Brit. J. Pharmacol. (1960), **15**, 235.

THE ABSORPTION, DISTRIBUTION AND EXCRETION OF PROTHIDIUM IN RATS, RABBITS AND CATTLE

BY

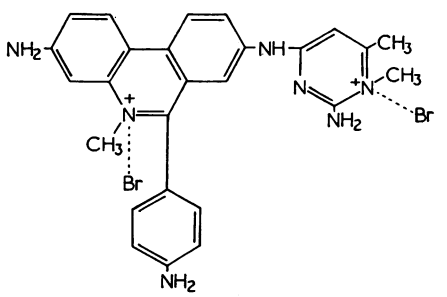
ANGELA E. R. TAYLOR

From the National Institute for Medical Research, Mill Hill, London

(RECEIVED AUGUST 19, 1959)

2 - Amino-7 - (2-amino-6-methylpyrimidin-4-ylamino)-9-*p*-aminophenylphenanthridine 10,1′ - dimethobromide (Prothidium), a prophylactic drug against cattle trypanosomiasis, was concentrated in the liver and kidneys of rats and rabbits after intraperitoneal or intracardial injection; it was detectable in these organs for 7 days in rats and 10 days in rabbits. The drug protected adult rats against *Trypanosoma vivax* for 8 weeks. Histological examination of the organs of rats treated with Prothidium indicated that no damage had been incurred from the treatment. When cattle were treated subcutaneously, elongated swellings appeared at the site of injection which disappeared within 6 weeks. Excretion of unchanged Prothidium occurred *via* the bile in rats and the drug was detectable in the bile for 9 days, but no Prothidium could be detected in the faeces or urine of rats or rabbits. No metabolic products of the Prothidium were found in the tissues or plasma of rats, rabbits, or cattle. In rat liver perfused for 6 hours with Prothidium only the unchanged drug was recovered. A depot of Prothidium was formed at the site of subcutaneous injection in cattle and this remained for at least 3 months. The prolonged prophylactic action was probably due to the formation of this depot since Prothidium injected intraperitoneally into a calf was excreted at a similar rate to that observed in rats and rabbits.

Prothidium (2 - amino - 7 - (2 - amino - 6 - methyl - pyrimidin - 4 - ylamino) - 9- *p* - aminophenylphen - anthridine 10,1′-dimethobromide) was introduced by Watkins and Woolfe (1956) as a powerful prophylactic agent against cattle trypanosomiasis.



An earlier paper (Taylor, 1960) has described the absorption of Prothidium by *Trypanosoma rhodesiense*. The present studies are concerned with the absorption, distribution and excretion of Prothidium when administered to rats, rabbits and cattle. The effectiveness of the drug as a prophylactic agent against *T. vivax* in rats was also investigated.

METHODS

Prothidium is an orange powder, readily soluble in water or acid. It is less soluble in alcohol and other organic solvents. Solutions are strongly fluorescent, the colour depending on *p*H: acid solutions fluoresce yellow, neutral solutions fluoresce orange, and the fluorescence is quenched by strong alkali. A specially purified sample of Prothidium (kindly supplied by Dr. G. Woolfe of Messrs. Boots Pure Drug Company, Nottingham) was used in all experiments with the exception of those in cattle, where commercial Prothidium was used.

The free base may be extracted into butanol from alkaline solution and quantitatively back-extracted into N sulphuric acid. The solution in sulphuric acid has a characteristic ultra-violet absorption spectrum (maximum at 315 mμ, Fig. 1) and this has been used throughout to identify the drug in tissue extracts. For the estimation of Prothidium at low concentrations it was more convenient to use the more sensitive fluorimetric method described below.

Chemical Methods

Extraction of Prothidium from Tissues.— Preliminary evidence suggested that Prothidium was strongly bound to proteins; dialysis of solutions of Prothidium in plasma or in serum at 4° against

normal saline extracted only 50% of the drug. When a solution of Prothidium in water was dialysed in a similar manner, almost all the drug passed into the dialysate.

