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Effect of Atabrine on the Urinary Porphyrin Output in the Rat.

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In view of the hepatoxic action of atabrine,[1] and the prolonged retention of this drug in the liver, bone marrow, kidneys, and spleen,[2] it seemed desirable to investigate the effect of the administration of atabrine upon porphyrin excretion.[3] In the present study, the rat was used as the experimental animal.

Experimental. We are indebted to Dr. Konrad Dobriner for a crystalline sample of coproporphyrin I-tetramethyl ester. This material in 5% hydrochloric acid solution, when examined in the Beckman spectrophotometer, showed a strong band at 4000 Å. Since the extinction at this wave-length is 25 times as great as the values obtained in the visible (Fig. 1), the Coleman spectrophotometer was calibrated at 4000 Å against solutions of this material.* At concentrations of 0.1 to 1.0 γ per cc these solutions obeyed Beer's Law and the concentrations could be measured within an error of ±3%. These solutions appear colorless to the naked eye, but show an intense red fluorescence when exposed to ultra-violet light.

The method of Rimington and Hemmings[5] was followed in detail. The final use of 0.5% hydrochloric acid as recommended by these authors for the separation of protoporphyrin

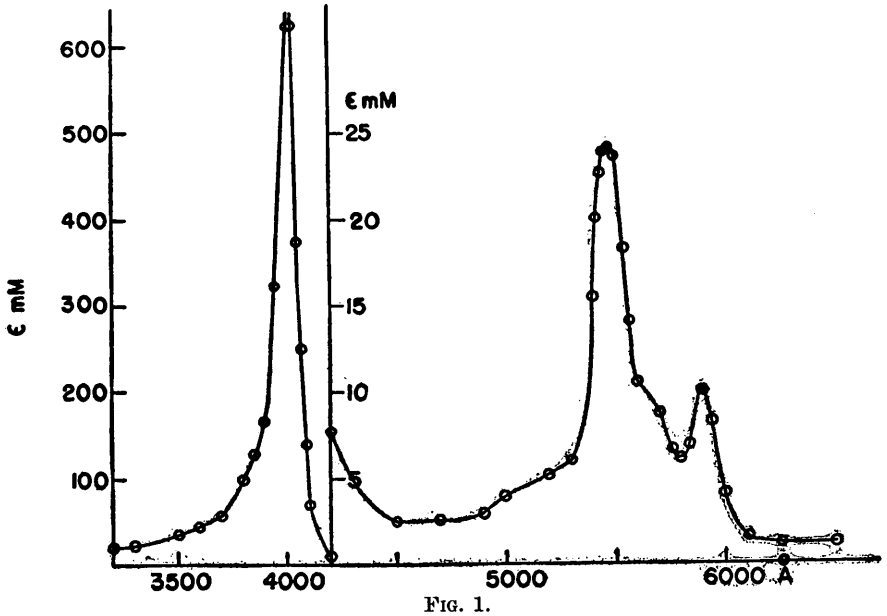

FIG. 1.
The absorption of coproporphyrin I-tetramethyl ester in 5% hydrochloric acid.

1 Clark, B. B., Cuminole, B., and Martin, S. J., *J. Pharmacol.*, 1939, **65**, 166.

2 Scudi, J. V., and Hamlin, M., preceding paper; Hecht, L., *Arch. Exp. Path. Pharmak.*, 1936, **183**, 87.

3 See Dobriner, K., and Rhoads, C. P., *Physiol. Rev.*, 1940, **20**, 416, for an excellent review of the subject.

* This intense absorption between 3900 and 4100 Å appears to be quite general for the porphyrins.[4]

4 Fischer, H., and Orth, H., Die Chemie des Pyrrols II, p. 587 *Akad. verlagsgesellsch*, Leipzig, 1934.

5 Rimington, C., and Hemmings, A. W., *Biochem. J.*, 1939, **33**, 960.

was highly important in the present work since it removed an interfering, green fluorescent material. This material appeared in the urine in increasing amounts as the animals continued on test. The method of Rimington and Hemmings gave excellent results when carried out with pure solutions of the crystalline coproporphyrin and with samples of urine to which 10 and 20 μg quantities of the coproporphyrin were added.

TABLE I.
Urinary Excretion of Atabrine.
Mg per rat per day.

	Dose 5% L.D. 50			Dose 10% L.D. 50		
May '42	1	2	3	4	5	6
7*	.2	.20	.25	.40	.41	.31
10	.41	.33	.29	.43	.50	.40
13	.29	.38	.35	.32	.46	.49
19	.55	.42	—	.65	—	—
26	.20	.30	—	.38	—	—

* Administration of drug begun 5/4/42; ceased 5/15/42. Pooled samples were analyzed, but these values were divided and are expressed in mg per rat per day.

