13. Koletsky, Simon, *Arch. Path.*, 1954, v58, 592.
14. Raaschou, F., and Trautner, K., *Scand. J. Clin. Lab. Invest.*, 1953, v5, 223.
15. Bradley, S. E., *Am. J. Med.*, 1948, v4, 398.
16. Spatt, S. D., and Rosenblatt, P., *Ann. Int. Med.*, 1949, v31, 475.

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Porphyrin Synthesis by Mouse Harderian Gland Extracts: Sex, Age, and Strain Variation.* (23501)

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It has been observed that the Harderian glands of very young mice, 2 to 4 weeks of age, were not red fluorescent(1). It had also been shown that the red fluorescence of Harderian glands of mice of different strains varied in intensity. In some respects the Harderian glands resemble sebaceous glands in human subjects. Both of these glandular structures elaborate free porphyrins in the secretory product at some stage of development. In the adult human subject, the sebaceous glands of the skin of the face and forehead are seen as tiny red dots when illuminated with near ultra-violet light because of the red fluorescence of the sebum(2,3). However, no red fluorescent sebum was observed during the examination of the facial skin of more than 200 immature children. Thus, red fluorescent sebaceous secretions appear in the human subject at about the time of puberty, persist throughout the period of maturity, and usually decrease in older people. While the porphyrin content and red fluorescence of the Harderian glands of mice follow a similar pattern, variations in red fluorescence may occur as a result of dietary and other factors. It has been shown that the Harderian glands and the livers of mice contained an abundance of porphyrin-producing enzyme(4). Muscle-bone and brain tissues in mice contained some porphyrin-producing enzyme, but were less than 20% as effective in producing porphyrin(4).

In these experiments, an attempt was made to ascertain whether there was a variation in the porphyrin-producing enzyme content of the Harderian glands of mice of different ages and in different strains of mice of both sexes. It was found that the porphyrin-producing enzyme activity did vary with age and strain. The observations on the enzyme activity were in agreement with the previous observations on the fluorescence of Harderian glands(2,3).

Materials and methods. The Harderian glands used in this series of experiments were taken from 74 male and 78 female mice of the C_3H, C_{57}, A, and LCSa strains, varying in age from 2 to 52 weeks. The animals were killed with ether and the Harderian glands dissected out in a cold room (6°C). After removal of any excess blood, 100 mg of the tissue was weighed and placed in a mortar with a small amount of coarse grinding sand and 2 cc of 50:50 buffer-saline solution. After the tissue was homogenized, the residue was rinsed from the mortar with 3 cc of the buffer-saline solution. This was centrifuged at 2500 r.p.m. for 15 minutes and the supernatant solution was used as the enzyme extract. To each of 148 tubes (37 for each age series of tissues to be tested), 1.4 cc of 0.01 M δ-aminolevulinic acid hydrochloride buffer-saline solution was added to act as the substrate. Various amounts (0-0.8 cc) of enzyme extract, from each Harderian gland to be tested, were then placed in their respective Klett tubes. An amount of buffer-saline was added to each tube to bring the volume to 3 cc. The Klett tubes were then closed with cellophane covered corks. The tubes were incubated at 37°C for 24 hours. Concentrated HCl (6.5 cc) was then added to

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TABLE I. Age, Strain, and Sex Variation in Porphyrin Producing Enzymes of Harderian Glands of Mice.

Strain	No. mice	Age, wk	Avg μg of porphyrin produced/10 cc of substrate solution by amounts of enzyme extract indicated ♀ .2	♀ .4	♀ .8	♂ .2	♂ .4	♂ .8
C_3H	8	2	.1	5.5	6.8	.7	5.7	6.1
	8	3	1.7	7.2	8.9	2.0	6.7	10.0
	6	5	5.2	14.0	18.0	5.7	15.0	18.7
	5	8	11.7	30.5	51.5	13.7	35.0	55.7
	5	16	15.0	33.3	55.4	15.1	33.9	55.1
	5	52	14.4	33.0	52.2	16.5	35.5	54.0
Total	37							
C_{57}	8	2	—	4.0	7.0	.4	3.1	5.0
	8	3	.7	5.7	8.5	.9	5.5	7.2
	6	5	4.6	12.0	16.8	5.1	14.2	18.1
	5	8	10.7	27.6	49.8	12.8	28.9	52.4
	5	16	13.3	30.1	53.1	13.9	33.7	55.0
	5	52	12.8	32.4	51.5	12.2	30.5	52.8
Total	37							
A	8	2	—	3.0	4.6	.2	3.5	4.2
	8	3	.7	5.0	7.2	.9	5.9	8.1
	6	5	4.0	13.5	15.9	5.4	12.8	14.4
	6	8	10.2	28.0	48.5	11.5	31.1	49.0
	6	16	11.3	29.4	48.1	14.4	31.8	49.0
	5	52	11.1	30.0	50.0	12.8	33.1	48.3
Total	39							
LCSa	8	2	—	2.6	4.4	.2	3.1	4.6
	8	3	.3	4.2	5.2	.4	5.1	6.7
	7	5	3.9	10.7	13.3	3.3	9.8	15.0
	6	8	9.4	25.9	44.6	10.1	27.4	45.9
	5	16	8.3	27.4	46.8	9.0	28.7	45.0
	5	52	9.0	28.1	45.9	10.9	31.1	47.9
Total	39							

each Klett tube to give a final concentration of 25% HCl, which stopped the enzyme reaction and cleared the solution of any protein or precipitate. 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 amount of porphyrin formed by each tissue.

