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.

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Received June 28, 1957. P.S.E.B.M., 1957, v96.

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-

* With the technical assistance of E. Van Winkle.

lem in the clinical use of BAS is that large doses bring about a feeling of drowsiness. It was thought possible that this was caused by penetration of the drug into the central nervous system. Even though it has been shown (in laboratory animals)(6) that BAS penetrates with difficulty into the brain, it was postulated that this property of the drug (difficulty in entering the brain) might be improved further by replacement of the methoxy-group with an hydroxyl. There is much empirical evidence to indicate that while methyl ethers act on the brain, the corresponding phenols do so less readily when given peripherally. In an effort then to avoid the drowsiness induced by BAS, BAS-phenol was studied.

The BAS-phenol was synthesized from BAS by cleavage of the methyl ether with pyridine hydrochloride. This method of cleavage of phenolic ethers was the only one of the several tried which was satisfactory. Thus the more common methods, which use aluminum chloride, HBr, or KOH, proved unsuccessful.

Methods. Synthesis of 1-benzyl-2-methyl-5-hydroxytryptamine (BAS-phenol) from 1-benzyl-2-methyl-5-methoxytryptamine (BAS). 1-Benzyl-2-methyl-5-methoxytryptamine hydrochloride† (7) (6.0 g) was dissolved in a solution of pyridine (30 ml) in *excess* ethanolic hydrogen chloride (150 ml of 3M). The ethanol was removed under reduced pressure and the resultant viscous syrup was warmed at 75° and 12 mm pressure for 20 minutes to complete the solvent removal. The flask containing the residue was then provided with an air condenser and heated at 200 ± 5° for 30 minutes. The yield decreased with longer heating. When cooled, the melt was dissolved in water (400 ml) and filtered. 6N Sodium hydroxide was added until the pH reached 9 (indicator paper) when the product, which initially separated as an oil, crystallized. Seeding was useful in bringing this about. Excess alkali caused the product to redissolve. The material was filtered, washed with water, and dried *in vacuo*. The yield of base was 4.4 g, 86%, m.p. 199-201°. Recrystallization from aqueous pyridine gave a constant m.p. of 201-203°

Anal. calc'd for $C_{18}H_{20}N_2O$:	C, 77.11; H, 7.19; N, 9.99
Found:	C, 77.01; H, 7.19; N, 9.86

The hydrochloride of BAS-phenol was obtained by suspension of the above base in boiling absolute alcohol (5 ml per g) and addition of ethanolic hydrogen chloride to effect solution. When cooled, the hydrochloride crystallized in an over-all yield of 76%, m.p. 244-245°.

Anal. calc'd for $C_{18}H_{21}N_2OCl$:	C, 68.24; H, 6.68; N, 8.84
Found:	C, 68.50; H, 6.90; N, 8.70

Testing for antiserotonin activity. The ability to prevent the pressor action of serotonin in dogs was used for testing for antiserotonin activity. Details of these methods have been given previously(8,9). The animals were calibrated as to their pressor responses to serotonin and graded doses of BAS-phenol, dissolved in Ringer's solution, were injected intravenously each followed by a serotonin challenge. The smallest dose was thus determined which would protect against the pressor response to serotonin‡ administered 15 minutes after the analog. The various precautions described earlier with respect to times of injection and methods of measurement were strictly heeded.

Antiserotonin potency by the oral route was determined as follows. Dogs were calibrated for their pressor responses to intravenous serotonin as described previously(8,9). They were then fed BAS-phenol for 4 days. The drug was given once daily, mixed with the food. On the fourth day of feeding, just after the final dose of drug, the animals were anesthetized again with Nembutal, and again challenged with serotonin intravenously.

Results. Antiserotonin activity of BAS-phenol as measured by intravenous injection into dogs. By the intravenous route, BAS-phenol was very effective in antagonizing the pressor action of serotonin. In fact, it was

† We wish to thank Merck and Co. for gifts of this compound.

‡ Serotonin was generously provided by Dr. K. E. Hamlin, Abbott Laboratories.

TABLE I. Rises in Arterial Blood Pressure of Dog 32 Given Serotonin* Intravenously after Injection of BAS-phenol.

Amt of analog (mg/kg)	Interval between analog and serotonin (min.)	Rise in pressure (mm of Hg)
0	—	52
.035	15	52
.120	15	28
.120	90	48
.350	15	6

* Challenging dose of serotonin was 0.12 mg of 5-hydroxytryptamine creatinine sulfate/kg body wt. BAS-phenol was 1-benzyl-2-methyl-5-hydroxytryptamine hydrochloride.

the most active compound encountered thus far among all the serotonin analogs examined in this laboratory. Data from a typical experiment are shown in Table I, where it can be seen that protection against 0.12 mg of serotonin per kg was afforded by 0.35 mg of BAS-phenol per kg. Similar results were found in 4 other dogs.

Comparison of BAS-phenol with BAS in this intravenous test showed that the phenol was considerably more active. Thus, 3.0 mg of BAS per kg of body weight was required by the intravenous route (avg of 3 dogs) whereas 0.3 mg of BAS-phenol was needed for similar protection (avg of 5 dogs). Furthermore, the phenol exerted its maximal effect within 15 minutes (results from 4 dogs). For these reasons (high potency and rapid action) BAS-phenol recommended itself over previous compounds where an intravenously administered antiserotonin was desired.

