terminations on these 2 diabetic groups were practically identical as far as mean, range and standard deviation of cholesterol fractions and lipid phosphorus were concerned; they are therefore considered as one unit.

At least 2 baseline total cholesterol, cholesterol esters and lipid phosphorus determinations were made on all diabetic patients before they were given inositol. They then began taking 3 g of inositol daily (two 0.5 g capsules after each meal); bloods were taken at intervals of 1, 2, 4, and 8 weeks thereafter.

Results. The range of normals is indicated by the enclosed area in Fig. 1. The mean, range and standard deviations are shown in Table I. No apparent variation was found with regard to age or sex and repeated determinations on the same subject showed little or no variation over a period of several months.

Before taking the inositol, the diabetic patients tended to show elevated serum total cholesterol, 18 (60%) being outside the range of normals as shown in Fig. 1, only 4 below the normal average, the rest high normal. Cholesterol esters were proportional to total cholesterol, the ratio of esters to total being relatively constant (68-73%). Lipid phosphorus tended to be high when the total cholesterol was high, but the correlation was not as consistent as with the cholesterol esters. No correlation between the level of cholesterol fractions or lipid phosphorus and the severity of diabetes or the accuracy of its control could be made.

In all of the diabetic patients fed the inositol a fall occurred in the serum cholesterol and lipid phosphorus. After 8 weeks (Fig. 1) all determinations were within the normal range. As shown in Table I, the total cholesterol tended to fall abruptly over the first 2 weeks, then to fall more slowly or become stationary. The higher initial total cholesterol showed the greater tendency to fall; thus, 17 diabetics with an initial total cholesterol above 300 mg% had an average drop of 69.2 mg% in 8 weeks, while the 13 diabetics below 300 mg% had an average drop of only 29.0 mg%. The proportion of esters to the total remained constant after inositol, and lipid phosphorus tended to fall in conjunction with fall in total cholesterol, contrary to Herrmann's findings. When the patients were taken off inositol, a gradual rise occurred in the determined serum lipids, but after 6 weeks none had returned to the baseline levels.

The only possible untoward effect of inositol observed occurred in one woman who complained of gastric distress; many of the patients reported a feeling of increased vigor and well-being. No consistent change in the diabetic status of any of the patients was noted.

Conclusions. Inositol is an effective agent in lowering serum cholesterol and lipid phosphorus in hypercholesteremic diabetics. Further study of its action and applications is warranted.

Received Sept. 14, 1949. P.S.E.B.M., 1949, **72**.

Metabolism of Some 9-Aminoacridine Derivatives. (17438)

EVAN W. MCCHESNEY AND JOHN P. MCAULIFF

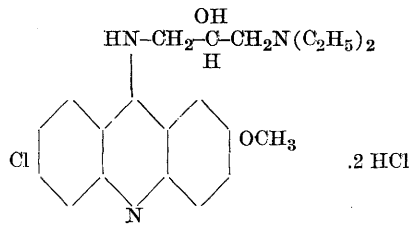
From the Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Many compounds in the 9-aminoacridine series have been synthesized for the purpose of studying their action against a variety of parasitic infections, and as disinfectants. The recent antimalarial survey[1] included a large number of these compounds. One of them was Acranil (SN-186), which was found to be only slightly active against avian malaria.[2] Previously[3-6] it had been used successfully for

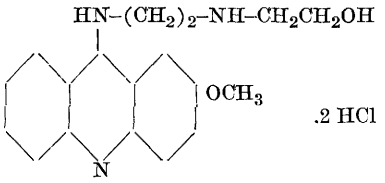
1 Wiselogle, F. Y., A Survey of Antimalarial Drugs, J. W. Edwards, Ann Arbor, 1946, pp. 1321-1377.

2 Gingrich, W. D., and Fillmore, R. S., *Am. J. Hyg.*, 1942, **36**, 276.

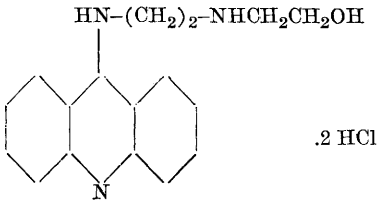
the treatment of human giardia infections and, more recently, against *Hymenolepis nana*.[7] Berberian has also found it quite effective for the treatment of human subjects infested with *Enterobius vermicularis*. Three closely related compounds which are superior to Acranil against oxyurids of mice,[8] and also as to acute toxicity for mice* are Win 2333, 2389, 2390. The structural formulae of these 4 compounds, to be discussed in this paper, are as follows:



