hypoglycemic activity of carbutamide must be explained on some other basis than inhibition of glucose-6-phosphatase.

Vaughan(16) has reported a similar lack of effect of 1-butyl-3-p-tolylsulfonyl urea on the glucose-6-phosphatase activity of a rat liver homogenate. However, Hawkins, *et al.*(17) reported that oral administration of carbutamide to rats for a three-week period decreased the glucose-6-phosphatase activity of a liver homogenate prepared from these rats. No information was given regarding an immediate effect of carbutamide on the glucose-6-phosphatase activity of liver. Since the effect of carbutamide on blood sugar is observed within 2 hours, the lowered level of glucose-6-phosphatase reported by Hawkins may have no direct relationship to the hypoglycemic activity of the drug.

Summary. The concentrations of carbutamide in the liver and plasma of rats receiving a wide range of doses of the drug have been determined. When carbutamide was added to a rat liver microsome preparation in these concentrations no inhibition of glucose-6-phosphatase activity was observed.

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Benzyldimethylbufotenin, a Powerful Antimetabolite of Serotonin. (22712)

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Although many antimetabolites of serotonin, capable of antagonizing specifically the actions of this hormone, have been discovered (1,2,3,4) only a few have proven to be active in whole animals. Most of them act only in isolated tissues, or exhibit only low potency in intact mammals(2,4). Even among those capable of affecting the whole animal many are only effective when administered parenterally, and fail by the oral route. Yet it is necessary to develop orally active compounds, as has been pointed out earlier(5), if useful drugs related to serotonin are to be achieved. Consequently we would like to report studies of a new antimetabolite of serotonin which is highly active in combatting the pressor action of this hormone, and one which is very effective even when fed.

The new antimetabolite is 1-benzyl-2, 5-dimethylbufotenin hydrochloride, which may be abbreviated to "benzyl analog of bufotenin" or BAB. The structural formula, as well as that of serotonin, is shown in Fig. 1. Here also is shown the benzyl analog of serotonin (BAS) which recently(4,6) has been found to be a highly active antiserotonin, capable of

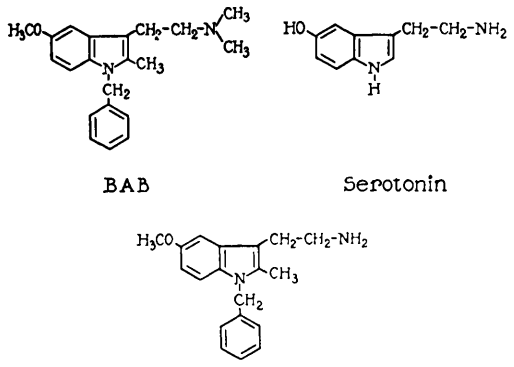

FIG. 1.

protecting dogs by the oral route from the pressor action of serotonin. BAS has also proved to be capable of lowering the blood pressure of humans suffering from hypertension(6.7), and to do so without producing marked side effects. The close relationship of the new antimetabolite to BAS is evident, hence some differences in the biological properties of BAB and BAS are noteworthy.

Preparation of BAB. BAB was synthesized as previously described(8). An unfortunate typographical error in reference(8) caused this compound to appear in the table without its methyl group at position 2. Thus, in Table II on page 4321 of the chemical paper the 12th compound should read 1-benzyl-2-methyl-5-methoxy-N, N-dimethyltryptamine hydrochloride instead of 1-benzyl-5-methoxy-N, N-dimethyltryptamine hydrochloride. A modification was introduced into the scheme of synthesis which facilitated large scale operations. This was to conduct the benzylation at the amide stage rather than at the hydrazine step. BAB in the form of its hydrochloride was soluble in water to about 40 mg per ml. In Ringer's solution or other chloride-containing aqueous solutions, it was soluble only to about 4 mg per ml. All solutions for biological testing were in Ringer's solution.

