Paper chromatography was performed after hydrolysis[11] of foetal proteins from the ethionine-injected animals and the radioactivity was determined on the chromatograms by means of autoradiography. The radioactivity was detected mainly in the spots corresponding to ethionine and ethionine sulphoxide.

Tab. II. Specific radioactivity in the T.C.A. (10% trichloroacetic acid) soluble fraction and in the protein fraction of foetus and maternal liver and pancreas after the intravenous injection of pregnant mice with C^{14}-methionine

Mouse No.	Time after administration of C^{14}-methionine	T.C.A. soluble radioactivity Cpm/mg tissue dry weight			Protein bound radioactivity Cpm/mg protein		
		foetus	liver	pancreas	foetus	liver	pancreas
5	15 min	27	31	311	40	215	780
6	1 h	32	22	66	165	187	519
7	4 h	26	23	24	329	191	215
8	24 h	55	13	32	144	141	114

Tab. III. Specific radioactivity in the T.C.A. (10% trichloroacetic acid) soluble fraction and in the protein fraction of foetus after the intravenous injection of pregnant mice with C^{14}-ethionine.

Mouse No.	Time after administration of C^{14}-ethionine	T.C.A. soluble radioactivity Cpm/mg tissue dry weight	Protein bound radioactivity Cpm/mg protein
9	15 min	109	8
10	15 min	1983	69
11	1 h	579	20
12	1 h	3045	60
13	4 h	643	20
14	4 h	994	59
15	24 h	4148	34
16	24 h	1163	74

These studies indicate that ethionine may freely pass the placenta barrier of the mouse and accumulate in the foetus. A very small part of the radioactive ethionine is incorporated into the foetal proteins. From this has been assumed that an abnormal protein is formed in which the naturally occurring amino acid has been replaced by the analogue. Such a replacement may in part explain the toxic effect of ethionine on the foetus. Most of the radioactivity, however, is found in the non-protein fraction, and earlier observations[12] have shown that ethionine can furnish ethyl radicals for synthesis of choline and thus produce triethylcholine which has proved to have a growth-inhibiting effect on rats. The present observations therefore support the concept that ethionine may exert its effect on the organism in other ways in addition to the formation of abnormal proteins.

Zusammenfassung. Trächtige Mäuse wurden zwei Tage vor dem erwarteten Werfen mit dem Antimetabolit des Methionins ^{14}C-*DL*-Aethionin intravenös behandelt. Aufnahme und Einbau des Antimetabolits wurden gleichzeitig autoradiographisch und chemisch untersucht. Die Plazentaschranke ist für Aethionin durchlässig; die Radioaktivität fand sich in den Foeten angehäuft, dabei war aber nur ein ganz geringer Teil des Aethionins in den Eiweisskörper derselben eingebaut. Der Einbau des Aethionins in den Organen (Leber und Pankreas) der Mutter war ebenfalls äusserst gering.

Aus den Ergebnissen wird der Mechanismus der toxischen Wirkung des Aethionins erklärt.

E. Hansson and T. Garzo[13]

Department of Pharmacology, Kungl. Veterinärhögskolan, Stockholm (Sweden), June 28, 1961.

[11] D. Gross and H. Tarver, J. biol. Chem. *217*, 169 (1955).
[12] J. A. Stekol and K. Weiss, J. biol. Chem. *185*, 577 (1950).
[13] Present address: Chemical Institute of the Medical University, Budapest (Hungary).

Mechanism of the Serotonin Depressor Response Following BAS-Phenol Administration in the Dog

The number of serotonin (5-hydroxytryptamine) antagonists which have been tested in both isolated tissues and intact animals is legion, but only a select few have been shown to be active against the cardiovascular effects of serotonin. The benzyl analog of serotonin, 1-benzyl-2-methyl-5-methoxytryptamine or BAS, is one of the more potent antagonists and it has been found to be effective against the pressor action of serotonin in the dog[1]. An even more powerful serotonin antagonist has been synthesized from BAS by cleavage of the methyl ether to form 1-benzyl-2-methyl-5-hydroxytryptamine or BAS-phenol[2]. In anesthetized dogs, intravenous injection of BAS-phenol blocks the pressor response to serotonin selectively while the pressor action of tryptamine remains unaffected, or may even be potentiated[3]. Possibly as a result of blockade of the pressor component of serotonin action, augmentation of the depressor phase in its activity occurs. The following studies were undertaken to investigate the mechanism of the depressor response to serotonin which is unmasked by the administration of BAS-phenol in the anesthetized dog.