Protection of dogs by the oral route against the pressor action of serotonin. The data of Table II show that doses of serotonin which had elicited good rises in arterial pressure in the unprotected animals, failed to do so after the ingestion of BAS-phenol. In the 6 experiments summarized there, this phenol at about 1 mg per kg per day protected all of the dogs, but at one-tenth of this dose there was no protection. By the oral route, therefore, BAS-phenol was approximately equal in potency to BAS. Previous trials(9) done under identical conditions (some of them on the same dogs as used for the BAS-phenol) had shown that BAS gave protection at 1 mg per kg per day by the oral route. Clearly then BAS-phenol was approximately 10 times more active than BAS by the intravenous route, but approximately equal to it in potency by the oral route. These comparisons of course apply only to the pressor test with serotonin and may not necessarily be valid for other test systems.

Serotonin-like action of BAS-phenol. When anesthetized dogs were injected intravenously with solutions of BAS-phenol, a sharp rise in arterial pressure was always observed (6 of 6 dogs). This was in contrast to the failure to find such rises with BAS when tested under identical conditions(9). The rise in arterial pressure was rapid and short-lived and in these respects resembled the response to serotonin. The pressures always returned to the original values within one to 2 minutes. Quantitative comparisons (in these 6 dogs) of graded doses of serotonin with graded doses of BAS-phenol showed that serotonin-creatinine sulfate was about 10 times more active than BAS-phenol (as the hydrochloride). However, one dog was found in which BAS-phenol was 3 times as active as serotonin.

The rise in pressure elicited by BAS-phenol was probably serotonin-like because it could be prevented by prior treatment of the animal with BAS. BAS is known to protect against serotonin but to be unable to do so against adrenaline or even against tryptamine(10). The BAS-protected dog is thus a very specific test object for serotonin-activity. A dog which, unprotected, had given a rise in arterial pressure of 26 mm of Hg following intravenous injection of BAS-phenol (0.6 mg per kg) was fed BAS (3 mg per kg per day) for 3 days.

TABLE II. Rises in Arterial Pressure of Various Dogs Given Serotonin Intravenously before and after Ingestion of BAS-phenol.

Oral dose of analog (mg/kg/day)	Serotonin response*	
	Before analog (mm of Hg)	After analog (mm of Hg)
3.2	30	6
1.4	36	7
1.1	52	8
1.2	23	8
.14	36	41
.11	52	40

* Challenging dose of serotonin was 0.06-0.12 mg of 5-hydroxytryptamine creatinine sulfate/kg body wt.

When challenged again with BAS-phenol (same dose) the rise in pressure was insignificant (8 mm of Hg). Tryptamine (1.1 mg/kg), however, elicited a full response (80 mm of Hg). This experiment was repeated in another dog with similar results.

Specificity. In the intravenous test with dogs as described above BAS-phenol was a remarkably specific antagonist of serotonin. It did not antagonize the action of adrenaline, noradrenaline, or even of tryptamine. Data illustrating these points are being published elsewhere(10).

Toxicity of BAS-phenol. Acute and chronic toxicities for adult mice (10 for each dose level) given BAS-phenol intraperitoneally were determined by the methods previously described for other members of this series(9, 11). All animals were killed by a single injection of 80 mg/kg and all survived a single injection of 40 mg/kg. A daily dose of 40 mg/kg for one week killed 2 of 10. Twenty mg/kg per day for one month was well tolerated. No animals died and none lost weight or showed changes which could be detected by visual inspection of the organs on autopsy.

Summary. A method was described for synthesis of 1-benzyl-2-methyl-5-hydroxytryptamine involving cleavage of the methyl ether of 1-benzyl - 2-methyl - 5-methoxytryptamine (BAS) by heating with pyridine hydrochloride. The phenol so produced exhibited powerful antiserotonin action either orally or intravenously in dogs. In the intravenous test it was the most potent antiserotonin thus far encountered. It also had considerable serotonin-like activity.

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Received July 15, 1957. P.S.E.B.M., 1957, v96.

Effect of a Brain Extract on Turnover Rate of Serum Cholesterol.*† (23503)

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At high levels of serum cholesterol a definite reduction will usually be achieved in human subjects with the feeding of an alcoholic extract of defatted brain(1). This closely simulates the effect of oral beta-sitosterol(2). However, when low normal levels of serum cholesterol already exist, a significant reduction of the total serum cholesterol may not be achieved with similar dosage. Thus the question arises whether any effect on cholesterol metabolism occurs in such cases, and further whether any effect exists in the absence of dietary cholesterol which had been used in our earlier chicken experiments(3). The following experiments were performed in an effort to elucidate the effect of this material upon the endogenous cholesterol metabolism in the experimental animal.

Methods. Chick experiment. An 8-week-old cockerel, on a diet of 100 g per day of starter mash, received an intravenous injec-

* This work was supported by grants from Amer. Heart Assn., Eli Lilly Foundation and the U.S.P.H.S.

† This work was presented in part at 28th Annual Meeting of Amer. Heart Assn. Oct. 1955.

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