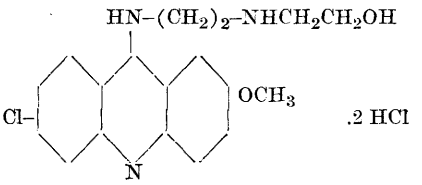
6-Chloro-2-methoxy-9-(2-hydroxy-3-diethylamino)-propylaminoacridine dihydrochloride
ACRANIL



9-(2-hydroxyethylaminoethylamino)-2-methoxyacridine dihydrochloride
WIN 2389



9-(2-hydroxyethylaminoethylamino)-acridine dihydrochloride
WIN 2333



6-Chloro-9-[3-(2-hydroxyethylamino)-propylamino]-2-methoxyacridine dihydrochloride
WIN 2390

Some information on the absorption, excretion, and tissue distribution of a typical 9-aminoacridine derivative is available as a result of the widespread interest in the use of Atabrine† for the therapy of human malaria.[9-12] It is known, for example, that the highest tissue concentrations for Atabrine, following oral administration to rats, are found in the liver, with lesser amounts in the spleen, lung, and kidney, in order of decreasing concentration.[9,10] In dogs, deposition in the tissues accounts for 10 to 30% of the oral dose.[10]

We have studied the 4 compounds listed above with respect to their absorption, excretion, and tissue-distribution, since these factors are important in determining their suitability for the treatment of susceptible parasitic infections.

Methods. The experimental subjects were male hooded rats from our colony, weighing 250-365 g each. They were kept in metabolism cages, one pair to each cage. In order to simulate as closely as possible the conditions used for the treatment of intestinal parasitic infections, they were allowed free access to our regular colony diet and water until the medication was given, but then only water was provided for the remainder of the experimental period. The drugs were given by

[3] deMuro, P., *Acta Med. Scand.*, 1939, **102**, 17.

[4] Grott, J. W., *Schweiz. Med. Wochschr.*, 1939, **69**, 683.

[5] Friederich, L. V., *Gastroenterol.*, 1940, **65**, 24.

[6] Berberian, D. A., *Am. J. Trop. Med.*, 1945, **25**, 441.

[7] Berberian, D. A., *Am. J. Trop. Med.*, 1946, **26**, 339.

[8] Berberian, D. A., and Dennis, E. W., manuscript in preparation.

* Laboratory data, courtesy of Dr. J. O. Hoppe.

† Registered trade-marked name of Winthrop-Stearns, Inc., brand of quinacrine.

[9] Barlow, O. W., Auerbach, M. E., and Rivenburgh, H., *J. Lab. Clin. Med.*, 1945, **30**, 20.

[10] Army Malaria Research Unit, *Ann Trop. Med. and Parasitol.*, 1946, **40**, 173, 181, 368, 372, 472, 482.

[11] Dearborn, E. H., Oldham, F. K., Geiling, E. M. K., and Kelsey, F. E., *J. Pharm. and Exp. Therap.*, 1943, **78**, 120.

[12] Annegers, J. H., Snapp, F. E., Paskind, L., Ivy, A. C., and Atkinson, A. J., *War Med.*, 1943, **4**, 176.

stomach tube in 5 cc of water per kg body weight. This was enough water to dissolve the drug except for some doses of Win 2389 and 2390, which remained partly in suspension.

Twenty-four hours after medication samples of blood were drawn into oxalate by heart puncture. The animals were then sacrificed; the carcasses were frozen and stored until they could be analyzed. Urine and feces were collected for the 24-hour experimental period, the samples thus representing the combined excretions of one pair of rats. The blood and tissue samples, however, were usually analyzed for each animal. The methods used for the extraction and analysis have been described by Brodie *et al.*,[13,14] with the exception that in this work ethylene dichloride was used as a solvent in place of petroleum ether. As a routine measure the extracts were washed with 10% NaOH to remove metabolic products of the drugs, but no colored material was removed in this way. Acranil, Win 2389, and Win 2390 fluoresce in the visible range; they were determined in the Coleman photofluorometer using the filter system B-2, PC-9A. Win 2333 fluoresces only in the ultraviolet range. However, its behavior proved to be too erratic to use this property for its determination, therefore a colorimetric method was used.‡ Since the sensitivity of the method was not as great as the fluorometric, it seemed inadvisable to attempt the determination of tissue concentrations of Win 2333 on any dose less than 200 mg/kg, and blood determinations were not made.