Good recovery of Prothidium from either tissue or from plasma was obtained when the material (5 g.) was homogenized with 2N sulphuric acid (30 ml.) in an all-glass Potter homogenizer. The resulting suspension was centrifuged at 16,000 *g* at 4° for 40 min., and the solids were discarded. The supernatant fluid was extracted with ether (10 ml.) to remove fats and subsequently made alkaline by the addition of sufficient 40% sodium hydroxide (7 ml.) to neutralize the sulphuric acid. The Prothidium base was extracted into butanol (10 ml.) and centrifuged to facilitate separation.

The loss on extraction was small and was accepted as a necessary defect of a simple routine method for use on large numbers of samples. For liver, the loss, estimated by recovery of Prothidium (100 μg.) added to a liver homogenate (5 g.) and extracted under the standard conditions described above, was 25 μg. (25%). Recovery from plasma (5 ml.) was 95%. No correction factor has been applied in the estimation of recovery in actual experiments since the percentage loss varies somewhat with different tissues and fluids. The distribution in different organs is unaffected by these losses.

Estimation of Prothidium by Fluorescence.—The butanol extract was washed once with an equal volume of distilled water and dried over anhydrous potassium carbonate (2.5 g.) for 30 min. Fluorescence of the dried butanol extract was measured using a Farrand fluorimeter with an orange secondary filter (Wratten No. 25). A blank was obtained by extracting normal tissue in a similar manner. The concentration of Prothidium present was estimated by comparison with a standard curve (Fig. 2). The performance of the fluorimeter was standardized using aqueous eosin solution at a final concentration of

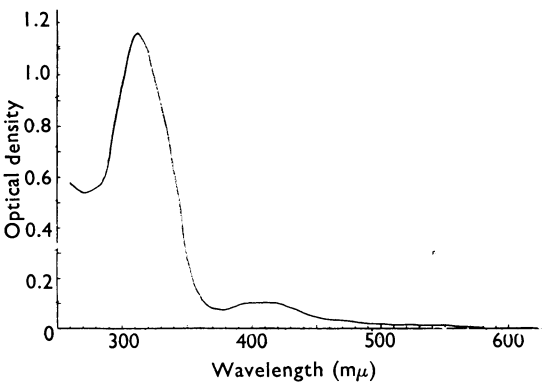


FIG. 1.—The ultra-violet absorption curve of Prothidium in N sulphuric acid.

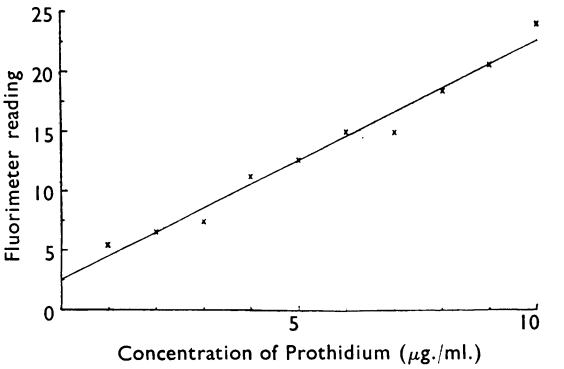


FIG. 2.—The calibration curve of Prothidium for the Farrand fluorimeter.

50 μg./ml. As a check that the fluorescence was due to Prothidium, a sample of the butanol extract was mixed with N sulphuric acid and separated by centrifugation. The ultra-violet spectrum of the sulphuric acid extract was measured in a Beckman spectrophotometer; the peak of the absorption spectrum curve (260 mμ–500 mμ) served to characterize the drug (Fig. 1).

Biological Methods

Tissue Concentrations.—Prothidium was injected into adult rats and rabbits intraperitoneally (7.5 mg./kg.) or intracardially (3.7 mg./kg.). Groups of 2 or 3 animals were killed at intervals and their tissues assayed for Prothidium.

Excretion.—Thirty adult rats, in 10 groups of three, were used in these experiments. Two rats in each group were injected intraperitoneally with Prothidium and the third rat reserved as a control. Immediately after the dose, the bile from the rats in the first group was collected through a cannula in the bile duct over a 24 hr. period, after which the rats were killed. Thereafter, each day for 9 days, a fresh group of rats was treated similarly. The abdomen was opened under ether anaesthesia; the bile duct was identified and freed from the lesser omentum along one inch of its length. The duct was then cannulated using polythene tubing (0.1 cm. diameter) which had been introduced through the side wall of the abdomen. The abdomen was closed and the rat maintained for 24 hr. in a restraining cage whilst the bile was collected. The Prothidium content of the bile (15 to 20 ml.) was estimated after the addition of an equal volume of 2N sulphuric acid and extraction in the usual way.

