.—The effect of carbidium ethanesulphonate upon isolated rabbit duodenum. Tracing (1) at (*a*), 1 in 10 million acetylcholine; at (*c*), 1 in 6,250 carbidium ethanesulphonate. The effect of acetylcholine was reduced in the presence of the drug. Tracing (2) at (*b*), 1 in 400,000 histamine; at (*c*), 1 in 25,000 carbidium ethanesulphonate. The drug caused relaxation of the gut and abolished the action of histamine. Both responses recovered after the carbidium ethanesulphonate had been washed out.

prevented by atropine and was therefore probably a direct action upon the muscle. The action was much less marked upon the duodenum of the rabbit than upon that of the cat, and sometimes a very slight relaxation occurred.

In the isolated organ bath, movements of the rabbit's duodenum were inhibited, and the muscle relaxed, at a concentration of 1 in 25,000. The drug also caused inhibition of contractions produced by acetylcholine or histamine (Fig. 7). With the isolated guinea-pig ileum, the action of the drug was to cause a slight, slowly developing contraction, and an increase of spontaneous movements. The action of histamine upon a piece of ileum treated with carbidium ethanesulphonate was reduced.

Effect upon the knee-jerk reflex.—Doses of drug which were sufficient to depress the respiration had no effect upon the knee-jerk. Doses which caused complete arrest of respiration gave the same effect, of stimulation followed by depression of the reflex, as was produced by simple asphyxia (Fig. 8).

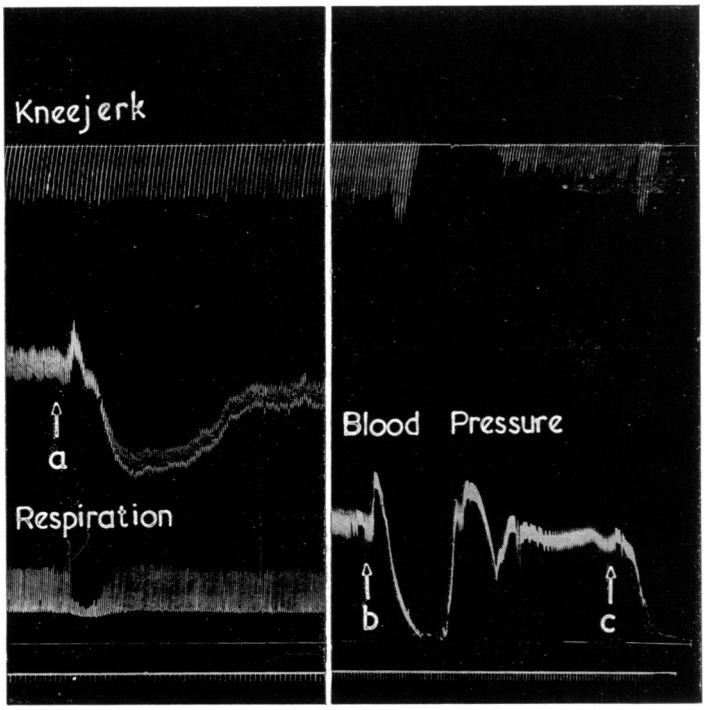


FIG. 8.—The effect of carbidium ethanesulphonate upon the knee-jerk, respiration, and blood pressure. Cat, ♂, 2.9 kg. Chloralose. Time marker = 10 sec. At (*a*) 10 mg. carbidium ethanesulphonate intravenously. There is a fall in blood pressure and inhibition of respiration but no effect upon the knee jerk. At (*b*) 25 mg. carbidium ethanesulphonate. The blood pressure fell to zero, and the respiration ceased. The knee-jerk was abolished after an initial increase. The cat was artificially respired for 3 min., and the blood pressure, respiration, and knee jerk recovered. At (*c*) the trachea tube was occluded. The effect of asphyxia upon the knee jerk was identical with the effect of carbidium ethanesulphonate at (*b*), showing that the drug had no specific action upon the reflex.

SUMMARY

1. 3-Amino-9-*p*-carbethoxyaminophenyl-10-methylphenanthridinium ethanesulphonate (" carbidium ethanesulphonate ") is active against *Trypanosoma cruzi* infections in mice. It usually prevents the death of the animals but does not always eradicate the infection.

2. The drug does not appear to cause any permanent damage to liver or kidneys, although the blood urea level is raised during treatment. It produces necrosis if injected subcutaneously or intramuscularly, but is well tolerated intravenously in repeated doses.

3. Carbidium ethanesulphonate is concentrated in the liver and kidneys; it is excreted principally in the bile.

4. The drug produces a fall in blood pressure and depression of the respiration. Although concentrated in the kidney tubules, it does not appear to exert any specific effect upon the renal circulation. It has no apparent action upon the nervous system, as judged by the knee-jerk reflex.

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