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MIRACIL D, ITS TOXICOLOGY, ABSORPTION, AND EXCRETION IN ANIMALS AND HUMAN VOLUNTEERS

BY

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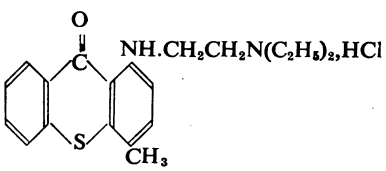
Miracil D is a new compound which has been devised for the treatment of schistosomiasis (bilharziasis). The present paper outlines its behaviour when given to laboratory animals and human volunteers. A subsequent paper will give information about clinical trials of this compound in patients infected with *Schistosoma haematobium* or *S. mansoni*.

History

The original discovery of the action of miracil against schistosomes was made at the Elberfeld Research Laboratories of the I. G. Farbenindustrie, where experimental study of infections with *S. mansoni* was begun by Dr. W. Kikuth in 1932. A series of compounds, synthesized by Dr. Mauss (known as the miracil series), was tested in 1938 by Kikuth and Gönnert and found to have considerable activity. Further investigation led in 1941 to the conclusion that the greatest activity against schistosomal infections of monkeys was reached in a member of the series designated miracil D (Kikuth, Gönnert, and Mauss, 1946). This compound was investigated pharmacologically by Dr. Hecht, who studied it in a limited number of mice, rabbits, and cats, and considered that it might lead to gradual fatty degeneration of the liver, kidney, and heart muscle. Owing to the war, clinical trials were impossible and no further progress was made in Germany, apart from tests carried out by Prof. Vogel, which showed that *S. japonicum* was not susceptible to these compounds. (*S. haematobium* could not be tried.) After the military occupation of Germany, this information was discovered by Allied investigators and the compounds were studied further in Britain and America. In America, Bueding, Higashi, Peters, and Valk (1947) found that a majority of mice were cured of infections with *S. mansoni* when treated with miracil D in doses of 36 mg. per kg., administered intraperitoneally every 8 hours for 18 doses; these doses killed one-quarter of the mice. In Britain the pharmacology of miracil D was studied in small animals by Wood (1947), who did not confirm the insidious toxicity reported by Hecht; and delicate methods for the estimation of the compound in body fluids were worked out by Coxon, Latner, and King (1947). Early in 1947 we began to study its toxicity and behaviour in rabbits, monkeys, and human volunteers; and in July (by the kindness of the Medical Director, Southern Rhodesia) we were able to begin clinical trials. Studies on the toxicity have also been carried out by workers at the Wellcome Research Institute under Dr. J. S. K. Boyd. To all the above workers we are grateful for kindly giving us confidential information of their results as they were obtained.

Chemistry

Miracil D is the hydrochloride of 1-methyl-4-β-diethylaminoethylaminothioxanthone



It is a crystalline orange-yellow powder which is soluble up to 1 to 2 per cent in water at room temperature. When present in body fluids it can be estimated by alkalinization and extraction first into ether and then into dilute hydrochloric acid. The yellow colour of the resultant concentrate is estimated at a suitable *p*H in a Spekker absorptiometer which has been calibrated by means of known concentrations of miracil (Coxon, Latner, and King, 1947). The sensitivity of this technique is sufficient to detect 0.05–0.1 mg. per litre.

Pharmacology

Miracil has an irritant action when applied locally to the tissues, and subcutaneous or intramuscular injection causes considerable inflammation and some necrosis. When it is injected intravenously, its toxicity is much greater than when it is given by mouth and it tends to cause thrombosis of the vein. Consequently, oral administration is to be preferred.