Perfusion of Rat Liver with Prothidium.—Adult rats were starved overnight and 5 to 8 ml. of blood were removed by cardiac puncture under ether anaesthesia. About 55 ml. of blood was collected in siliconed glass tubes for each perfusion. The plasma was separated from the erythrocytes by centrifugation.

To this plasma was added 30 mg. of powdered Prothidium and the solution continuously stirred using a glass rod. Ten minutes later a clot had formed on the rod; the volume of the clot was decreased by rolling it around the inside of the tube for about 5 min. The plasma was then centrifuged at about 600 *g* for 2 min.; 1 ml. was removed for fluorimetric estimation of Prothidium, and the *p*H of the remainder adjusted to 7.4 with sodium bicarbonate. The treated plasma was then remixed with the erythrocytes, which had been washed once with saline to remove any traces of plasma, and the whole used as the liver perfusate.

The technique of liver perfusion used was that described by Cohen and Gordon (1958), and the surgical technique and the perfusion apparatus were basically as described by Miller, Bly, Watson, and Bale (1952). The liver donor (hooded male rat) was starved overnight. The abdomen was opened along the mid-line, the bile duct and the portal vein identified and cannulated without previous ligation of the gastric and duodenal blood vessels. Perfusion of the liver was commenced as soon as the superior vena cava had been cannulated; the liver was then removed from the animal. Initially, the liver was perfused with Ringer solution to wash out any traces of the donor blood, and the Ringer was then replaced with the treated blood described above. The perfusion was allowed to run for 5 hr., and during this time the bile was collected.

At the end of the experiment the blood was collected for extraction of Prothidium. A 3 mm. cube of liver was frozen in dry ice for histological sectioning at −20°; a 5 mm. cube of liver was fixed in Carnoy and another fixed in formal saline, both for histological sectioning. The remainder of the liver was homogenized in 2N sulphuric acid and the Prothidium was extracted and determined. In some experiments, plasma and bile were extracted by various methods in an attempt to detect any metabolites. Samples were also subjected to paper electrophoresis and chromatography.

A homogenate of the perfused liver was subjected to electrodialysis using the method of Molle (1956). An acid liver brei (8 g. liver in 30 ml. 0.1 N sulphuric acid) was placed in the central cell and the two outer cells were filled with water. Platinum electrodes were used and 100 mA. was passed through the apparatus until the current had fallen to a steady minimum (after about 2 hr.) indicating that all the ions present had travelled to the electrodes. The contents of the cathode cell were removed into 2 ml. of 40% sodium hydroxide, extracted with 10 ml. butanol and back-extracted into N sulphuric acid, and the ultra-violet absorption spectrum determined.

Distribution of Prothidium in a Calf.—Liver biopsies were carried out on a 6-months calf. I am indebted to Mr. Ford of the A.R.C. Unit, Babraham Hall, Cambridge, who kindly demonstrated the technique of liver biopsy. The method used was that described by Loosmore and Allcroft (1951). The tissue samples thus obtained were homogenized in acid and assayed for Prothidium in the usual way. The first liver biopsy, taken 3 days after intraperitoneal injection of 140 mg. of Prothidium in 7 ml. water, yielded 650 mg. of liver. A second liver biopsy (700 mg.) was taken 18 days after injection. Thirteen days later (1 month after injection) the calf was slaughtered and the liver, kidney, and a sample of bile were removed. Samples of the liver, kidney, spleen, muscle, lung, and heart were fixed in formal saline and examined histologically.

Absorption in Cattle.—Three adult barren Ayrshire cows were each given a subcutaneous injection of a 4% solution of Prothidium in water (2 mg./kg.). The site of injection was about a 6-in. square over the eleventh and twelfth ribs about 8 to 10 in. down from the dorsal mid-line.

