was suggested as a possible cause by the work of Bellows and Chinn.[5]

Ocular Findings. After decompression, there is a marked hyperemia of the iris. The light beam in the aqueous, although difficult to observe, is visible. With oblique illumination small diffuse gray opacities are observed in the superficial cortex. The most striking finding is the fact that the anterior superficial suture becomes more readily visible. By use of the higher magnification of the slit-lamp the changes are seen to begin with development of opacity of the anterior sutures from which fine fibrillar opacities extend. In optical section it is seen that the entire opacity is thin and lies in the superficial layers of the cortex. Gradually the opacity extends towards periphery. Similar changes, although less marked, occur in the posterior cortex. In severe cases the fiber structure is lost and a shiny white total cataract is formed.

The cataract lasts from about ¾ to 1 hour and then gradually regresses, exactly reversing the course of its formation, so that the last opacities to disappear are those about the sutures.

Although the author has examined the lenses of human volunteers subjected to decompression to the equivalent of 18,000 feet, it must be pointed out that opacities due to anoxia have been observed only in animals.

[5] Bellows, J. G., and Chinn, H., *Arch. Ophthal.*, 1941, **24**, 979; 1941, **25**, 796.

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Distribution and Excretion of Atabrine in Experimental Animals.

JOHN V. SCUDI AND MARGARET T. HAMLIN.

From the Research Laboratories of Merck & Co., Inc., and the Merck Institute for Therapeutic Research, Rahway, N.J.

Although a number of workers have reported upon the absorption, distribution, and excretion of atabrine in experimental animals, current interest in this antimalarial drug suggested an extension of these studies.

Methods. The method used for the extraction of atabrine is a modification of Hecht's procedure[1]: One gram samples of tissue or feces were warmed in 10 cc of 30% sodium hydroxide on a water bath for 5 to 15 minutes to emulsify solids. The mixture was cooled and extracted 3 times with 5 cc portions of isoamyl alcohol, centrifuging after each extraction. Ten cc samples of urine were rendered strongly alkaline and extracted directly with isoamyl alcohol without prior heating. Oxalated blood samples were precipitated with 9 volumes of acetone and the acetone filtrates were evaporated to dryness and the residues were taken up in 10 cc of isoamyl alcohol.

Four volumes of benzene were added to the isoamyl alcohol extracts and the basic acridines were extracted with two 5-cc portions of 0.5 N hydrochloric acid. Sodium hydroxide was added to the aqueous extract and the strongly alkaline solution was extracted with an equal volume of isoamyl alcohol. After centrifuging, the alcoholic extract was dried briefly with anhydrous sodium sulfate.

For determinations in blood, the alcoholic extract was read directly in the Pfalz and Bauer fluorophotometer. The instrument was equipped with the Corning filter, red ultra No. 584, across the incident beam, and between the fluorescent solution and the photocell a yellow filter (Corning noviol No. 38) and a blue-green filter (Corning No. 553) were used to permit maximum transmission of the yellow-green fluorescence. Concentrations of atabrine between 0.1 and 1.0 γ per cc can be measured within the limits of instrumental error. For all other determinations, one drop of 6 N hydrochloric acid was added

[1] Hecht, G., *Arch. Exp. Path. Pharmak.*, 1936, **183**, 87.

TABLE I.
Blood Levels of Atabrine Given in mg %.

Rats					Dogs			
Time after dosing, hr	Group 1	Group 2	Days	Group 3	Time after dosing hr	No. 221	No. 97	No. 111
Control	.0	.0	Control	.0	¼	.15	—	—
½	.1	1.3	1	.0	½	.11	.16	.26
1	.1	.5	2	.1	1	.23	.18	.34
2	.3	.8	3	.1	2	.12	.22	.32
3	.6	.2	4	.15	5	.04	.10	.34
4	.4	.4	5*	.1	24	—	.13	.50
6	.4	.3	6	.2				
24	.3	—	7	.15				
			8	.15				

2 rats were used at each interval in Groups 1 and 2, and 4 rats were used for each analysis in Group 3. Average figures are given.
* Daily administration of the drug stopped.

to the alcoholic extract and the color was measured in the Evelyn colorimeter equipped with a No. 420 filter. Concentrations of atabrine between 1 and 10 γ per cc can be measured within instrumental error.