According to Kikuth and Gönnert (1945, 1948), Hecht (1945), and Wood (1947), the maximum single doses tolerated by mice are 300 to 1,000 mg. per kg. by mouth, 340 to more than 500 mg. per kg. by subcutaneous injection, and 20 to 30 mg. per kg. by intravenous injection. Rabbits tolerate single doses of 600 to 800 mg. per kg. by mouth, but only 15 to 20 mg. per kg. by intravenous injection. With repeated oral doses, mice tolerate 125 mg. per kg. daily for 10 days. Wood found that rabbits died after 4 daily doses of 150 mg. per kg. or 6 daily doses of 50 mg. per kg. In our own experiments rabbits tolerated 28 daily oral doses of 50 mg. per kg. but died after 12 or 14 daily doses of 100 mg. per kg. One monkey (No. 70) weighing 3.5 kg. survived a total dose of 5.45 g. per kg. by mouth, given as 3 doses of 50 mg. per kg. in 6 days, 13 doses of 100 mg. in 30 days, and 20 doses of 200 mg. in 30 days. Another monkey (No. 105) weighing 4.6 kg. survived a total dose of 3.4 g. per kg., given as 17 doses of 200 mg. per kg. in 26 days. A third monkey (No. 94) weighing 3.6 kg. died after 4.8 g. per kg. given as 12 doses of 400 mg. in 18 days. A fourth (No. 72) weighing 3.2 kg. died after 5 g. per kg. given as 13 doses of 200 mg. in 30 days and 6 doses of 400 mg. in 9 days. Apparently the maximum tolerated dose for monkeys is about 200 mg. per kg. four times a week. The minimum curative dose for mice infected with *S. mansoni* is 120 mg. per kg. on 6 successive days, while monkeys have been cured by two oral doses of 10 mg. per kg. (Kikuth and Gönnert).

Wood reports that intravenous injections into rabbits of more than 20 mg./kg. quickly cause convulsions, similar to those of picrotoxin or strychnine; often there is head retraction and extension of the limbs. In anaesthetized rabbits or cats small doses cause no particular effect on the cardiovascular system; larger doses cause depression of the heart and dilatation of the peripheral vessels. Other investigations on isolated organs have shown no significant pharmacological actions, except a mild spasmolytic action on intestinal muscle.

Absorption, Distribution, and Excretion

For this work we have estimated miracil in the body fluids by the method of Coxon, Latner, and King (1947). In one rabbit which had received 0.8 g. per kg. by mouth, the blood concentration of miracil was 1.6 mg. per litre at 24 hours, 1.1 mg. at 48 hours, and 0 at 144 hours. Fig. 1 shows the curve of the blood concentration after a single dose of 0.4 g. per kg. by stomach tube to a monkey (No. 94). Absorption was rapid and the concentration in the blood was sustained for at least 21 hours.

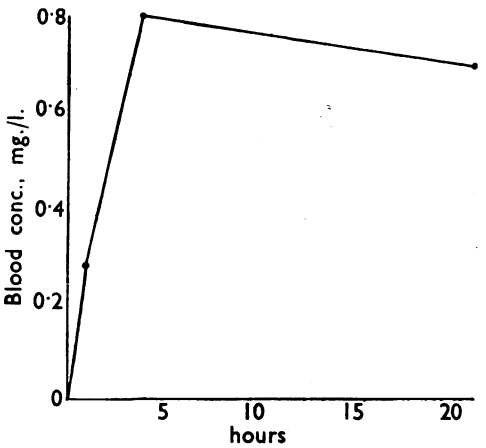


Fig. 1.—Blood concentrations of miracil in a monkey (No. 94) after a single oral dose of 0.4 g. per kg.

In 3 monkeys which were receiving oral doses of 0.1 to 0.4 g. per kg., daily or every other day, the blood concentration 24 hours after the previous dose was 0.65, 0.80, and 1.60 mg. per litre respectively, the concentrations not being in proportion to the dosage administered. In a monkey (No. 70) which had been receiving 0.2 g. per kg. (20 doses in 30 days) the blood was examined 3 days after the last dose, and it contained only a trace of miracil (0–0.1 mg. per litre). Since 10 mg. per kg., repeated once, is stated by Kikuth and Gönnert to be the minimum curative dose for monkeys, this dose was given to a monkey weighing 2.3 kg.; at 2¼ hours the blood concentration was just detectable (about 0.1 mg. per litre), and at 6 hours miracil could not be detected. Another monkey weighing 2.7 kg. was given 20 mg. per kg.; at 2½ hours the blood concentration was 0.45 mg. per litre. In a third monkey, given 20 mg. per kg., no miracil could be detected in a 2.5 c.c. blood sample (i.e. probably less than 0.1 mg. per litre). These concentrations should be compared with those obtained in the blood of human subjects (below).