Prophylactic Activity Against T. vivax *in Rats.*—Adult or young (60 to 80 g.) hooded rats bred in this Institute were given 7.5 mg./kg. of Prothidium by intraperitoneal injection. This dose is close to the toxic range. A group of 3 treated rats, and 2 control rats, were infected immediately with 8 to 12m. parasites. Further groups were inoculated at weekly intervals. Tail blood films were taken daily for six

TABLE I

THE DISTRIBUTION OF PROTHIDIUM AFTER INTRAPERITONEAL AND INTRACARDIAL INJECTION INTO RATS

Route	Intervals after Injection	Percentage of Dose Recovered (Mean Values in Brackets)	
		Liver	Kidneys
Intraperitoneal	2 hr.	11, 26, 24 (20·1)	
	6 ,,	21, 15, 17 (17·5)	
	9 ,,	16, 25, 21 (20·5)	
	12 ,,	18, 23, 25 (21·8)	
	24 ,,	30, 28, 38 (29·0)	5, 2 (3·45)
	48 ,,	16, 9, 11 (11·5)	4, 2 (3·0)
	72 ,,	11, 5, 11 (8·7)	3, 2 (2·5)
	96 ,,	5, 7, 1 (5·3)	0·5, 3 (1·75)
	120 ,,	Trace	3, 3 (3·0)
	192 ,,	0	3, 2 (2·5)
	216 ,,	0	2, 1 (1·5)
Intracardial	2 ,,	23, 15, 12 (16·6)	
	6 ,,	21, 18, 20 (19·5)	
	9 ,,	26, 42, 29 (32·2)	
	12 ,,	37, 48, 27 (36·2)	
	24 ,,	26, 29 (31·0)	
	48 ,,	22, 15 (22·0)	
	72 ,,	3, 10 (3·2)	
	96 ,,	7, 4 (5·4)	
	120 ,,	Trace	
	192 ,,	0	
	216 ,,	0	

days after infection and thereafter weekly for 7 to 11 weeks, and examined for the presence of trypanosomes. The strain of *T. vivax* in this work was the Ilorin (unsupplemented line) strain described by Desowitz and Watson (1953); this strain was made available to me by the courtesy of Mr. Reed of the Wellcome Laboratories of Tropical Medicine, London. The parasite produces a severe and usually lethal infection in laboratory rats and mice.

RESULTS

Absorption and Excretion in Rats and Rabbits

The fluorescence of Prothidium was used to follow visually the fate of the drug after injection. Freshly killed rats were observed under an ultra-violet lamp 2 to 6 hr. after intraperitoneal injection of Prothidium. Under these conditions the liver and kidneys took on an orange fluorescence; the xiphisternum and a small area of the abdominal wall around the site of injection also fluoresced. No orange fluorescence was observed elsewhere in the animals.

The percentages of the dose recovered from rat liver after intraperitoneal and intracardial injections and from rat kidneys after intraperitoneal injections are recorded in Table I; the rates of disappearance of Prothidium from the liver and kidneys of rats and rabbits are shown in Figs. 3 and 4. There were considerable differences between individual animals, but it is clear that the drug appeared in considerable quantities in the liver and kidneys quite soon after injection. Little or no drug was detectable in the gut, spleen, heart, lung or muscle of rats. The rate of disappearance of the drug from the liver and kidneys of both rats and rabbits was rapid for the first 24 hr. after injection, but the drug was detectable in these organs for 7 days in rats and for 10 days in rabbits. A typical ultra-violet absorption curve of these extracts is shown in Fig. 5, demonstrating that unchanged Prothidium was present in the tissues.