Results. The variation in amount of porphyrin produced by different Harderian gland enzyme preparations is shown in Table I. The amount of porphyrin produced by the extracts of Harderian glands of mice in the 4 strains tested varied consistently with the strain and also with the age of mice. The Harderian gland extracts of mice of the C_3H strain produced the greatest amount of porphyrin, while the extracts of Harderian glands of mice of the other strains (C_{57}, A, and LCSa in this order) produced less porphyrin.

There was a great variation in the porphyrin-producing enzyme activity in the different stages of development. The Harderian glands of mice of 2 weeks of age had the lowest enzyme activity. The maximum enzyme activity was observed in Harderian glands of mice approximately 8 weeks of age. After 8 weeks, the Harderian glands showed an uniformly high enzyme content, but showed little or no increase in porphyrin-producing enzyme activity in older animals. The small variation in the amounts of porphyrin produced by the extracts of Harderian glands of mice of different sexes was not significant.

Discussion. One of the most striking observations in these experiments was the fact that enzymes for producing porphyrin are practically absent in the Harderian glands of

very young animals. As the animals become sex mature (2 to 5 weeks) the porphyrin-producing enzymes appear and increase in concentration in the Harderian gland. From this time on, there is a relatively rapid increase in porphyrin-producing enzyme activity, which reaches a practical maximum at about the age of 8 weeks. This is uniform for all of the mice of the various strains studied and is the same in both males and females. The porphyrin-producing enzyme activity then remains relatively constant until the animals are at least a year old. It is possible that if animals older than one year had been tested, that a decline in the porphyrin-producing enzyme would have been observed after the active reproductive period. While no definite correlation can be made between the sex hormones and sex activity and the fluctuations in these, the possibility exists that the porphyrin metabolism in the Harderian gland (and sebaceous glands in human subjects) may be in some way linked with sexual development and sex hormone patterns.

Summary and conclusions. 1. The porphyrin-producing enzyme activity of the Harderian glands of mice of different strains, ages, and sex was determined. 2. The Harderian gland of the C_3H strain displayed the greatest porphyrin-producing enzyme activity, while the C_{57}, A, and LCSa strains showed lower activity in the order listed. 3. The age of the animals was the most important variable. From the age of 2 weeks until the age of 8 weeks, there appeared to be a steady increase in the activity of porphyrin-producing enzyme. After this age, porphyrin-producing enzyme activity remained uniformly high in all animals up to one year of age. 4. The small differences in amounts of porphyrin produced by Harderian glands of the mice of 2 sexes was not great enough to conclude that there is a sex difference with respect to porphyrin-producing capacity in the Harderian glands of mice of the strains tested.

1. Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A., *Cancer Research,* 1942, v2, 335.
2. Figge, F. H. J., *Bull. Sch. Med. Univ. of Md.,* 1942, v26, 165.
3. Figge, F. H. J., *A.A.A.S. Research Conf. on Cancer,* 1944, p117-128.
4. Davidheiser, R. H., and Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 461.

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Synthesis and Demonstration of Antiserotonin Activity of 1-Benzyl-2-methyl-5-hydroxytryptamine (BAS-phenol) (23502)

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The recent demonstration(1,2) that a suitably constructed antimetabolite of serotonin may be useful in clinical treatment of essential hypertension together with the discovery, through the use of other antimetabolites of this hormone, that serotonin may play a role in the maintenance of normal mental processes (3,4,5) have focused attention on the potentialities of the antiserotonins. We have therefore continued to examine new members of this series in the hope of finding more active and otherwise more useful compounds. This report will describe the synthesis of a new analog of serotonin, *viz.* 1-benzyl-2-methyl-5-hydroxytryptamine, or 1-benzyl-2-methyl-serotonin. A demonstration will also be made that it is a very powerful antimetabolite of the hormone, and one which shows certain desirable pharmacological properties. The preceding antiserotonin which was said to be effective in hypertension was 1-benzyl-2-methyl-5-methoxytryptamine, called the benzyl analog of serotonin, or BAS. The new compound is the phenol of this methyl ether, or BAS-phenol.

The following considerations prompted synthesis and study of BAS-phenol. One prob-

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