Samples of urine collected from some of these monkeys contained 33 to 64 mg. per litre. The monkeys in these experiments passed about 100–150 c.c. of urine per day. When the administration of miracil to two of the above monkeys was discontinued the excretion of miracil in the urine diminished rapidly and ceased after 3 days or 4 days respectively. The faeces of the monkeys which had received these repeated high doses contained 3.8 to 10 mg. miracil per g. moist weight.

The distribution of miracil in the different organs was studied in two monkeys which died from prolonged overdosage. Monkey No. 94 had received 12 doses of 400 mg. per kg. during 18 days, and monkey No. 72 had received 13 doses of 200 mg. per kg. in 30 days, and 6 doses of 400 mg. per kg. in 9 days. The concentrations of miracil extracted are shown in Table I. In addition, some of the organs yielded considerable quantities of a yellow pigment which was extracted by ether but did not pass from ether into hydrochloric acid ; presumably this was a degradation product of miracil in which the basic character of the molecule had been masked or destroyed. The high concentration of miracil in the kidney is presumably due to excretion of the compound in the urine. The concentrations in the lung and heart muscle are higher than might have been expected, while that in the liver is lower. The high concentration of presumed degradation product in the liver is easily understood, since the catabolism of miracil probably takes place in this organ ; its high concentration in the brain is less easy to explain.

TABLE I

THE CONCENTRATION OF MIRACIL AND ITS DEGRADATION PRODUCT IN THE ORGANS OF TWO MONKEYS

Concentrations in mg. per kg.

$$\text{Ratio} = \frac{\text{Concentration of degradation product}}{\text{Concentration of miracil}}$$

Organ	Miracil		Degradation product		Ratio	
	No. 94	No. 72	No. 94	No. 72	No. 94	No. 72
Brain	3.5	1.3	60	24	17	18
Muscle	30	5.4	1.8	2.2	0.006	0.41
Liver	25	12	80	140	3.2	12
Heart	21	16	6.0	9.8	0.29	0.61
Kidney	93	56	8.0	13	0.09	0.23
Lung	98	63	3.0	5.0	0.03	0.08

Miracil was given to six volunteers, one receiving it on two occasions. The miracil was taken as solution, preferably after a meal, and a piece of bread was eaten to provide extra material in the stomach and protect the gastric mucous membrane. The blood concentration curves during the first six hours are shown in Fig. 2 and the dose schedules and blood concentration of different subsequent times are given in Table II. Fig. 2 shows that absorption of miracil is rapid, a relatively high blood concentration being reached in 2¼ hours ; during the next 4 hours, removal of the drug from the blood (by degradation, excretion, etc.) may or may not be greater than the continued absorption from the intestine. There are considerable differences between different individuals in the height and persistence of the blood concentration after a