Histological sections of treated liver (cut at $-20°$), when viewed with the fluorescence microscope, showed a concentration of fluorescent material in the nucleoli, nuclear membrane and basophilic granules of the hepatic cells. The Kupffer cells were loaded with fluorescent

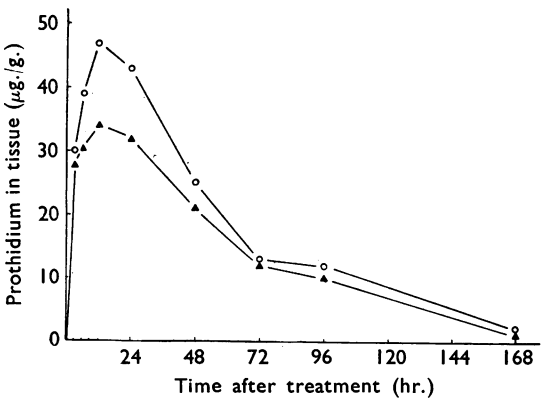


FIG. 3.—The rate of elimination of Prothidium by rat liver and kidneys. O—O = liver (each point on the curve represents the average of 6 rats; 3 rats were injected intraperitoneally and 3 were injected intracardially, but, as the results from each route were similar, the average of the 6 rats was used). ▲—▲ = kidneys (each point on the curve represents the average of 3 rats after intraperitoneal injection).

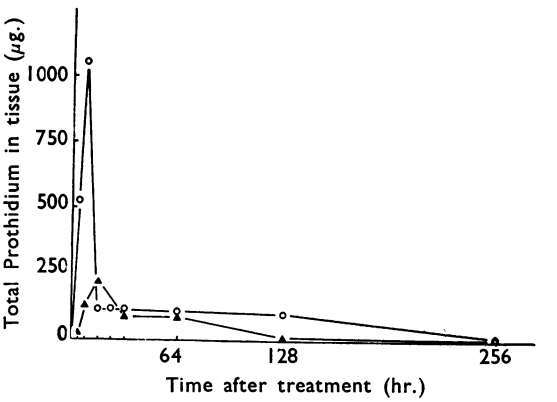


FIG. 4.—The rate of elimination of Prothidium from rabbit liver and kidneys. Each point on the curve represents the average of 2 rabbits, after intraperitoneal injection. O—O = liver. ▲—▲ = kidneys.

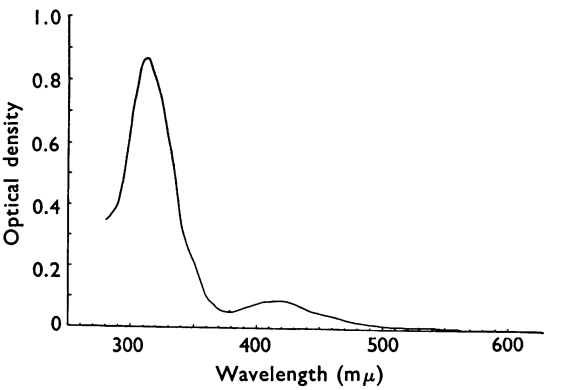


FIG. 5.—The ultra-violet absorption curve of a rat liver extract in N sulphuric acid. The liver of a treated rat was homogenized in 2N sulphuric acid, extracted into butanol and back-extracted into acid.

TABLE II

EXCRETION OF PROTHIDIUM IN THE BILE OF RATS

Each result represents the amount of Prothidium extracted from the bile excreted by one rat in 24 hr. One animal was used for each time point.

Time after Injection (hr.)	Prothidium in Bile (μg.)	Prothidium Given (μg.)	Percentage of Prothidium Recovered/ 24 hr.	Mean Percentage Recovery/ 24 hr.
0–24	135·3 209·0	2,000 1,900	6·75 11	8·9
24–48	285·0 104·0	1,500 2,300	19 4·5	11·7
48–72	46·0 137·0	1,600 2,500	2·85 7·0	4·9
72–96	39·6 43·0	1,800 1,900	2·20 2·25	2·2
96–120	25·6 25·0	1,600 1,550	1·6 1·6	1·6
120–192	24·0 24·2	2,200 2,200	1·1 1·1	1·1
192–216	8·64 10·0	1,600 2,300	0·54 0·44	0·5
	Total Prothidium recovered (mean of each 2 rats)= 558·2 μg.	Average μg. of Prothidium given =1,920 μg.	Percentage of Prothidium recovered in 216 hr.= 30·9%	

material and were frequently swollen with it. Sections of treated liver stained with haematoxylin and eosin appeared little damaged by the drug.

