the incidence of diabetes from 52 to 100%.

2. The potentiating effect of methylene blue is attributed, in part at least, to its ability to reoxidize dialuric acid back to alloxan and thereby in effect it is equivalent to increasing the effective dose of alloxan.

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Absorption, Excretion, and Toxicity of Milibis* (Bismuthoxy-p-N-glycolyl-arsanilate) Following Oral Administration. (17671)

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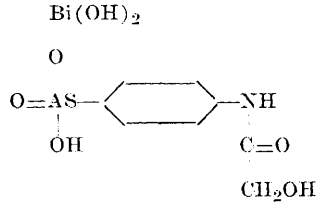
Milibis, a drug which promises to become important in the treatment of amebiasis, is a salt-like complex derived from bismuth trioxide and p-N-glycolyarsanilic acid. It has the approximate composition $C_8H_9AsBiNO_6 \cdot xH_2O$, and was first studied by Hauer(1) under the name of "Wia". The probable structure of its principal component, bismuthoxy-p-N-glycolylarsanilate, is as follows:



In the product as supplied, a small amount of the di-substituted compound $(C_8H_9AsNO_5)_2BiOH$, is probably also present. Milibis has been found quite effective as an amebicide in both experimental animals(2,3) and human subjects(1,2,4-6) as well as for the treatment of experimental yaws and syphilis in rabbits(2). From the very low aqueous solubility of the product† it could be predicted that only small amounts would be absorbed following oral administration, but obviously the product must dissolve to some extent if microorganisms are to be attacked. In view of the toxic potentialities of As and Bi compounds, it has been of interest to determine specifically the amounts of these elements absorbed following oral administration of the drug, and to ascertain whether appreciable quantities tend to accumulate in the tissues.

Acute toxicity. No toxic manifestations were apparent following single oral doses of 2.5, 5, or 10 g/kg, in either mice or rats. Groups of 3 rats, and 9 mice, were used at each dose level.

Subacute toxicity. Four groups of 5 rats weighing initially 130-140 g were given single oral doses of 2.5, 5, 7.5, and 10 g/kg by stomach tube, on 6 days of each week for 3 weeks. The drug was administered as a suspension in 0.5% aqueous gum tragacanth, and in a volume of 2 cc/100 g. Food (Purina Dog Checkers) and water were provided *ad lib.* The rats were weighed frequently so that comparisons could be made with a control group of 5 animals. One rat in the 5 g/kg and 2 rats in the 10 g/kg group did not survive the experiment. Two of these deaths were obviously accidental, due to difficulties in ad-

* Registered trademark of Winthrop-Stearns Inc.

1. Hauer, A., *Deutsche Trop. Z.*, 1943, v47, 153; *O. P. B. Report* No. 237, 1945, p. 38.

2. *O. P. B. Report* No. 981, 1945, p. 28.

3. Dennis, E. W., Berberian, D. A., and Hansen, S., *Am. J. Trop. Med.*, 1949, v29, 683.

4. Berberian, D. A., *J. Clin. Invest.*, 1948, v27, 525.

5. Garza Brito, A. de la, and Treviño Villaseñor, A., *Rev. Med. Mex.*, 1949, v29, 261.

6. Dennis, E. W., Berberian, D. A., and Tainter, M. L., *Prensa Med. Mex.*, 1949, v14, 115.

† The solubility of Milibis in water, ethanol, acetone, and propylene glycol is less than 0.025% at room temperature.

Some specific solubilities (per 100 cc of solvent) are as follows: 20% ethanol, 0.2 mg; 30, 50, and 95% ethanol, each 0.3 mg; 0.1 N HCl, 1.5 mg. These figures were obtained by determining the Bi content of a solution which had been saturated at room temperature, and calculating the Milibis content therefrom.

ministering the heavy suspensions of the drug and only the one death at the highest dose level could be attributed to the drug. The average % weight gains of the surviving animals during the 21 days were as follows:

Control group	51
2.5 g/kg/day group	48
5.0 " "	51
7.5 " "	43
10.0 " "	33

Thus only at the highest dose level was the growth of the animals significantly inhibited, and this may simply have been the result of the administration of such a large bulk of indigestible material. At autopsy no gross or microscopic pathological changes attributable to the drug were found in any of the animals.‡ It may be concluded, therefore, that rats are completely tolerant to oral doses of 5 g/kg/day for 3 weeks, under the conditions of the experiment.