Protection of dogs intravenously against pressor action of serotonin. The tests were conducted exactly as described previously for other antiserotonins(4). Normal dogs, anesthetized with Nembutal were connected to a mercury manometer via the femoral artery, and were calibrated as to their pressor responses to graded doses of serotonin administered intravenously. Small doses of BAB (10 μg per kg) were injected intravenously into the jugular vein. The animals were then challenged again with a dose of serotonin, which, in their untreated state, had been sufficient to evoke a pressure rise of 20-40 mm of Hg. Changes in blood pressure were recorded, and if there had been no protection against the pressor effect, or if the protection was not complete, the dose of BAB was increased and the challenging repeated. Thus an estimate of the amount of BAB required to protect each dog was made. Data for 3 dogs so tested are shown in Table I. Very small doses indeed (8-12 μg per kg) were capable of causing partial protection. However complete protection was difficult to achieve, although doses of 1 mg per kg approached this goal.

In 2 additional dogs (not shown in the table) which had given abnormal responses to serotonin, the effect of BAB was less easy to assess. For example dog 35 had shown only small pressor responses to serotonin.

TABLE I. Changes in Arterial Pressure of Dogs Given BAB and Serotonin Intravenously.

Dog No.	I.V. serotonin —μg/kg—	I.V. analog —μg/kg—	Interval between analog & serotonin, min.	Initial pressure —mm Hg—	Maximal pressure increase —mm Hg—
7	62	0	—	102	28
	62	12	15	—	14
	62	120	15	—	12
	62	120	65	—	20
	62	1200	60	—	10
29	40	0	—	92	38
	40	8	15	—	24
	40	80	15	—	30
	40	80	75	—	38
	40	800	15	—	18
	40	800	94	—	38
34	80	0	—	116	18*†
	80	8	15	—	12
	80	1200	15	—	6

* All responses in this dog were obtained after intrav. inj. of atropine sulfate 1.2 mg/kg which was administered to obliterate the marked hypotension caused by serotonin alone.

† After each inj. of serotonin there was a very fleeting spike of pressure both before and after BAB. This response was not included in the results shown. Only sustained rises in pressure were considered.

TABLE II. Changes in Blood Pressure in Dogs Challenged with Serotonin before and after They Had Been Fed BAB.

Dog No.	Analog, mg/dog/day	I.V. serotonin challenge, μg/kg	Initial pressure	Maximal fall in pressure	Maximal rise in pressure
			mm Hg		
19	0	37	76	8	36
	10	37	78	0, 8*	26, 4*
	10	74	—	2	14
29	0	40	92	0	38
	10	40	106	20, 12*	2, 14*
32	0	71	96	0	24
	0	142	—	0, 8*	24, 30*
	10	71	70	0, 20*	22, 6†
	10	142	—	20	10

* The 2 numbers represent responses to 2 successive challenges with serotonin.
† The response to the first inj. of serotonin was followed by a rising baseline (Fig. 4, ref. 5). Subsequent challenges gave only slight rises.

These consisted of a tiny sharp spike (16 mm, 15 sec. duration) followed by a small sustained rise lasting several minutes. Larger doses of serotonin (up to 222 μg per kg) did not produce a more typical response. Administration of BAB (1.2 mg per kg) protected this dog against the sustained rise caused by serotonin, but greatly increased the height of the sharp spike (72 mm).

The protecting action of BAB appeared to be shortlived by the intravenous route. Data of Table I show that the protection had largely disappeared after about an hour. This was in contrast to the action of BAS in which case it has been found that full protection developed slowly and required about an hour after intravenous administration to become evident.

Protection of dogs by the oral route. The design of the experiments was the same as that used by Shaw and Woolley(4,5). Dogs which had been calibrated with serotonin were fed BAB once daily for 4 days. They were then challenged with serotonin again, and changes in arterial pressure were recorded and compared to those in the unprotected state. A dose of 10 mg of BAB per dog per day was found adequate to protect the animals from the pressor effects of serotonin. Data from 3 such experiments are shown in Table II.

Toxicity of BAB. The dogs which had eaten, or had been injected intravenously with BAB, showed no discernible toxic effects.