Experiments were performed on 14 mongrel dogs of both sexes, weighing from 8 to 18 kg. The dogs were anesthetized with sodium pentobarbital[4], 30 mg/kg, administered intravenously. Arterial blood pressure was recorded with a mercury manometer from a femoral artery. Drug injections were made into the cannulated ipsilateral femoral vein. The agonist drugs and corresponding doses used were serotonin creatinine sulfate, 10–120 μg/kg, tryptamine hydrochloride, 125–500 μg/kg, histamine phosphate, 3–5 μg/kg, and acetylcholine bromide, 5–10 μg/kg. The antagonist drugs used were 1-benzyl-2-methyl-5-hydroxytryptamine hydrochloride (BAS-phenol)[5], 2.5 mg/kg, and diphenhydramine hydrochloride[6], 5 mg/kg. Doses for the agonist drugs are expressed in terms of the base while those for the antagonists are in terms of the salt.

[1] E. Shaw and D. W. Woolley, J. Pharmacol. *116*, 217 (1956).
[2] E. Shaw and D. W. Woolley, Proc. Soc. exp. Biol. Med. *96*, 439 (1957).
[3] D. W. Woolley and E. Shaw, J. Pharmacol. *121*, 13 (1957).
[4] Trade name Nembutal.
[5] Generously supplied by Dr. K. Pfister, Merck Sharp & Dohme Research Laboratories, Rahway, N. J.
[6] Trade name Benadryl.

In all the dogs tested, where the response to serotonin was initially pressor, after the administration of BAS-phenol the arterial pressure response to serotonin became purely depressor. As compared with the control responses, the serotonin depressor response elicited after BAS-phenol administration was greater in degree and duration. This depressor response to serotonin can be blocked with diphenhydramine, an antihistaminic drug. To test specificity of the blockade, control injections of acetylcholine were given before and after administration of diphenhydramine. Figure 1 shows the results obtained from such an experiment. Blockade of depressor responses to serotonin and histamine is attained with diphenhydramine in doses which do not have any significant effect on similar responses to acetylcholine. The tracing also shows that section of both vagi nerves at the cervical level to eliminate the Bezold reflex, does not alter the depressor responses to serotonin, histamine or acetylcholine.

Following the administration of various serotonin antimetabolites, including BAS, SHAW and WOOLLEY consistently observed that 'the initial fall in pressure after serotonin injection was both large and persistent', however, no attempt was made at elucidation of the mechanism involved in this depressor response[7]. The effect of single injections of serotonin on canine blood pressure usually consists of three phases: an initial fall with concurrent slowing of the heart rate, followed by a rise and then finally by a prolonged fall in pressure[8]. The initial fall has been ascribed to a Bezold-like reflex since it can be abolished by either vagotomy or atropinization[9], while the final fall is believed to be due to other mechanisms. The release of endogenous histamine as a mechanism to explain the sustained fall in pressure produced by serotonin in anesthetized dogs, has previously been demonstrable only when serotonin is given by continuous infusion[10]. Antihistaminic drugs have been reported to have no effect on the late depressor phase of the response to single

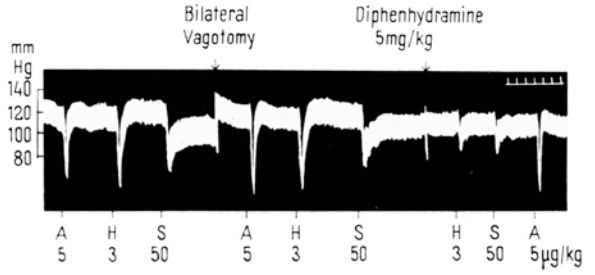

Fig. 1. Blockade by diphenhydramine of depressor responses to serotonin following BAS-phenol administration. Femoral blood pressure in a dog, 8.6 kg, under pentobarbital anesthesia. BAS-phenol, 2.5 mg/kg, was given i.v. prior to recording of responses. H, histamine, A, acetylcholine, and S, serotonin. Note the absence of effect of bilateral cervical vagotomy on the responses to the agonist drugs. Time interval: 1 min.

