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An Experimental Study of the Cerebral Coproporphyrin in Rabbits.*

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Klüver's discovery of the presence of small amounts of coproporphyrin in the central nervous system of warm-blooded animals[1,2] raised the question whether there might be a relationship between this porphyrin and the increased urinary coproporphyrin in conditions such as lead and arsenic poisoning, which are characterized both by injury of the nervous system and by marked coproporphyrinuria. It is well known that in heavy metal and chemical poisoning the excess urinary coproporphyrin is the type III isomer.[3] Preliminary studies by Klüver,[4] and in this laboratory, with the differential precipitation, or "fluorescence quenching" technique[5,6] indicate that the coproporphyrin of the nervous system is likewise the type III isomer. Hence it seemed desirable to determine the coproporphyrin concentration in the brains of rabbits suffering from acute lead poisoning.

Hitherto, quantitative data on the coproporphyrin of the central nervous system have not been reported, nor has a quantitative method of determination been described. The method devised for use in the present study combines certain features of procedures previously described for urinary coproporphyrin[6] and erythrocyte protoporphyrin.[7] The procedure was briefly as follows: The entire rabbit brain was ground in a mortar, washed repeatedly with physiological saline solution to remove most of the blood, and then mixed with 10 ml of glacial acetic acid and 100 ml of acetone. The mixture was allowed to stand overnight with repeated shaking. It was filtered through cheesecloth and the residue was ground with additional amounts of glacial acetic acid and acetone, and finally with ethyl acetate.† The residue was pressed as dry as possible. The combined filtrate was mixed with an equal volume of distilled water and extracted 4 times with ethyl acetate. The latter was extracted 4 times with 15 ml portions of 10%HCl. The acid extract was made negative to Congo red paper by addition of saturated aqueous sodium acetate solution, and the solution was extracted 4 times with ethyl acetate after a few ml of glacial acetic acid had been added. The combined ethyl acetate extracts were washed with water and

TABLE I.
Cerebral Coproporphyrin in Normal Rabbits.

No.	Wt of brain in g	γ of coproporphyrin (Total content)	γ/g brain
1	8.1	.3	.037
2	9.1	.225	.025
3	8.5	.57	.067
4	8.3	.30	.036
5	8.0	.27	.034
6	7.6	.345	.045
7	9.0	.225	.025
8	8.7	.225	.026
9	8.0	.30	.038
10	7.7	.18	.023
		Avg	.0356

† More recently it has been found advantageous to grind and extract the brain with glacial acetic acid and ethyl acetate (1:10), on a sintered glass filter; several such extractions obviate the necessity of preliminary extraction with acetone and the period of standing in contact with it. (C. J. W.)

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1 Klüver, H., *J. Psychol.*, 1944, **17**, 209.

2 Klüver, H., *Science*, 1944, **99**, 482.

3 Watson, C. J., and Larson, E. A., *Physiol. Rev.*, 1947, **27**, 478.

4 Klüver, H., personal communication.

5 Schwartz, S., Hawkinson, V. E., and Watson, C. J., *Science*, 1946, **103**, 338.

6 Schwartz, S., Hawkinson, V. E., Cohen, S., and Watson, C. J., *J. Biol. Chem.*, 1947, **168**, 133.

7 Grinstein, M., and Watson, C. J., *J. Biol. Chem.*, 1943, **147**, 675.

TABLE II.
Urinary and Cerebral Coproporphyrin in Lead-Poisoned Rabbits.

No.	UCP* in γ/day at time of killing	Wt of brain in g	γ of coproporphyrin (total content)	γ/g brain
1	12.6	7.5	.3	.04
2	16.0	9.1	.48	.052
3	20.0	8.0	.195	.024
4	3.7	8.5	.30	.035
5	11.5	9.3	.195	.021
6	19.8	9.2	.345	.037
7	43 0	8.3	.15	.018
8	58.0	8.6	.195	.023
9	49.0	8.0	.345	.043
10	44.0	9.1	.345	.038
11	15.0	7.7	.30	.040
12	26.0	8.0	.405	.05
			Avg	.035

* UCP = total urinary coproporphyrin.

extracted 4 times with 2-3 cc portions of 1% HCl. This was washed with $CHCl_3$, separated and filtered. The determination of total coproporphyrin was then made on the 1% HCl extract in the Klett fluorophotometer, as previously described.[6] The concentration and individual content was determined for the brains of 10 normal rabbits, data for which are given in Table I.

Twelve rabbits were poisoned with lead acetate, the first 6 receiving 50 mg per kilo, the second six 100 mg per kilo, in a single intraperitoneal injection. The animals were killed 7-10 days later. The analytical data are given in Table II. The urinary coproporphyrin was determined by the method of Schwartz and associates[6] which gives an upper limit of 5 γ per day for normal rabbits.

No increase in the brain coproporphyrin occurred following the acute lead poisoning. Thus it is evident that the present study does not provide evidence of a relationship between the coproporphyrin content of the brain and that of the urine which was much increased. The possibility cannot be excluded that an accelerated formation and release of coproporphyrin by the brain could have maintained the actual concentration at a constant level. Other studies are in progress in which various substances affecting the nervous system are being used, both in acute and chronic experiment.

Summary and Conclusions. 1. A method is described for the quantitative determination of coproporphyrin in the brain. 2. The concentration and total content of the coproporphyrin of normal rabbit brains was compared with that of brains from rabbits, in which acute lead poisoning was induced. No variation was observed.