A more extensive study of toxicity was made in mice. For the acute tests, groups of 5 to 12 adult Swiss mice of the Webster strain were injected intraperitoneally with graded doses of BAB. With 100 mg/kg a flaccid paralysis of the hind legs appeared a few minutes after injection, succeeded by convulsive seizures which passed off after about an hour, followed by a period of lethargy, then complete recovery. Repetition of this dose on the following day again precipitated the signs which were again followed by recovery. After 5 days of this treatment, one of the 5 mice was unable to walk 24 hours after the last injection. Autopsy showed swollen and hemorrhagic intestines in this mouse but not in others. With 40 mg per kg only minimal signs were seen, most prominent of which were some difficulty in use of hind legs, some hyperventilation and lethargy or sleepiness which developed about half an hour after the injection. The behavioral changes caused by the large doses (100 mg/kg) could be summarized as excitement of the central nervous system, quickly followed by tranquilization.

Tests of chronic toxicity on groups of 12 adult mice were made by daily injection of 40 mg/kg intraperitoneally. Aside from slight motor difficulties with the hind legs which were noticeable for a short time after each injection, these animals showed no ill effects. They maintained their weights for the duration of the experiment (4 weeks). At this time autopsy revealed no changes which could be recognized on gross examination. The acute and chronic toxicities of BAB were therefore somewhat less than for BAS(4).

Antiserotonin action of BAB on isolated rat

uterus. The antiserotonin action of BAB was tested in the way described for other substances(4) on the isolated uterus of estrogen-treated rats. In this system the compound prevented the contractions induced by serotonin. At 0.1 μg per ml BAB prevented the effects of 0.01 μg of serotonin per ml. Quantitative comparison of the effectiveness of BAB with that of BAS was difficult because the action was irreversible, and the potency depended on the time of exposure of the tissue to the analog before the challenging dose of serotonin was applied. These difficulties also were a prominent feature of the action of BAS, as has already been described in detail (4). The chief conclusion to be drawn from these studies was that BAB was a potent and irreversible antagonist of serotonin on the rat uterus.

Differences in action between BAB and BAS.

(a) *Taste.* BAS when applied to the tongue as solid crystals had little if any taste. BAB, on the other hand, when similarly applied had a most pronounced taste, and caused a numbing sensation which persisted and spread to adjoining areas of the tongue, and which lasted for several hours. It was reminiscent of the similar sensation caused by pyribenzamine. When one considers that BAS is a primary amine which should be attacked by amine oxidase, whereas BAB is a tertiary amine which should not be thus attacked, one may suggest that amine oxidase may play a role in the protection of nerves from the primary amine. Several of the older antimetabolites of serotonin such as the 5-aminoindoles(1) which should not be subject to amine oxidase numbed the tongue just as BAB did. The need for keeping antiserotonin out of the nervous system, for example in order to prevent psychiatric disorders, has been clearly recognized(6). A clue as to how this is to be done may be furnished by the present example which shows that, at least in the tongue, the presence of a primary aliphatic amine is necessary in an analog to keep it away from the neural structures.

(b) *Hypotension on intravenous injection.* Intravenous injection of BAS into dogs(4) or into men(7) did not result in hypotension. By contrast, doses of BAB 10 mg or greater per dog (1 mg per kg) always caused a sharp fall in arterial pressure. This was 10-40 mm of Hg (average of 6 dogs, 24 mm of Hg). The hypotension was short-lived (1-2 minutes).

(c) *Hind leg paralysis.* Mice given more than 40 mg of BAB per kg exhibited the transient paralysis of the hind legs already described. This has not been seen after treatment with BAS. This provided further evidence for an effect of BAB on nerves.

(d) In a preliminary clinical trial of BAB given orally to hypertensive patients, Drs. Robert Wilkins and William Hollander of Boston have not found it to possess the significant hypotensive effect shown by BAS at comparable dosage.

These differences in two compounds so similarly constituted, and so similar in several other biological properties tended to focus attention on the role of the primary amino-group in the designing of antiserotonins for specified pharmacological effects.

Summary. The benzyl analog of bufotenin, BAB, or 1-benzyl-2,5-dimethylbufotenin (1-benzyl-2-methyl-5-methoxy-N, N-dimethyltryptamine hydrochloride) has been found to be a highly active antagonist of serotonin. Not only was it effective on isolated tissues, but, unlike many others, was highly active as an antagonist to the pressor effects of serotonin in dogs, even when given by the oral route. Some differences in action from the corresponding primary amine (benzyl analog of serotonin) were found.

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