Methods for metabolic studies. Male hooded rats weighing 240-270 g were kept in metabolism cages, one pair to each cage, with food§ and water being provided *ad lib*. The drug was incorporated in the diet at a level of 0.15%. It was estimated that the daily food consumption of the rats would be about 20 g, hence the expected intake of drug was slightly in excess of 100 mg/kg/day. The animals remained on the drug-containing diet for 12 days, with an accurate record of their food consumption being kept. For the next 24 hours they received the same diet without drug, then they were killed, frozen, and stored for subsequent analysis. Excreta were collected during the 13-day period, with the feces being separated from the urine on wire screens. For analytical purposes the excreta were combined for the first 6, and for the last 7 days of the experiment. The urine samples were centrifuged; the precipitated solids were added to the feces, which were then thoroughly dried, ground, and mixed before sampling. An aliquot of the clear supernatant urine was taken for analysis.

Methods used for the determination of As and Bi in food, urine, and tissues have been described elsewhere(7,8). A sample of the drug-containing diet was found on analysis to contain 0.557 mg Bi and 0.212 mg As per g. Since the particular lot of drug used in this work contained 36.8% Bi and 14.3% As,‖ the calculated amounts for 0.15% Milibis in diet are 0.552 mg Bi and 0.215 mg As per g. The procedure used for the digestion of feces represents a slight modification of those previously described. It gave the most consistent and most nearly quantitative recoveries of As and Bi:

Transfer 1.00 g dry, finely powdered feces and a few glass beads to a 300 cc Kjeldahl flask. Add 25 cc conc. C.P. nitric acid and boil very gently for about 20 min. Cool briefly, add 17 cc conc. C.P. sulfuric acid, then boil more actively. Add further small quantities of nitric acid as needed so that an excess is always present. Finally remove the last traces of color in the digest with a few drops of perchloric acid. Cool, add 30 cc water and boil it off; repeat the boiling-off process. Cool the digest and dilute it accurately with water to 100 cc. It is suitable for the determination of both As and Bi by the methods specified. When known amounts of Milibis were digested in the presence of 1 g dry rat feces the recoveries were as follows: As, 96%; Bi, 97.5%.

For analysis of representative tissues of the animals, the livers, kidneys, and spleens were removed, the tissues of each animal of a pair being treated separately. Digests were made of 2 to 3 g liver, of both kidneys, and of the whole spleen. At the same time the gastrointestinal tracts were dissected out, and the entire contents were removed for analysis. The results of this experiment are presented in Table I.

‡ Pathological examinations were made by Dr. F. C. Goble.

§ Percentage composition: Ground yellow corn and ground whole wheat, each 28.5; whole milk powder, 23.0; linseed meal, 8.0; commercial casein, 4.1; alfalfa and brewer's yeast, each 3.0; cod liver oil, 0.9; calcium carbonate and sodium chloride, each 0.5.

7. McChesney, E. W., *Anal. Chem.*, 1949, v21, 880.

8. McChesney, E. W., *Ind. Eng. Chem., Anal. Ed.*, 1946, v18, 146.

‖ Data supplied through the courtesy of Mr. M. E. Auerbach.

TABLE I.

Absorption and Excretion of Arsenic and Bismuth in Rats Receiving Milibis in the Diet at About the Level of 100 mg/kg/day for 12 Days.

		Drug intake		Arsenic metabolism*					Bismuth metabolism*				
Pair	Interval, days	Total, mg†	mg/kg/day‡ avg	Intake§	Urinary output	Fecal output	Digestive tract¶	Total	Intake‖	Urinary output	Fecal output	Digestive tract¶	Total
1	1- 6	333	105	48.0	1.7	37.2	—	38.9	123.0	0.4	102.3	—	102.7
	7-13	340	117	48.8	2.2	46.4	—	48.6	125.4	0.4	132.5	—	132.9
	1-13	673	111	96.8	3.9	83.6	0.1	87.7**	248.4	0.8	234.8	0.3	235.9
2	1- 6	338	104	48.4	1.2	35.8	—	37.0	124.0	0.4	98.6	—	99.0
	7-13	403	134	57.7	1.6	55.6	—	57.2	148.1	0.5	160.0	—	160.5
	1-13	741	119	106.1	2.8	91.4	0.2	94.5**	272.1	0.9	258.6	0.5	260.0
3	1- 6	350	111	50.0	1.5	39.6	—	41.1	128.5	0.5	108.0	—	108.5
	7-13	357	123	51.2	1.7	47.0	—	48.7	131.5	0.5	132.0	—	132.5
	1-13	707	117	101.2	3.2	86.6	0.2	90.1**	260.0	1.0	240.0	0.5	241.5
4	1- 6	232	80	33.6	0.9	25.3	—	26.2	86.0	0.3	71.0	—	71.3
	7-13	315	117	45.2	1.5	42.5	—	44.0	116.0	0.7	116.0	—	116.7
	1-13	547	98.5	78.8	2.4	67.8	0.4	70.7**	202.0	1.0	187.0	0.7	188.7
5	1- 6	345	112	49.5	2.1	39.5	—	41.6	126.8	0.6	106.5	—	107.0
	7-13	359	122	51.4	1.9	50.4	—	52.3	132.0	0.6	143.0	—	143.6
	1-13	704	117	100.9	4.0	89.9	0.1	94.0**	258.8	1.2	249.5	0.5	251.2