Urine and most tissues gave recoveries of 92% (± 3, maximum deviation 6%). Results were therefore multiplied by the factor 1.09. Liver and spleen gave slightly high values when the atabrine concentration fell below 0.1 mg per gram of tissue. This error was eliminated by using extracts of normal tissue for the instrument blank. Recoveries from blood were 76.5% (±5, maximum deviation 10%). Final values were multiplied by 1.3. Recoveries from feces were 80% (±6, maximum deviation 10%). Final values were multiplied by 1.25. Weise[2] encountered similar difficulties in obtaining complete extraction of atabrine from feces.

Atabrine is detoxicated differently in different animal species, and as many as 4 or 5 isoamyl alcohol-soluble excretion products may occur in the urine of experimental animals.[3] All known detoxication products are extracted by the procedures described above. Such products may occur in blood and tissues, and since we have not attempted to separate these products in the present study, the concentration of atabrine recorded in subsequent tabulations must be regarded as "total" concentrations, including those acridines and related products which occur in the animal organism as the result of atabrine administration.

Animal Experiments. To determine the blood levels of atabrine in the rat following single or multiple doses of the drug, 3 groups of rats were used: 14 rats were given a single, 90 mg per kg dose of the drug; another 14 rats were given a single 225 mg per kg dose; and 32 rats were given 5 daily doses (45 mg per kg). The rats, maintained on a stock diet and water, were deprived of food for 12 hours prior to the administration of the drug by stomach tube. Animals were sacrificed and blood samples were analyzed at the time intervals shown in Table I. The results indicate that large single doses of the drug produce very low blood concentrations, but that these levels remain quite constant over a 24-hour period. The 5 daily doses produced a concentration of only 0.1 to 0.2 mg %, and this level was reached only after 24 hours. This level was maintained rather steadily for at least 3 days after the discontinuation of the drug.

To determine the blood levels of atabrine in the dog following the oral and intravenous administration of the drug, 3 animals were used as follows: Dog No. 221, a male, weighing 5.9 kg, was given 12.5 mg of the drug per kg body weight intravenously over a 2-minute period in the form of a 3% aqueous solution. Dogs Nos. 97 and 111 were followed for a 24-hour period in the course of a study in

[2] Weise, W., *Arch. Schiffs. Tropen. Hyg.*, 1937, **41**, 715.

[3] Scudi, J. V., and Jelinek, V. C., *J. Biol. Chem.*, in press.

TABLE II.
Elimination of Atabrine from Liver and Spleen of the Rat.

Days after last dose of Atabrine	Group 1		Group 2		Group 3	
	Liver	Spleen	Liver	Spleen	Liver	Spleen
1	1.2	.2	*C 6.2	2.9	2.0	2.5
	0.6	.15	*N 2.9			
	0.9	.2	C 4.4	4.2	C 4.5	2.5
			N 1.5		N 1.5	
3	1.5	.3				
	0.8	.4	—	—	—	—
	1.5	.5				
6	—	—	2.2	1.2	—	—
			3.4	1.2		
			2.2	0.8		
8	0.3	.2				
	0.2	.3				
	0.3	.2				
13-15	0.5	.2	C 1.1	0.9	C 2.0	1.8
	0.7	.1	N 1.2		N 1.1	
	0.2	.1				
	0.1	.2	1.5	1.3	C 2.9	2.2
					N 3.7	

*C = Areas showing congestion with prominent lobular architecture.
*N = Necrotic areas. These were dissected out and analyzed separately.
Data given in mg per g of wet tissue.

which the animals received 50 and 100 mg per kg body weight of the drug by stomach tube per day, respectively. The results are also shown in Table I. In agreement with the findings of Gentzkow,[4] the blood levels were never high. Nor did these levels reflect the dose administered, but the blood concentrations were notably constant.