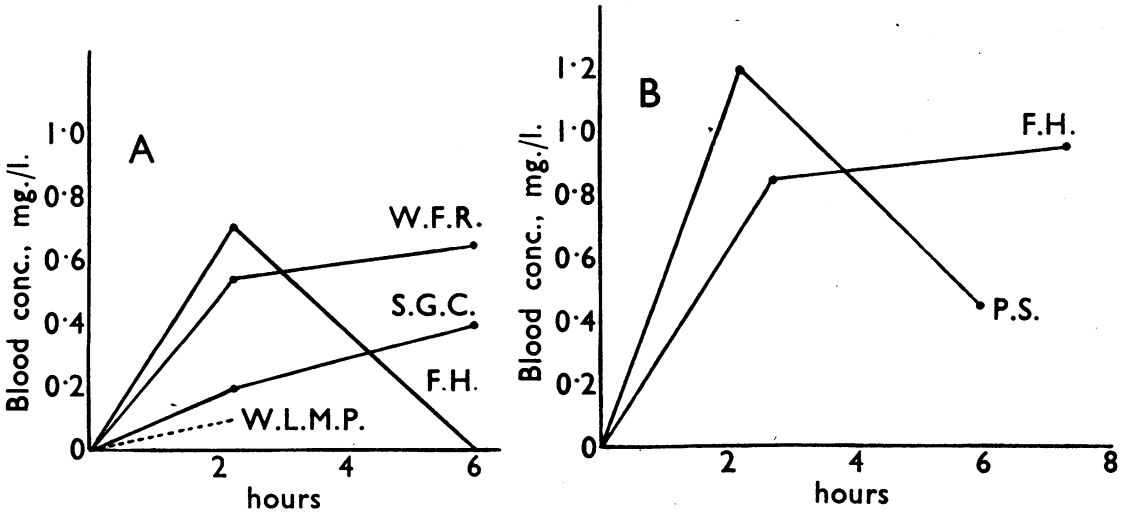


FIG. 2.—Blood concentrations of miracil in volunteers after single oral doses. A—100 mg. (volunteer W.L.M.P., shown by dotted line, received 50 mg.). B—200 mg.

TABLE II

THE BLOOD CONCENTRATION OF MIRACIL ON VARIOUS DOSE-SCHEDULES

The figures in bold type give the individual doses in g. The other figures give the blood concentrations of miracil in mg. per litre. The crosses mark the approximate times when symptoms were felt

Subject and dosage	1st day				2nd day					3rd day				4th day			5th day			6th	7th	8th	Symptoms
	0	hours 2¼	hours 6	10	0	hours 2¼	hours 4	hours 6	8	0	hours 2¼	hours 6	10	hours 0	hours 2¼	hours 6	hours 0	hours 2½	6	hours 0	0	0	
F.H. 0.1 g. once	**0.1**	0.70	0																				None
F.H. 0.2 g. once	**0.2**	0.85	0.95		×	×	×	×	×														Slight abdominal uneasiness
R.H. 0.3 g. once	**0.3**				×	×	×	×	×														Nausea, vomiting, and fatigue
W.F.R. 0.1 g. twice daily	**0.1**	0.55	**0.1** / 0.65		**0.1** / 0	1.50		**0.1**		**0.1**	0.90	**0.1**		**0.1**	0.90	**0 1**	**0.1**	0.80	**0.1**				None
P.S. 0.2 g. twice daily	**0.2**	1.20	**0.2** / 0.45		**0.2**	1.00		**0.2**		**0.2**	1.04	**0.2**		**0.2**	1.20	×	0.90 ×	×	×	0.30 ×			Marked nausea and fatigue
W.L.M.P. 0.05 g. 3 times daily	**0.05**	**0.05** / 0.10	**0.05**		**0.05**	0.75	**0.05**		**0.05**	**0.05**	**0 05** / 0.95	**0.05**	×	×	0.90 ×								Slight nausea
S.G.C. 0.1 g. 3 times daily	**0.1**	0.20	**0.1** / 0.40	**0.1**	**0.1**	1.00	**0.1**		**0.1**	**0.1**	0.65	**0.1**	**0.1** ×	×	×	×	0.10 ×						Marked nausea, malaise, etc.

given dose. Absorption from the intestines is almost complete, and little appears in the faeces. In one volunteer (F.H., after a single dose of 100 mg.) the faeces during 48 hours contained less than 3 mg.; in another (W.F.R., taking 100 mg. twice daily) the faeces during the first 30 hours contained about 2 mg.; in a third (P.S., taking 200 mg. twice daily) the faeces collected during the first 48 hours contained 33 mg.; some of this may have been excreted into the bowel. In volunteers taking repeated doses the blood concentrations on the second day were usually much higher than the corresponding ones on the first day; but after that there was no constant tendency for the blood concentration to rise higher, or for accumulation to occur (Table II). When the drug is stopped, it disappears from the blood in two or three days. The amount of miracil excreted in the urine often amounts to about 7 per cent of the dose ingested. Since less than 10 per cent of the dose can be recovered from the urine and faeces, it appears that most of the drug absorbed is broken down in the body so that it is not recognized by the test employed. The concentration in the urine may be as high as 30 mg. per litre, but usually it is much lower. When administration of repeated doses of the drug is stopped, miracil ceases to appear in the urine after 3 days.

To study the distribution of miracil between the different elements in the blood two successive samples were taken from volunteer P.S. after the 5th and 7th doses respectively. In the first experiment, the concentration of miracil in the different components was:—

Plasma sample (approximately 44 per cent of total volume), 1.2 mg. per litre.