Eighteen white rats, weighing 145 to 190 g each, were placed in metabolism cages in groups of 3, and 72-hour samples of pooled urine were collected. The funnel-shaped lower portions of the cages were coated with paraffin to prevent the urine from coming in contact with the metal. The animals were removed from their cages for 1 hour in the morning and 1 hour in the evening. (A single 2-hour period was used on Sundays). During these periods the animals were allowed free access to water and the stock laboratory diet. During the feeding periods the cages were cleaned. A small amount of urine was unavoidably lost during these feeding periods. Particular care was exercised to prevent fecal contamination of the urine samples.

The animals, maintained under these test conditions for a control period of 9 days, made weight gains of 10 to 42 g indicating that the food intake was sufficient to permit growth. Each of 9 rats was then given 45 mg of atabrine per kilo body weight per day by stomach tube, and the remaining animals were given 90 mg per kilo body weight per day. The experiments were continued for 12 days at which time the animals began to die. During the test period there was a marked loss in body weight.

The pooled samples of urine were analyzed colorimetrically for their atabrine content.[2] The data shown in Table I are given in mg per rat per day. Because of deaths it was not possible to maintain the original groups of rats, but by using the surviving animals individually it was shown, in agreement with previous work,[6] that the drug was excreted for long periods of time after its administration was stopped.

All animals were autopsied as soon as they were found dead. On gross examination all livers appeared severely damaged. Chemical analyses of these livers showed concentrations of atabrine averaging about 3.5 mg per gram of wet tissue with a maximum concentration of 6.5 mg per gram. The spleens showed concentrations averaging about 3.0 mg per gram, with a maximum concentration of 4.2 mg per gram. Three of the 7 surviving animals were sacrificed one week after the administration of the drug was stopped, and the remaining four animals were sacrificed an additional 5 days later. Analysis of tissues from these animals showed about 2.0 mg of atabrine per gram of wet liver, and concentrations in the spleen were about 1.0 mg per gram. Thus, these tissues retain large amounts of the drug for long periods of time.

TABLE II.
Urinary Coproporphyrin Output.

	5% L.D. 50			10% L.D. 50		
Date	1	2	3	4	5	6
4/8/42	17	32	38	12	27	16
5/1	13	28	—	12	21	15
4*	21	27	27	16	31	16
7	32	36	39	24	48	26
10	28	30	34	11	39	14
13	8	18	16	4	15	6

*Drug begun 5/4/42.

The coproporphyrin output by these animals is given in Table II. The values are given in micrograms per group of 3 rats per 3-day collection period. Although liver damage was produced by the massive doses of atabrine

[6] Tropp, C., and Weise, W., *Arch. Exp. Path. u. Pharm.*, 1933, **170**, 339.

used, and high concentrations of the drug were found in the liver and spleen, the data indicate that the urinary coproporphyrin output is not increased by the administration of atabrine. It may be noted that, in comparable experiments with the sulfonamides, Rimington and Hemmings[5] were able to demonstrate a marked porphyrinuria.

Conclusion. The absorption of acidic solutions of the coproporphyrins at 4000 Å is 25 times that observed at 5480 Å, suggesting that measurements should be made at the former wavelength.

Daily oral administration of toxic doses of atabrine does not produce an increased urinary coproporphyrin output in the rat.

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Effects of Exposure to Oxygen at High Barometric Pressure on Higher Functions of the C.N.S.*

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Observations have been previously made by one of us (J. W. B.) that intermittent exposure of rats to high oxygen pressure not only causes motor dysfunction but also what appears to be a disruption of some of the higher functional activities of the C.N.S. and that these alterations persist for considerable periods after the animal's return to the normal atmosphere. This suggested that less severe exposures to increased O_2 pressure might appreciably affect certain mental processes without causing any gross manifestations in motor function. Experiments were therefore carried out to determine what effect, if any, successive short exposures to O_2 at increased pressure made over a period of several days might have on learning and memory in rats.

The pressure chamber employed was essentially that described earlier (Bean[1]). A continuous circulation of O_2 and removal of CO_2 was maintained by means of a power blower and a canister of soda lime. The O_2 pressure used was 65 pounds (gauge); the temperature was held at 25°C. The experimental animals selected were young adult rats; throughout their exposures to high O_2 pressure they were continuously under the observation of the operator stationed outside the chamber. Convulsive seizures were avoided as far as possible by shortness of the exposures (about 15 minutes) and decompression was carried out so as to insure freedom from the formation of oxygen emboli. Learning and memory tests were made with the Lashley Maze III (Lashley[2]). In the preliminary trials it was found that the O_2 exposures so affected some of the rats that they frequently failed to run the maze for several hours after their removal from the pressure chamber. For this reason an interval of one day was provided between the removal of the animals from the chamber and their subsequent test in the maze.

Learning. The facility shown in learning the maze was determined by two measures, *viz.*, the number of trials, and the number of errors, made before reaching the learning criterion. No significant difference was found in either respect between those animals which had been exposed to increased O_2 pressure and in the control animals which had been kept at normal atmospheric conditions.

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1 Bean, J. W., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 832.

2 Lashley, Karl Spencer, *Brain Mechanisms and Intelligence*, Chicago, University of Chicago Press, 1929.