9387 P

Excretion of Porphyrin by Dogs.

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Certain qualitative and quantitative studies concerning the excretion of porphyrins have been published previously.[1-5] The existence of a constant ratio between the production of coproporphyrin I and of the Type III porphyrin compounds as reflected by hematopoietic activity has been suggested by those studies. A series of experiments on animals has been made which supports further this suggestion.

The methods of separation and quantitative measurement of the excreted porphyrins are those which have been described previously.[1,2,4] For the studies, fistulæ between the gall-bladder and renal pelvis were made in 4 dogs by the method of Harvey.[6] The animals were fed a constant diet and were kept in metabolism cages. All the urine and feces passed was collected, using toluol or chloroform as a preservative. The blood values were normal according to the figures of Scarborough.[7] Experience has shown that determinations of porphyrin excretion made during periods of less than 10 successive days are valueless.

In all the animals coproporphyrin as well as a small amount of protoporphyrin was excreted in both urine and feces. The coproporphyrin was identified in all instances as coproporphyrin I by melting point determinations. From these facts the dualism of the porphyrins as shown by the constant excretion of a different type of porphyrin from that which is required for the construction of the respiratory pigments is established for dogs.

Quantitative determinations of the total urinary and fecal coproporphyrin excretions were made on 2 normal dogs with bile-renal

1 Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

2 Dobriner, K., *J. Biol. Chem.*, in press.

3 Dobriner, K., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 175.

4 Dobriner, K., Strain, W. H., and Localio, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 752.

5 Dobriner, K., Strain, W. H., and Localio, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 755.

6 Kapsinow, R., Engle, L. P., and Harvey, S. C., *Sur. Gynec. and Obstet.*, 1924, **39**, 62.

7 Scarborough, R. A., *Yale J. Biol. and Med.*, 1930, **3** and **4**.

fistulæ. Although the daily total excretion varied greatly, the daily averages of 10-day periods fell within the same range. The first animal was observed from May, 1936, to August, 1936, a total of 110 consecutive days, and showed daily averages coproporphyrin I excretion of 445, 332, 304, 316, 330, 291 micrograms during the first 6 consecutive 10-day periods. The high first value is attributable to the fact that during the first 10-day period the animal was fed dog bile containing coproporphyrin.* During the remainder of the experiment the animal received ox bile which did not contain coproporphyrin. Simultaneous bile-pigment studies showed an average daily total pigment excretion of 92, 83, 86, 97, 128, 118 mg. The second animal showed comparable daily average total coproporphyrin I excretion values for 10-day periods 195, 187, 181, 189 micrograms. Since the animals were of different sizes and the total circulating hemoglobin in the first dog was greater than that of the second animal, hemoglobin construction and destruction in the first animal, although normal, was actually greater. Since the coproporphyrin I excretion seems to be proportional to the total construction of hemoglobin under normal conditions, it is not surprising that the first dog excreted a larger amount of coproporphyrin I. This is similar to the previously reported findings in normal adults and children.

These experiments on animals maintaining a constant hemoglobin level, and consequently a constant respiratory-pigment destruction and construction, show constant daily average total coproporphyrin I excretion. These findings further demonstrate the direct proportion between Type I porphyrin excretion and Type III porphyrin construction in normal individuals.

In order to prove further that coproporphyrin I excretion is not related to blood destruction, but is parallel to normal hematopoietic activity, the following experiment was undertaken. At the beginning of the seventh period the first animal was given a total of 1450 mg. of acetylphenylhydrazine over the period of 18 days. The hemoglobin gradually decreased from 120% at the onset of treatment to 55% on the twentieth day, then gradually rose to 112% during the succeeding 35 days during which time treatment was omitted. Shortly after the administration of phenylhydrazine was begun the bile-pigment excretion increased from a daily average of 118 to 263 and 548 mg. during the two 10-day periods of treatment. The bile-pigment excretion then slowly decreased, averaging 307,

* About 70% of intravenously administered coproporphyrin I is excreted in 24 hours.

136, and 66 mg. daily respectively during the remaining three 10-day periods without treatment.

Coproporphyrin I excretion remained constant during the first 12 days of phenylhydrazine therapy and then increased suddenly on the thirteenth day to twice its normal value. During the first 10 days of therapy the average was 306 micrograms per diem. There was an increase to 655 and 608 micrograms per day during the eighth and ninth 10-day periods respectively and a decrease to 348 and 269 during the tenth and eleventh 10-day periods. The porphyrin excreted during the period of increased blood construction was characterized as coproporphyrin I. A rapid increase in hemoglobin production was observed simultaneously with the increased porphyrin excretion, making it necessary to increase the phenylhydrazine dosage in order to maintain the anemia level.

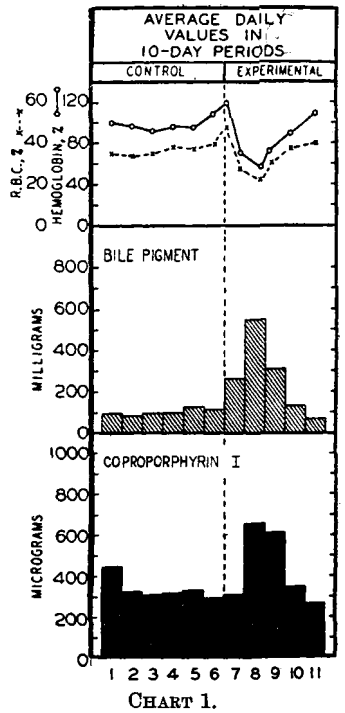

CHART 1.

In chart I the experimental results are summarized and indicate that there is no relation between coproporphyrin-I excretion and blood destruction; on the other hand they demonstrate that there is a very close relationship between coproporphyrin I excretion and hematopoietic activity (Type III porphyrin compound construction).

Conclusions—1. Coproporphyrin I is excreted at a constant rate in normal dogs, proving the existence of the dualism of the porphyrins in that animal. 2. Experimental evidence is given that coproporphyrin I excretion seems to be directly proportional to and a measure of hematopoietic activity. 3. Coproporphyrin I is not a product of or related to blood destruction.

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9388

Inactivation of Bacteriophage by Ethyl Alcohol.

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General agreement has prevailed among investigators that phage freed from a major portion of extraneous materials is more susceptible to inactivation by physical and chemical agents than is crude broth phage. One exception to this statement has been noted. Bronfenbrenner[1] reported that phage free from nitrogen as determined by Nessler's reagent or ninhydrin was not weakened in lytic activity when mixed with 10 volumes of alcohol and left for 8 days at 22 to 25°C., although broth phage was inactivated under similar conditions in a very short time. Since Kligler and Olitzki[2] have reported that the presence of protein delays the inactivation of phage by alcohol and Callow[3] found that both purified and broth phages were inactivated by alcohol after 3 days in the refrigerator, corroboration of Bronfenbrenner's findings appears to be worth recording.

Bacteriophage for a strain of *B. coli* was purified from lysogenic cultures by a method recently described.[4] Purified phages used in these experiments contained between 0.3 and 0.5 mg. of nitrogen per 100 cc. as determined by the microkjeldahl method of Koch and McMeekin.[5] Homologous crude broth phage was adjusted to approximately the same titer and pH (6.3). Each kind of phage was

1 Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 372.

2 Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 393.

3 Callow, B. R., *J. Infect. Dis.*, 1927, **41**, 124.

4 Colwell, C. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 100.

5 Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.