Excretion

Urine from rats and rabbits which had received Prothidium contained little if any of the drug. No Prothidium could be detected by heating the urine samples with acid or alkali to hydrolyse any metabolic derivative, by electrophoresis, by continuous ether extraction or by paper chromatography. If any Prothidium is excreted by this route it can only be a very small amount.

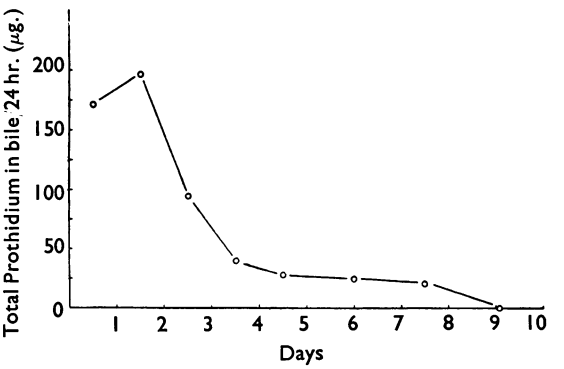


FIG. 6.—The rate of elimination of Prothidium in rat bile. Each point on the curve represents the average of 2 rats.

Since the liver was heavily loaded with Prothidium and since excretion of the drug in the urine appeared to be negligible, the possibility was considered that secretion took place via the bile.

Table II shows the results of experiments in which bile was collected from treated rats; the rate of elimination of Prothidium from the bile is shown in Fig. 6. The drug was rapidly excreted in the bile for the first 3 days and was detectable for 9 days. About 31% of the initial dose of Prothidium was recovered from the excreted bile in 9 days. The ultra-violet absorption curves of the bile extracts corresponded closely with that of the pure drug.

Absorption by Perfused Rat Liver

In view of the concentration of Prothidium in the liver and its rapid elimination from this organ, it was considered possible that this was the site of metabolism of the drug. Plasma, bile and liver tissue taken after 5 hr. of perfusion and examined by various methods of extraction and by paper electrophoresis and chromatography failed to yield evidence of metabolites of Prothidium. A butanol extract of the cathode cell after electrodialysis of a homogenate of perfused liver gave an ultra-violet absorption spectrum which corresponded closely with that of pure Prothidium; no trace of metabolic products could be found.

Free Prothidium was also found in the plasma perfusate and in the bile, but no metabolic product could be detected. Almost all the Prothidium used in the liver perfusion was recovered at the end of the experiment.

TABLE III

THE DISTRIBUTION OF PROTHIDIUM IN CATTLE AFTER INTRAPERITONEAL OR SUBCUTANEOUS INJECTION

i.p.=intraperitoneal; s.c.=subcutaneous.

Animal	Prothidium Given (mg.)	Route	Time after Treatment	Sample	Wt. or Vol. Sample	Conc. of Prothidium in Sample	Total Prothidium Recovered(μg.)	Fluorescence
Calf	140	i.p.	3 days	Liver	0·65 g.	5 μg./0·1 g.	35	
			18 ,,	,,	0·7 ,,	Trace	Trace	
			31 ,,	,,	3·1 kg.	,,	,,	Yellow
				Kidney	660 g.	Nil	Nil	Nil
				Bile	9 ml.	Trace	Trace	
				Muscle	Section			,,
				Lung	,,			,,
				Heart	,,			,,
Cow I (4–5 yr.)	1,026	s.c.	3 weeks	s.c. tissue	28·3 g.	35 μg./g.	994	
				,, fluid	12 ml.	20 μg./ml.	240	
,, II (4–5 yr.)	988	,,	3 ,,	,, ,,	23 ,,	9·9 ,,	227	
,, III (2–3 ,,)	978	,,	3 ,,	,, ,,	31·5 ,,	10·1 ,,	318·2	
,, II			6 ,,	,, tissue	18·2 g.	33 μg./g.	600·6	
,, III			12 ,,	,, ,,	17·3 ,,	15·4 ,,	267	

The Absorption of Prothidium in Cattle

The results of experiments in cattle treated with Prothidium are shown in Table III. The first liver biopsy, taken 3 days after injection of the drug in a calf, contained Prothidium, but no trace of the drug was found in a second specimen taken 18 days after injection. When the calf was slaughtered one month after injection, traces of Prothidium were present in the bile and liver but none was found in the kidneys. The histological sections of all the tissues examined appeared normal; only the (unstained) liver sections showed a yellow fluorescence when viewed with ultra-violet light.