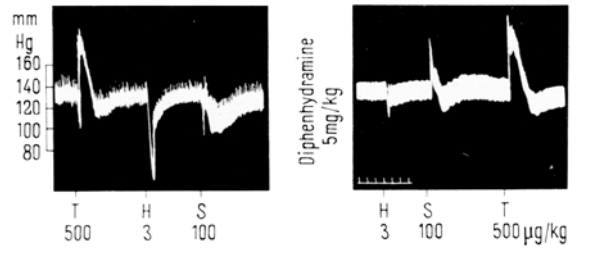

Fig. 2. The effect of diphenhydramine on arterial pressure responses to serotonin, histamine and tryptamine. Dog, 12 kg, under pentobarbital anesthesia. T, tryptamine, H, histamine, and S, serotonin. Time interval: 1 min.

injections of serotonin and histamine release as a possible mechanism in this situation was therefore discarded[11]. The present findings however indicate that if the dog is protected from the other cardiovascular effects of serotonin, a histamine mediated mechanism can be shown even with single injections of serotonin. Serotonin is known to release endogenous histamine from living tissues[12] and this mechanism has been shown to be responsible for the depressor response elicited by serotonin in the chicken[13–15].

It has been reported that various antihistaminic drugs, including diphenhydramine, reduce the pressor response to serotonin in dogs[16]. Although the predominant effect of serotonin in the dog is usually a rise in arterial pressure, occasionally the response is markedly depressor to begin with. The results obtained in our laboratory from an animal in which the initial response to serotonin is depressor, is shown in Figure 2. Our results indicate that diphenhydramine, given in a dose sufficient for antihistaminic blockade, augments the pressor component and diminishes the depressor phases of serotonin action. The present data might possibly be explained as an augmentation of the pressor component due to the absence of opposition from the histamine which is released since a block has been established at the histamine receptor sites.

The complex cardiovascular situation occurring after serotonin injection as affected by BAS-phenol may well be analogous to the well documented reversal of epinephrine effects on blood pressure brought about by adrenergic blocking agents. Intravenous injections of epinephrine produce a rise in blood pressure but when the same injections are given after adrenergic blockade has been established, epinephrine produces a fall in pressure. In a similar fashion, upon injection of serotonin, the cardiovascular effects manifested actually represent an interplay of several mechanisms, some which would elevate blood pressure and others which would produce a fall. The latter are usually obscured by the stronger pressor activity in the untreated anesthetized dog and can be unmasked only when such pressor actions have been prevented by suitable blocking agents.

Résumé. Après l'administration de BAS-phénol à des chiens anesthésiés, l'effet usuel de la sérotonine d'augmenter la pression artérielle se convertit constamment en un abaissement prolongé de la pression. Cet effet d'abaissement n'est pas influencé par la section bilatérale des deux nerfs vagues, mais on peut le bloquer avec la diphénhydramine. Les données présentées suggèrent que la réponse d'abaissement de la pression artérielle de la sérotonine qui survient dans les chiens ayant été traités avec du BAS-phénol est dûe à une libération d'histamine endogène.

R. D. BUÑAG and E. J. WALASZEK

Department of Pharmacology, University of Kansas Medical Center, Kansas City (Kansas), July 10, 1961.

[7] E. SHAW and D. W. WOOLLEY, J. Pharmacol. *116*, 217 (1956).
[8] I. H. PAGE, in *5-Hydroxytryptamine* (Ed. by G. P. LEWIS, Pergamon, New York 1957).
[9] I. H. PAGE, J. Pharmacol. *105*, 58 (1952).
[10] I. H. PAGE and J. W. MCCUBBIN, Amer. J. Physiol. *184*, 265 (1956).
[11] G. REID, J. Physiol. *118*, 435 (1952).
[12] F. FELDBERG and A. N. SMITH, Brit. J. Pharmacol. *8*, 406 (1953).
[13] R. D. BUÑAG and E. J. WALASZEK, Fed. Proc. *20*, 11 (1961).
[14] R. D. BUÑAG and E. J. WALASZEK, J. Pharmacol. *133*, 52 (1961).
[15] R. D. BUÑAG and E. J. WALASZEK, Arch. int. Pharmacodyn., in press.
[16] I. H. PAGE and J. W. MCCUBBIN, Amer. J. Physiol. *174*, 439 (1953).