When the animals were dissected, the procedure was as follows: Aliquots of about 2 g of liver and lung were accurately weighed for extraction, also the whole spleen, and one whole kidney. If the stomach contents were sufficiently solid, a 2 g aliquot was taken, otherwise the entire contents were washed with water into the vessel to be used for the extraction. The intestines were washed out in a similar way, and to these washings were added the cecal contents. The analytical results are presented in Table I.

Discussion. It is evident that the number of experimental animals used was not sufficient to establish the tissue concentrations of each drug at each dose level with great precision. In about 2/3 of the paired analyses the agreement was very close; in the other third the variations were greater than one might wish (*i.e.*, 100% or more). In about half the aberrant figures the extent to which absorption had proceeded was clearly a factor (for example, Acranil, 500 mg/kg pair). In some other cases the extent of absorption alone could not account for the differences between paired animals (for example, Win 2389, 500 mg/kg pair). However, the purpose of the work was only to establish general trends, and with this in mind, the following generalizations can be made:

1. The tissue concentrations tended to increase with the dose level, as would be expected.

2. The urinary and fecal excretions did not show any consistent relationship to the dose level. Doubtless this is a reflection of the fact that considerable, but variable, amounts of the drugs remained in the gastrointestinal tract. However, it can be said that Acranil was excreted to the least extent in the urine, and Win 2389 to the greatest. Acranil was also excreted in the feces to the least, and Win 2389 to the greatest extent.

3. The blood concentrations of Acranil and Win 2390 increased with the dose level, and were of the same magnitude for equivalent doses. The blood concentrations of Win 2389 averaged as high, but were in a mixed order.

4. At any dose level chosen for comparison, Acranil accumulated in the tissues more than any of the other compounds, and Win 2333 accumulated the least; the affinity of liver for Acranil was particularly marked. In order of decreasing over-all tissue affinity, and of decreasing concentration in the individual tis-

[13] Brodie, B. B., Udenfriend, S., and Baer, J. E., *J. Biol. Chem.*, 1947, **168**, 299.

[14] Brodie, B. B., Udenfriend, S., Dill, W., and Downing, G., *J. Biol. Chem.*, 1947, **168**, 311.

‡ The extraction, washing, and transference into 0.1 N HCl were carried out in the specified way except that 10 cc of the HCl was used instead of 6 cc. The color of the HCl solution was then read in an Evelyn colorimeter using a 420 filter, comparing with standards containing 0.005-0.5 mg of Win 2333 in 10 cc of 0.1 N HCl.

TABLE I.

Absorption, Tissue Distribution, and Excretion of Some 9-aminoacridines 24 Hr after Oral Administration to Rats.
Amounts in tissues,† gastrointestinal tract, excreta, and blood.

Compound, dose		Blood conc.,	Stomach cont.,	Intestinal and cecal	Liver,	Kidney,	Spleen,	Lung,	% of dose in tissues	% of dose in g. i. con-
mg/kg	mg	mg/l	mg	cont., mg	mcg/g	mcg/g	mcg/g	mcg/g	and urine	tents and feces
			Acranil							
50	13.0	1.1	0.07	0.52	430	75	100	105	36.0	7.4
50	13.5	0.6	1.03	0.02	450	88	130	42	34.0	11.0
100	27.6	1.3	4.35	3.57	485	163	400	150	20.0	29.0
100	30.4	1.3	0.52	1.46	172	166	300	330	8.6	6.5
500	124.0	3.8*	24.3	10.0	2000	368	1040	535	2.8	15.0
500	139.0		86.0	9.9	880	378	565	310	8.4	69.0
			WIN 2333							
200	66	—	3.1	11.2	53	17	54	12	2.2	27
200	64	—	17.6	1.9	33	15	40	11	1.8	36
500	182	—	80.6	4.9	47	49	41	11	2.0	50
500	140	—	18.0	1.4	98	33	54	30	2.9	17
			WIN 2389							
50	13.8	1.6	0.02	4.60	23	7	2	2	3.3	74
50	14.0	1.4	0.07	4.62	18	10	16	4	3.3	74
100	29.7	0.8	1.72	6.55	17	30	43	14	6.4	39
100	27.6	0.7	0.01	1.53	17	32	86	32	7.0	17
500	135.0	3.2*	73.2	5.2	147	86	364	117	2.3	63
500	138.0		57.0	5.9	61	56	71	58	1.4	51
			WIN 2390							
50	14.2	0.2	0.01	2.20	47	47	45	14	4.2	24
50	14.6	0.2	0.001	3.64	25	55	71	8	2.7	33
50	14.0	0.3	0.09	4.20	72	37	65	16	6.5	37
50	15.0	0.4	3.10	1.70	238	67	125	17	18.0	38
100	30.2	0.8	5.36	6.42	143	145	90	26	7.3	52
100	29.5	1.0	7.16	5.80	123	100	390	35	5.6	53
100	27.4	0.4	0.01	1.75	147	38	37	16	6.9	22
100	27.4	0.4	0.00	6.24	148	42	37	20	3.7	38
200	52.0	0.5	20.1	10.30	227	139	143	163	7.2	63
200	52.8	1.0	18.1	10.30	568	174	148	100	10.0	58
500	124.0	3.8*	17.6	75.4	256	124	233	100	2.7	76
500	133.0		51.6	26.0	279	81	70	80	2.7	60