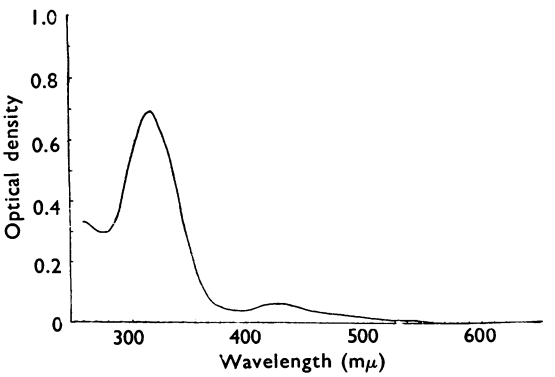


FIG. 7.—The ultra-violet absorption curve of a subcutaneous tissue extract in N sulphuric acid from cow 1. The subcutaneous tissue was homogenized in 2N sulphuric acid, extracted into butanol and back-extracted into acid.

When Prothidium was injected subcutaneously into cows, elongated swellings appeared at the site of injection in all 3 animals; the swellings remained for 6 weeks. They contained a fluid exudate with a high concentration of Prothidium 3 weeks after injection. The subcutaneous tissue at the site of injection also contained a high concentration of drug (Table III; Fig. 7). Six weeks and 12 weeks after injection the subcutaneous tissue at the injection site still contained much unchanged Prothidium, but the swelling had disappeared and there was no fluid.

Prophylactic Action in Rats

A single dose of 7.5 mg./kg. of Prothidium protected rats against *T. vivax* for about 8 weeks and young rats for at least 5 weeks. Young rats developed a transient infection before becoming permanently cured; infected control rats died 2 to 4 days after inoculation. Adult rats were more resistant to infection and untreated infected animals sometimes survived.

DISCUSSION

A single dose of Prothidium will protect cattle against trypanosomiasis for six months (Watkins and Woolfe, 1956). Suramin (Bayer 205) has a

similar prophylactic action in human trypanosomiasis and in this instance the prolonged protection is due to retention of the drug. This retention has been demonstrated for the rat, guinea-pig and monkey (Findlay, 1930). It seemed likely therefore that the protection afforded by Prothidium might also be due to the retention of the drug or its metabolites.

In the present investigation the method used for the estimation of Prothidium was capable of detecting the drug, added to plasma, down to a level of about 2 μg./ml. This is the same order of sensitivity as that of the method used by Boursnell and Wormall (1939) for the estimation of suramin. In contrast to their results with suramin, Prothidium was not detectable in rat blood 1 hr. after intraperitoneal injection of the drug. However, the Prothidium was strongly bound to liver proteins so that the blood concentration was not an adequate measure of the amount of drug retained in the body. When rats were given Prothidium either intraperitoneally or intracardially, the drug could be detected in liver and kidneys for 7 days, and significant levels of Prothidium were detected in the bile secreted between the 7th and 8th days. From a series of experiments in which bile was collected from rats over 24 hr. periods, it was evident that the bile was a major route for the excretion of Prothidium. Examination of the faeces yielded no evidence of the presence of the drug or its metabolites. Prothidium may be broken down by the gut flora or it may be reabsorbed by the small intestine. The concentration of Prothidium in rat's liver and kidney and its excretion *via* the bile show that it is similar in action to another phenanthridinium compound, carbidium ethanesulphonate, which has been shown to have a similar distribution in mice (Goodwin, Goss, and Lock, 1950).