* All figures given under these headings are in mg.
† 0.15% of net food consumption for the period.
‡ Drug intake in g, divided by 6 and by avg wt of the 2 rats during the period, in kg.
§ Drug intake × 0.1433.
‖ Drug intake × 0.368.
¶ Analysis of entire contents at autopsy on 13th day (stomach, intestines, and cecum).
** Includes 0.1 mg in liver, kidney, and spleen.

TABLE II.
Urinary Excretion of Arsenic* (as mg) by Human Subjects Taking Doses of 0.5 g Milibis 3 times daily for 10 Days.†

Day of exp.	Subjects				
	A.A.	D.B.	C.B.	E.F.	G.W.
1	0.5	1.1	2.9	1.6	0.5
4	2.6	1.3	1.9	2.6	0
8	2.2	2.5	3.2	3.1	0
10	3.4	1.4	1.4	2.3	0
11	1.3	0.2	0.4	1.2	0
12	0.9	0	0	1.4	0

* No bismuth was found at any time. The urine of all subjects was demonstrated to be arsenic and bismuth-free on a sample collected during the 24 hr before the first dose.

† The daily dose contained 215 mg As, 552 mg Bi.

Excretion of arsenic and bismuth by human subjects following the oral administration of Milibis. Five adult males weighing 62-84 kg took 2 tablets of 0.25 g each 3 times daily (total of 1.5 g) for 10 days. This is the recommended therapeutic course of Milibis, and amounts to about 20 mg/kg/day, or slightly less than 20% of the daily dose received by the rats. Twenty-four-hour urine samples were collected for Days 1, 4, 8, 10, 11, and 12 following the first dose. The urine samples were analyzed for As and Bi as described above. No Bi was detected in the urine at any time, which means that less than 0.2 mg was being excreted through the kidneys daily. The amounts of As excreted are presented in Table II; it will be noted that in 4 subjects the output was from 1 to 3 mg/day while the fifth subject excreted almost none. Two of the subjects were still excreting small amounts or arsenic 2 days after the last dose. Routine and microscopic analyses of the urine samples were conducted¶ with entirely negative results. The only untoward effect resulting from the medication was an occasional extra bowel movement.

Discussion: Arsenic metabolism. From 3 to 4% of the ingested As was excreted in the urine of the rats. Although urine samples were not collected from the human subjects for all of the entire experimental period, it is evident that they excreted a somewhat smaller proportion. In rats the fecal output accounted for almost all of the drug. A very small amount remained in the gastrointestinal tract 24 hours after the drug was removed from the diet. Analysis of the tissues revealed that the amounts of As present were so small as to be almost undetectable by the method used. For example, the whole liver of a single animal contained 0.04-0.08 mg, the spleen contained about 0.01 mg, and the 2 kidneys, 0.006-0.008 mg.

The recovery of arsenic as against the amount ingested, however, was not quantitative. Even after making allowance for the fact that only 96% of As added to feces as Milibis could be recovered by the analytical method used, it was still not possible to account for about 5% of the amount ingested. However, losses of greater magnitude have been observed in As balance studies conducted by others(9). In view of the known affinity of tissues for As following the administration of arsenicals(10), it was considered unlikely that significant amounts would be found in sites other than those mentioned above. Nevertheless, in order to establish this point more positively, the remainders of the carcasses of pair 3 were brought completely into solution in hot concentrated nitric acid, and small aliquots of these solutions were further digested as described for feces. It was found that an aliquot representing 1/400 of a carcass contained no detectable Bi (*i.e.* less than 0.005 mg), while an aliquot representing 1/40 of a carcass contained 0.007-0.008 mg As. If all the ingested As and Bi which was not accounted for had been contained in the carcasses, the amounts found in these aliquots (assuming equal distribution between the 2 animals) would have been 0.023 mg Bi and 0.137 mg As.