To study the influence of single and multiple doses of the drug upon the tissue concentrations, and the influence of time upon the elimination of the drug from certain tissues, the following experiments were performed: Group 1—Each of 15 rats were given a single dose of the drug (225 mg per kg body weight) by stomach tube. At intervals of 1, 3, 8, and 15 days after the administration of the drug groups of 3 rats were sacrificed and the livers and spleens were analyzed. Group 2—Rats receiving 12 daily doses of the drug (45 mg per kg) were sacrificed 1, 6, and 13 days after the last dose. Group 3—Rats receiving 12 larger daily doses of the drug (90 mg per kg) were sacrificed 1 and 13 days after the last dose of the drug. In Group 1 of this series the results, shown in Table II, indicate that the concentration of the drug in the liver was not greatly changed 1 to 3 days after the administration of the drug. The concentration dropped appreciably after 8 days but the livers still retained large amounts of the drug 13 days after the last dose. The concentration in the spleen was not greatly lowered even after 15 days. At autopsy the livers and spleens of these animals of Group 1 appeared normal on gross examination, but the animals which received 12 daily doses of the drug (Groups 2 and 3) showed badly mottled livers.

The urinary excretion of atabrine in the rat was studied with the above groups of animals. The animals were housed in metabolism cages in groups of 3 with access to food and water. Urine samples were collected over the time intervals shown in Table III. Each figure represents the analysis of pooled urine from a group of 3 rats.

The data (Table III) indicate that a single dose as large as 225 mg per kg results in a very small urinary output (about 0.25 mg per

[4] Gentzkow, C. J., *Am. J. Trop. Med.*, 1938, **18**, 149.

TABLE III.
Urinary Excretion of Atabrine in the Rat.

Group 1			Group 2	Group 3
Days after dosing	Atabrine output	Time of urine collection	Atabrine output	Atabrine output
1	.45	First 3 days pooled	0.6	1.2
	.59		0.6	1.2
	.75		0.7	0.9
	.62			
	.99	Second 3 " "	1.2	1.3
			1.2	1.5
3	.98		0.8	1.2
	.66			
	.41	Third 3 " "	0.9	1.0
	.84		1.1	1.4
			1.0	1.5
8	.43	Drug administration stopped		
	.47			
	.42			
13	.24	3 days after last dose. 24 hr urine	0.5	0.5
			0.4	
		10 " " " " " " " "	0.3	0.5

Group 1: Animals received a single dose of 225 mg per kg body weight.
Groups 2 and 3: Animals received 12 daily doses of 45 and 90 mg per kg respectively. The data are given in mg of atabrine per day.

TABLE IV.
Tissue, Urinary, and Fecal Concentrations of Atabrine.

Tissue analyses (mg per g wet tissue)				Urine and fecal analyses in the dog (mg per 24-hour sample)					
Organ	Monkey	Dog No. 111	Dog No. 104	Days	No. 94 (Feces)	No. 248 (Urine)	No. 247 (Urine)	No. 246 (Urine)	No. 104 (Urine)
Liver	1.4	4.9	0.09	1	†	10	29.5	27.0	25
									(30)
Kidney	0.23	1.2*	0.02*	2			12	21.5	16
Small intestinal wall	0.10	1.1		3	18	16	8.5	3.1	16
Brain	0.10	0.07		4	16†				
Thigh muscle	0.30			5	†	6			
Lung	0.30			6	†				
Red Marrow Femur			0.12	7		2.9	2.6	2.5	2.7
								(18.5)	
Spleen			0.10	8	22		2.4		4.4
							(2.3)		
				9	30				
				10	31		1.9		
				16			0.3	0.7	0.5

* Equal concentrations were found in the cortex and the medulla.
† 200 mg of atabrine per kg was administered to this dog on these days.
Figures in parentheses are the fecal atabrine output.