The results of the distribution and excretion experiments in rats suggested that Prothidium would protect these animals against *T. vivax* infection for at least 10 days. Experiments have shown that a single dose of Prothidium protected adult and young rats against *T. vivax* for about 7 weeks in spite of the fact that the young rats developed a transient infection before being fully protected. It may well be that sufficient Prothidium remained bound within the rat tissues to afford protection for this period of time, but there seemed a disinct possibility that Prothidium was converted to an active metabolite which was responsible for the prolonged action of the drug. No metabolite was detected in urine, in blood or in extracts of a liver which had been perfused *in vitro* with the drug, but this evidence is inconclusive because low concentrations of unknown metabolites would almost certainly have escaped detection. In order to obtain significant results on the excretion of metabolites it would be necessary to use radioactive Prothidium and this has not, so far, been available.

The more prolonged prophylactic action of Prothidium in cattle suggested that there might be marked species differences in the rate of excretion of the drug, and accordingly some of these experiments were repeated using rabbits. In these animals Prothidium was detectable in liver and in kidneys for 10 days after a single intraperitoneal injection ; the drug also disappeared at about the same rate from the tissues of a calf after it had received an intraperitoneal injection of the drug. The situation was entirely different when cattle were given Prothidium subcutaneously, the route of injection which is used in the field. The drug formed a depôt at the site of injection and was readily detectable near the site of injection for at least 3 months after treatment. It seems likely therefore that the prolonged prophylactic action of Prothidium is a result of the formation of a local depôt and to the powerful binding of the drug by liver tissue. This would explain the failure to find significant concentrations of Prothidium in the blood of animals which are resistant to trypanosome infection.

Williamson and Desowitz (1956) have shown that a mixture of Prothidium and suramin is less toxic to cattle and shows a greater prophylactic action than either compound alone. When Prothidium and suramin are mixed in the proportions 1:0.77 an insoluble complex is formed and it is probable that the effectiveness of the mixture is due to the formation of a better local depôt at the injection site by such an insoluble salt. Several similar complexes were prepared by these workers by mixing either quinapyramine sulphate, homidium bromide, berenil or RD 2902 with suramin. Extensive field trials were carried out on these substances by Desowitz (1957), who found that the homidium bromide-suramin complex was the most efficient prophylactic in cattle. However, he found that this substance was still highly toxic, whereas no severe local reactions or obvious toxic symptoms were found in the few animals treated with the Prothidium-suramin complex. Since the 3 cows used in the present work also showed little sign of toxic symptoms after treatment with Prothidium, it may be that the Prothidium-suramin complex could be used more safely in the field than the complex of homidium bromide and suramin. No direct comparisons have so far been made.

I would like to thank Dr. F. Hawking for suggesting this problem, and I am deeply grateful to Dr. T. S. Work for his unfailing advice and encouragement in these experiments. I am grateful to Dr. P. Walker, Dr. J. Hitchcock, and Mrs. V. Mijoric for their advice with some of the experiments. I am also much indebted to Miss B. C. Staehelin for her willing and skilful technical assistance.

References

Boursnell, J. C., and Wormall, A. (1939). *Biochem. J.*, **33**, 1191.
Cohen, S., and Gordon, A. H. (1958). Ibid., **70**, 544.
Desowitz, R. S. (1957). *Ann. trop. Med. Parasit.*, **51**, 457.
—— and Watson, H. J. C. (1953). Ibid., **47**, 62.
Findlay, G. M. (1930). *Recent Advances in Chemotherapy*. London: J. and A. Churchill.
Goodwin, L. G., Goss, M. D., and Lock, J. A. (1950). *Brit. J. Pharmacol.*, **5**, 287.
Jancsó, N., and Jancsó-Gabor, A. (1952). *Nature, Lond.*, **170**, 567.
Loosmore, R. M., and Allcroft, R. (1951). *Vet. Rec.*, **63**, 414.
Miller, L. L., Bly, C. G., Watson, M. L., and Bale, W. F. (1952). *J. exp. Med.*, **94**, 431.
Molle, L. (1956). *Ann. Soc. roy. Sci. med. nat. Brux.*, **9**, 5.
Taylor, A. E. R. (1960). *Brit. J. Pharmacol.*, **15**, 230.
Watkins, T. I., and Woolfe, G. (1956). *Nature, Lond.*, **178**, 368.
Williamson, J. (1957). *Ann. trop. Med. Parasit.*, **51**, 440.
—— and Desowitz, R. S. (1956). *Nature, Lond.*, **177**, 1074.