Red blood corpuscle sample (approximately 44 per cent of total volume), 0.7 mg. per litre.

Intervening sample, including buffy coat W.B.C. (approximately 12 per cent), 3.0 mg. per litre.

In the second experiment, the concentrations were:—

Whole blood, 1.2 mg. per litre.

Plasma (63 per cent of the volume), 1.5 mg. per litre.

Red blood corpuscles (36 per cent of the volume), 0.5 mg. per litre.

Buffy coat layer (1 per cent of the volume), 7.0 mg. per litre.

The figure for the buffy coat (leucocyte) layer in the second experiment was obtained by calculation from the preceding figures and is not reliable. These provisional results indicate that the concentration in the plasma is approximately double that in the red blood corpuscles and that the concentration in the leucocytes (or platelets) is probably much higher; however, the leucocytes contain only a small proportion of the total amount present in the whole blood, since their volume is small.

Toxic Effects of Miracil

In rabbits, repeated large oral doses of miracil, e.g., 0.4 g. per kg. daily, cause death after eight or more days. In the rabbits which eventually died there was marked loss of weight, haemo-concentration, and occasionally albuminuria. There was no anaemia or leucopenia. At autopsy the body fat and other tissues were often stained yellow (presumably with miracil), the kidneys sometimes appeared unhealthy, and the liver might be yellow and might contain white patches. Histological investigation revealed, in one rabbit, marked degeneration (sometimes going on to necrosis) of the cells of the renal tubules and areas of necrosis in the liver; in most of the rabbits, however, the degenerative changes in the kidneys and liver were quite mild, and in some they were inapparent. The other organs, including the heart, showed no pathological changes.

Four monkeys were given repeated oral doses. Vomiting often occurred after the early doses, but it ceased later when the same amount of miracil as before was given with a smaller volume of fluid (suspension in gum acacia). The monkeys showed intermittent diarrhoea, but the significance of this is doubtful as many other monkeys in the same room also showed it. Two of the monkeys gained 3–6 per cent in weight, two lost about 10 per cent while under treatment. Two of the monkeys died. One had shown no particular clinical symptoms except that on the day preceding death there had been marked lassitude and anorexia. The other one developed epileptiform fits, recurring at intervals; and, as death seemed imminent, it was killed. There was no anaemia, leucopenia, or albuminuria in these monkeys. At autopsy, the abdominal organs were stained yellow, especially the liver. Histological examination revealed slight degenerative changes in the renal tubules of one monkey; in the other monkey the kidney showed nothing abnormal. The other organs, including the liver and heart, showed no significant pathological changes.

In man, overdosage of miracil seems to produce a different picture. It was given to six volunteers as described above, the dosage being raised until symptoms appeared; their weights ranged from 64

to 80 kg. A single dose of 0.2 g. (F.H.) caused some uneasiness of the stomach during the *second* twenty-four hours after it. A single dose of 0.3 g. was taken by R.H. at 10 a.m., and during the first day he felt tired; he suffered from insomnia during the second part of the night. Next morning he felt nauseated and at midday he vomited. Later he had slight diarrhoea and felt tired and irritable, but he slept well that night. The third day he was all right again. (Dr. J. S. K. Boyd has kindly given information about two other volunteers on single doses. One took 0.2 g. with no ill-effects. The other took 0.4 g. about 2 p.m. That evening there was slight gastric discomfort which continued the next day. Sleep was disturbed. Forty-eight hours after taking the dose severe nausea, diarrhoea, and retching set in and persisted until the following morning, after which gradual improvement occurred.) Volunteer S.G.C. received 0.1 g. three times daily for three days. On the second night he had insomnia. On the evening of the third day he felt very tired with some aching of the legs and dizziness. The sclerotics were yellow. During the third night he awoke with headache, pains in the limbs, nausea, and great restlessness; he felt very ill. During the fourth day he had headache, extreme lassitude, nausea, and stayed in bed all day, but his appetite was fair. These symptoms passed off gradually and by the sixth day (third day from the last dose) he was normal again. Volunteer P.S. took 0.2 g. twice daily for 7 doses. He slept badly on the second night. On the fourth day he felt increasing nausea and fatigue and that night slept badly. His skin and sclerotics were yellow. On the fifth day the general nausea and malaise continued; on the sixth day his condition improved and by the eighth day he was normal again.