rat per day), and that this output does not drop significantly within 3 days after discontinuing the drug. While the urinary output drops after 8 days, the decrement is not large and the drug continues to be excreted for at least 13 days after administration of the drug. The daily urinary output following this single dose is not larger than the output following 3 daily doses totaling only 135 mg per kg. This aberrant elimination of the drug is also evident in the comparison of Groups 2 and 3 where it can be seen that 90 mg of the drug per kg per day does not produce a significantly greater output than 45 mg per kg per day. Thus, the urinary output of the drug does not parallel the dose administered, but tends to reach comparatively low levels. Although these levels diminish with time, as previously

reported,[5,6] the drug continues to be excreted by way of the urinary system long after the administration of the drug has been stopped.

Other animals were used to study the distribution of the drug in the animal organism: a monkey which had been given 7 massive doses of atabrine (300 mg per kg body weight) by stomach tube over a 3-week period, Dog No. 111, which received 100 mg of the drug by stomach tube per day, and Dog No. 104, which received an intravenous infusion of the drug (100 mg per kg), were sacrificed and the tissues were analyzed. The data are shown in Table IV. To investigate the localization of the drug in bone marrow, each of 3 rabbits were given a single dose (225 mg per kg body weight) and 6 days later red marrow taken from the femur contained 0.55, 0.71, and 0.79 mg of atabrine per gram of wet tissue. Relatively large concentrations of the drug are found in organs of the reticulo-endothelial system.

The excretion of the drug by the dog was studied as follows: Dog No. 94, given four 200 mg doses of the drug over a period of 1 week, was used to study the fecal output of the drug. Since the dog vomits after the oral administration of large experimental doses of the drug, collection of the fecal samples was arranged on alternate days to avoid contamination with vomitus. Dogs numbered 248, 247, 246, and 104 received a single intravenous infusion of the drug equivalent to 50, 75, 100, and 100 mg per kg body weight, respectively. The urinary and fecal output of this single dose of the drug was then studied over an extended period. All samples taken were analyzed at the time intervals shown in Table IV.

The data shown in Table IV indicate that following oral or intravenous administration of the drug, the urinary excretion of the drug is slow, and urinary concentrations are never high. As previously shown,[5] the drug is excreted by way of the bowel. Relatively large amounts of the drug are excreted in the bowel and this excretion continues for more than a week after the intravenous administration of single large doses of the drug.

Summary. The distribution and excretion of atabrine is similar in the rat and dog. The highest concentrations of the drug were found in the liver. These concentrations were not diminished 3 days after the administration of the drug and although the concentrations were diminished after 8 days, appreciable concentrations were found in the livers as late as 2 weeks after the administration of single or multiple doses of the drug. Intermediate concentrations were found in the spleen and kidney. These concentrations were not significantly diminished 2 weeks after administration of the drug was ceased. Intermediate concentrations of the drug were found in bone marrow and the wall of the small intestine. All other tissues studied showed low concentrations of the drug.

The elimination of the drug is slow. The urinary concentration of the drug never reaches high values, nor does the total output per day reflect the size of the dose administered. Large amounts of the drug are excreted by way of the bowel, and this mode of excretion continues long after the administration of the drug has been discontinued.

Following oral or intravenous administration of atabrine the concentration of the drug in the blood stream remains very low, never exceeding 1 mg % even after the administration of large multiple doses, but these low concentrations are maintained for days after administration of the drug is stopped. The blood concentration bears no apparent relationship to the dose administered. These results together with findings on the distribution and excretion of the drug, suggest that the drug is deposited in the tissues and is slowly liberated, thereby maintaining a rather constant blood concentration of the drug.

[5] Tropp, C., and Weise, W., *Arch. Exp. Path. Pharmak.*, 1933, **170**, 339.

[6] Chopra, R. N., and Roy, A. C., *Ind. J. Med. Res.*, 1938, **25**, 455.