Other particular sites of localization which are worthy of some consideration are muscle and blood. We have previously(11) carried out experiments identical in every respect with

¶ These observations were made by Dr. D. A. Berberian.

9. Clements, R., Hill, J. E., Tree, H. G., Ewing, P. L., and Emerson, G. A., *Fed. Proc.*, 1949, v8, 282.

10. Raulston, B. O., and Magnuson, H. J., *Trans. Assn. Am. Physicians*, 1940, v55, 254.

11. McChesney, E. W., unpublished observations.

those reported here except that the medications were given daily by stomach tube, suspended in aqueous gum tragacanth. The rats were killed 24 hours after receiving the eleventh consecutive dose of either 100 or 200 mg/kg/day. There was no detectable As in the muscles of these animals, and only 0.3 mg in the whole blood volume of a pair. The amount of 0.3 mg applied very closely to all pairs of animals on the test, and is almost the least detectable by the method used. Also, by way of confirmation of the present work, the entire livers of 2 animals contained 0.04 - 0.11 mg As, the 2 spleens contained 0-0.46 mg, and the kidneys, none.

Bismuth metabolism. Practically no Bi was absorbed as a result of ingesting Milibis. The average urinary output of the rats was only 0.4% of the intake, and in human subjects the amount (if any) was too small to be measured. Fecal excretion accounted for almost the entire intake of rats; making due allowance for the amounts of bismuth as Milibis known to be recoverable from feces, about 2% of the intake was not accounted for in most of the pairs. In the previously conducted experiments(11) the kidneys from a pair of animals contained only a trace of bismuth; *i.e.*, 0.1-0.5 mg, while the livers, spleens, muscles and blood contained none. These are the tissues which are most likely to contain Bi(12), and its absence from the remainders of the carcasses has been demonstrated (see above).

The data on both As and Bi metabolism support the idea that bismuthoxy-mono-p-N-glycolylarsanilate remains largely unabsorbed in its passage through the gastrointestinal tract. It is nevertheless clearly necessary that some solution, however slight, should occur if there is to be any amebicidal action. It is also evident that solution does occur. In this connection there are two apparent possibilities: First, the small amount of As^{5+} which is absorbed may originate in the bismuthoxy-di-p-N-glycolylarsanilate which is evidently present in the complex, since the second arsanilate radical would be more loosely held. In this sense the bulk of the drug would act as a carrier for the small amount of the amebicidally active component. Second, the compound may undergo slight hydrolysis in the stomach, releasing Bi^{3+} (which then becomes insoluble Bi_2O_3 in the gut) and the soluble arsanilic acid, which acts against the microorganisms. As a result of hydrolytic action, for example, Milibis seems to have a slight solubility in 0.1 N HCl,† while in N HCl it is very soluble.

In any case, the amount of As absorbed following the oral administration of Milibis is much less than that observed following the administration of Carbarsone(13) or of the thioarsenites C.C. 937 and C.C. 1014(14), compounds which have also been reported to be effective for the treatment of amebiasis. The method by which Milibis has been administered to the rats in this work; namely, the almost constant introduction of small amounts, should result in maximal absorption. The relatively minor amounts of As and Bi which were absorbed, at a level 5 times the human therapeutic dose, are in harmony with the demonstrated low laboratory and clinical toxicity of the drug.

Summary. Milibis is almost completely non-toxic when administered orally to rats. A dose of 10 g/kg/day for 18 of 21 days inhibited growth only slightly, and at this dose level the mortality was 20%; with smaller doses there was no inhibition of growth, and no mortality attributable to the drug. Absorption was studied in human subjects and rats, during and following repeated oral administration. Both species excreted in the urine at most 2 to 4% of the ingested As. Human subjects excreted no detectable Bi in the urine, but the rats excreted about 1% of the amount ingested. In the rats tissue concentrations of both As and Bi were negligible, as was the As concentration in blood.

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13. Anderson, H. H., Hansen, E. L., Sah, P. P. T., and Cafiso, J. R., *J. Pharmacol. and Exp. Therap.*, 1947, v91, 112.

14. Anderson, H. H., Johnstone, H. G., Bostwick, W., Chevarria, A. P., and Packer, H., *J. A. M. A.*, 1949, v140, 1251.

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