Summary of the symptoms in volunteers.—After single doses there was no immediate effect on the stomach or intestines and the symptoms usually did not appear until after a latent period of 18–24 hours. As can be seen from Table II, the occurrence of symptoms is not related to the concentration of miracil in the blood. The principal symptom was nausea, often profound; but vomiting was rare. There was also tiredness, prostration, and headache. Insomnia occurred in most volunteers, suggesting cerebral excitation. The skin and sclerotics were often yellow (apparently direct staining) and the urine was bright yellow. Two of the volunteers noted pains in the back or limbs. Diarrhoea was rare, in fact most of the volunteers tended to be constipated. There was no evidence of leucopenia, albuminuria, or jaundice. As it has later been found that patients can tolerate 0.6 g. or more daily without severe ill-effects, it is possible that some of the above symptoms in volunteers may have had a psychological element.

Discussion

The above work was undertaken in order to obtain a provisional picture of the behaviour of miracil in monkeys and man so that the therapeutic action of the compound in patients infected with schistosomes could be examined safely and easily; more detailed investigation of its pharmacology would depend on the outcome of these clinical trials. The following account of the behaviour of miracil must be regarded as only tentative.

Miracil is rapidly and completely absorbed from the alimentary canal; only a small proportion appears in the faeces, and this may be due to excretion by the bile or intestine. Excretion in the urine accounts for up to 7 per cent (approx.) of the dose. Degradation of the compound in the body is fairly rapid, and on repeated doses there is no obvious accumulation of the compound in the blood after the first day. When administration of the drug is stopped, the body gets rid of the remaining drug in about three days. In the blood, the concentration in the plasma is approximately twice that in the red blood corpuscles; the concentration in the leucocytes is probably much higher.

The toxic effects, produced by deliberate overdosage in animals, involve principally the kidneys and liver; they occur only after large and repeated doses, and even then they are often slight. In our experiments we have seen no evidence of the toxic effects on the heart which Hecht reported, and we believe that he was mistaken. In man the symptoms are apparently not due to a direct action of miracil on the stomach or intestines; they seem to be due rather to some degradation product of miracil acting perhaps on certain parts of the nervous system so as to produce nausea and the other disturbances described. The symptoms are unpleasant rather than dangerous.

Summary

1. Miracil is a new compound, synthesized by Mauss and stated by Kikuth and Gönnert to be highly effective in the treatment of mice and monkeys experimentally infected with *Schistosoma mansoni*. Chemically it is the hydrochloride of 1-methyl - 4-β-diethylaminoethylaminothioxanthone. It is administered by mouth.

2. Rabbits tolerate repeated daily doses of 50 mg. per kg. and monkeys tolerate 200 mg. per kg. four times a week.

3. Its behaviour after oral administration has been examined in six volunteers. Apparently it is rapidly absorbed from the alimentary canal, and, after single doses of 0.2 g., the concentration in the blood rises to about 1.0 mg. per litre at 2¼ hours. Over 90 per cent of the drug is degraded in the body and only about 7 per cent is excreted in the urine. There is little tendency for the drug to accumulate in the body.

4. The concentration in the plasma is about twice that in the red blood corpuscles; the concentration in the leucocytes is probably much higher.

5. In animals, deliberate prolonged overdosage may produce degenerative changes in the liver and the renal tubules, but these are usually less than would be expected.

6. In volunteers the maximum tolerated dose for repeated administration was about 0.2 g. per day. Overdosage produced nausea and general prostration; insomnia and yellow discoloration of the skin and sclerotics also occurred. These symptoms came on after a latent period of about one day.

Grateful acknowledgment is due to the previous workers on miracil, mentioned in the paper, for confidential information about their results, and especially to Dr. R. V. Coxon, Dr. A. L. Latner, and Prof. E. J. King for the instruction in the technique of estimating miracil; to the volunteers who experienced unpleasant discomfort in order to assist these investigations; and to Mr. R. Hunt for technical assistance.

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