* These animals were so close to a state of collapse that it was not possible to obtain sufficient blood for individual analyses.
† Tissue concentrations are based on wet weight.

sues the several compounds may be rated about as follows:

a. Acranil: liver > spleen > kidney > lung

b. Win 2390; liver > kidney > spleen > lung

c. Win 2389; spleen >liver > kidney > lung

d. Win 2333; liver > spleen > kidney > lung

It is of interest to compare the metabolism of these drugs with that of Atabrine, although the data available for comparison[9] were obtained with a higher dose than any used in this work: 675 mg/kg. None of these 4 drugs was absorbed as fast as Atabrine. Only at the lowest dose levels were as small (absolute) amounts found in the gastrointestinal tract as following Atabrine, and even less than 50 mg/kg of Win 2390 would have been required to produce an equivalent result. The reported tissue-distribution of Atabrine resembles that of Win 2389 in item[4] above. However, the latter compound was still only about half absorbed when the animals were killed. Win 2390 differed from Atabrine chiefly in giving higher liver concentrations. Even at the lowest dose level Acranil produced much higher liver concentrations than 675 mg/kg of Atabrine, and the other tissues contained amounts of the same order as following Atabrine.

It was noted that these compounds undergo some chemical alterations, probably in the liver, followed by excretion through the bile. This was shown by the fact that the ethylene dichloride extracts of both the intestinal (plus cecal) contents and of the feces contained an intensely yellow non-fluorescent substance which remained in the organic solvent, even when the latter was extracted with N HCl. This indicates that the basic side chain had been lost, leaving a neutral compound, possibly an acridone. A similar metabolic process has been observed by Hawking and Frazer in their studies of Miracil D.[15]

Summary. The absorption, excretion, and tissue-distribution of 4 compounds of the 9-aminoacridine series have been studied in rats at oral dose levels ranging from 50 to 500 mg/kg. At any dose level Acranil concentrates in the tissues to the greatest extent, while the other compounds follow in this order: Win 2390, 2389, 2333. For any single compound, the tissue concentrations ordinarily increase with the dose, the largest amount being found in the liver. In order of increasing rate of absorption the compounds are Win 2390, Win 2389, Acranil, Win 2333 but none of these is as completely absorbed within 24 hours as has previously been reported for Atabrine.

[15] Hawking, F., and Ross, W. F., *Brit. J. Pharm. and Chemotherap.*, 1948, **3**, 167.

Received Sept. 16, 1949. P.S.E.B.M., 1949, **72**.

Regeneration of the Neural Portion of the Retina from Pigment Cells in Adult Urodele Eyes.* (17439)

L. S. STONE

From the Department of Anatomy, Yale University School of Medicine, and the Osborn Zoological Laboratory, Yale University.

In previous experiments on grafted adult salamander eyes, it has been shown by the author,[1-3] that degeneration of the neural elements of the retina is followed by regeneration. The retina can be regenerated with sub-

* Aided by grants from the James Hudson Brown Fund and the United States Public Health Service.

[1] Stone, L. S., and Zaur, I. S., *J. Exp. Zool.*, 1940, **85**, 243.

[2] Stone, L. S., and Farthing, T. E., *J. Exp. Zool.*, 1942, **91**, 265.

[3] Stone, L. S., and Ellison, F. S., *J. E. Zool.*, 1945, **100**, 217.