Enzymatic Porphyrin Synthesis in Harderian Glands and Other Organs and Tissues of Mice.* (22065)

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The fact that the Harderian gland of mice of certain strains(1-3), rats(4), and hamsters (5) contain high concentrations of porphyrin is well established. Some investigators assumed that the red fluorescent gland was a secretory or porphyrin-synthesizing structure (6,1,4). On the basis of injection experiments(3,5), one of us stated that the Harderian gland appears to be an excretory structure and that its red fluorescence thus reflects the level of porphyrin metabolism of other tissues. The object of the experiments reported in this paper was to compare the porphyrin-synthesizing potentialities of the Harderian gland with those of other tissues and organs. It was hoped that this would definitely determine whether the Harderian gland is a secretory or an excretory structure.

The testing of the porphyrin-synthesizing potentialities of Harderian gland was made possible by the recent discoveries that certain cellular enzymes have the ability to synthesize porphobilinogen, uro, copro, and proto porphyrins from δ aminolevulinic acid(7,8). In these experiments, measured amounts of aqueous extracts of tissue homogenates were tested to determine their content of porphyrin synthesizing enzymes. It was found that the Harderian gland of mice contained a high concentration of porphyrin-producing enzyme as compared with other tissues, and it thus appears to be a porphyrin-synthesizing organ.

Materials and methods. The tissues to be tested were taken from 12 male mice of the Strong A strain. The mice were raised and housed in glass cages containing wooden shavings. They were fed Purina Laboratory Chow and given water without restrictions. The mice were anesthetized with ether and the tissues dissected out in a cold room (6°C). The Harderian glands were removed as rapidly as possible with care being taken to exclude extraneous tissues. One hundred milligrams of Harderian gland tissue was weighed out and placed in a mortar with a small amount of coarse grinding sand. To this was added 2 cc of 50:50 buffer-saline solution. The mixture was homogenized, rinsed from the mortar with 3 cc of the buffer-saline solution, and centrifuged at 2500 r.p.m. for 15 minutes. The supernatant material was used as the enzyme solution. The same procedure was followed to prepare enzyme solutions of brain, liver, and the femur with its attached muscles. To each of the 24 Klett tubes (6 for each tissue to be tested) 1.4 cc of 0.01 M δ aminolevulinic acid buffer-saline solution was added to act as the substrate. The buffer-saline solution consisted of equal volumes of isotonic sodium chloride solution and buffer solution - pH 7.2. The amounts of enzyme solution from each tissue to be tested were pipetted into the 6 tubes as follows: Tube No. 1-none (control); Tube No. 2 - 0.1 cc; Tube No. 3 - 0.2 cc; Tube No. 4 - 0.4 cc; Tube No. 5 - 0.8 cc and Tube No. 6 - 1.6 cc. Buffered-saline was added to each tube to bring the volume to 3 cc. The tubes were then stoppered with cellophane-covered corks and incubated at 37°C for 24 hours. At the end of this period, 6.5 cc of concentrated HCl was added to each tube to stop the enzymatic reaction and to clear the solution of any precipitate. This produced a 25% HCl solution. The tubes were then allowed to stand overnight in a cold room (6°C). A solution of hematoporphyrin hydrochloride in 25% HCl was prepared as a standard to be used in a Klett-Summerson filter photometer to determine the amounts of porphyrin formed. These experiments were repeated 4 times.

Results. The variation in the amount of porphyrin produced by different tissue enzyme preparations is shown in Table I. From this table, it may be seen that the amount

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TABLE I. Porphyrin Production by Aqueous Extracts of Harderian Gland and Other Mouse Tissue Homogenates. 12 mice in each series.

Tissues	μ of porphyrin/10 cc of reaction mixture/24 hr* Enzyme concentration .1 cc	.2 cc	.4 cc	.8 cc	1.6 cc
Harderian gland	7.1 ± .6	12.8 ± .4	32.3 ± .6	49.5 ± .9	100.7 ± 1.5
Liver	5.5 ± .3	9.5 ± .9	28.5 ± 1.6	34.60 ± .6	73.4 ± 1.8
Muscle and bone	1.8 ± .01	3.4 ± .2	6.7 ± .3	8.7 ± .2	16.1 ± .4
Brain	.8 ± .01	2.5 ± .2	4.4 ± .3	5.2 ± .4	9.7 ± .4

* Mean ± S.E.

of porphyrin produced in the tube containing the extract of the Harderian gland homogenate was much greater than in any other tissue tested. This was first noted when the tubes with the various enzyme concentrations were examined in ultraviolet light.

Other tissues tested (liver, muscle-bone, and brain) also showed a definite ability to produce porphyrin. The amount of porphyrin produced by the enzyme preparation from these tissues was not as high as that found for the Harderian gland. The porphyrin-producing potentialities of liver tissue was less than that of Harderian gland but 5 and 7 times as great as muscle-bone and brain respectively.

Discussion. The fact that the Harderian gland homogenate enzyme preparation produces a greater amount of porphyrin than the other tissues examined indicates that the Harderian gland is a porphyrin-secreting structure; at least the enzymes for manufacture of porphyrin from simple compounds are abundant in it. Porphyrinogenic enzymes are even more concentrated in the Harderian gland than they are in the liver or kidney. Gibson, Neuberger, and Scott(9) had reported that the liver and kidney of rabbits and the erythrocytes of birds contained considerable quantities of δ aminoleuvulinic acid dehydrogenase. Since the liver is one of the primary organs involved in porphyrin and heme degradation, the presence of large amounts of porphyrin-synthesizing enzymes in this organ seems paradoxical.

The much lower quantities of porphyrin-producing enzyme in the muscle-bone marrow homogenates and in the brain may be related to the low percentage of cellular material involved in the homogenate. Also, our results may have been influenced to a slight degree by the presence of variable amounts of blood in the tissues, as no attempt was made to wash the blood out before the tissues were homogenized. However, this could not explain the great variation which we observed. Analysis of these results showed, as might be expected, that the differences observed are highly significant ($P<0.01$).

Summary and conclusions. 1. Buffered saline extracts of equal weights of mouse Harderian gland, liver, muscle-bone, and brain homogenates were prepared. 2. Various amounts of these extracts were tested for their capacity to produce porphyrin by adding 1.4 cc of a 0.01 M solution of δ aminolevulinic acid and incubating the mixture in a stoppered Klett tube at 37°C for 24 hours. 3. The Harderian gland displayed the greatest porphyrin-producing enzyme activity, whereas the liver was less than that of Harderian gland but 5 and 7 times as great as muscle-bone and brain respectively. 4. These results led to the conclusion that the Harderian gland is a porphyrin-secreting structure and probably not an excretory type of gland.

1. Derrien, E., and Turchini, J., *Compt. rend Soc. de biol.*, 1924, v91, 637.

2. Figge, Frank H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A., *Cancer Res.*, 1942, v2, 335.

3. Figge, Frank H. J., *ibid.*, 1944, v4, 465.

4. Thomas, J., *Bull. Soc. Chim. biol.*, 1938, v20, 1058.

5. Figge, Frank H. J., *A.A.A.S. Research Conference on Cancer*, 1945, 117-128 (Forest Ray Moulton, editor).

6. Grafflin, A. L., *Anat. Rec.*, 1941, v79, 25.

7. Dresel, E. J. B., and Falk, J. E., *Nature*, 1953, v172, 1185.

8. Granick, S., and Vander Schrieck, H. G., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 270.

9. Gibson, K. D., Neuberger, A., and Scott, J. J., *Biochem. J.*, 1954, v58, XLI.

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