PHARMACOLOGY OF INDOLEALKYLAMINES

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I. INTRODUCTION

Indolealkylamines have so far been considered as a group of active substances of rather slight pharmacological interest. Renewed attention has been focussed on them since the discovery that one of the indolealkylamines, 5-hydroxytryptamine, is identified with both serotonin, the serum vasoconstrictor, and enteramine, the specific secretion product of the enterochromaffin cell system.

Owing to its widespread occurrence in nature, its intense pharmacological activity, and its presumably high biological significance, 5-hydroxytryptamine constitutes by far the most important member of this group of substances. It is not surprising, therefore, to find most of the space in the present paper devoted to it.

This seems to be the first review on indolealkylamines. It calls attention to a number of experimental data and well established facts, but, more important, it shows the vast amount of work that remains to be done in this extremely fascinating field and the many problems which await solution.

Isolation of 5-hydroxytryptamine has been carried out independently by two groups of research workers.

1) Erspamer and his colleagues started their investigation with the purpose of extracting, characterizing and isolating the substance which imparts their peculiar histochemical properties to the enterochromaffin cells of the gastrointestinal mucosa (45, 169, 173, 269).

In the period from 1937 to 1940 a series of color reactions of the substance, called *enteramine*, was established and at the same time a number of pharmacological actions of extracts of mammalian gastrointestinal mucosa and of posterior salivary glands of octopods were described (64, 65, 272, 276). In subsequent years the chemical and biological characterization of enteramine became more and more precise (66, 278, 279), partly as a result of progress in our systematic search for new examples of the enterochromaffin system and of new tissues containing enteramine.

The presumably indolic nature of enteramine was pointed out as early as 1946 (73) and 1948 (75).

At present the enterochromaffin cell system, speaking broadly, includes:

(a) the typical enterochromaffin cells of the gastrointestinal mucosa (16, 45, 55, 115, 195, 269), biliary ducts (62) and the pancreas (63) of vertebrates and ascidians (171). These cells constitute the enterochromaffin cell system in the strict sense.

Among the vertebrates only *Cyclostomata* and *Teleostei* are lacking in typical enterochromaffin cells. However, these groups of fishes contain in their intestinal mucosa numerous cells morphologically indistinguishable from the enterochromaffin cells, but with no enteramine in their granules. We also include these last cells which we call "preenterochromaffin argentophil cells", in the enterochromaffin system and now consider them as cells which have lost their 5-hydroxytryptamine during the phylogenesis of *Cyclostomata* and *Teleostei*.

(b) the chromaffin cells of the frog urinary bladder (275) and the lizard oviduct (274);

(c) the chromaffin cells of the thymus of some birds (43, 44, 222);

(d) the chromaffin cells of the posterior salivary glands of octopods (169, 268, 273);

(e) the giant chromaffin cells of the hypobranchial body of some prosobranchiate molluscs (172, 228, 271);

(f) some of the secretory cellules of the cutaneous glands of amphibians, where these cellules contain a chromaffin secretion (170, 270, 277).

In addition to extracts of all the above tissues, enteramine was detected also in spleen extracts. This was at first rather surprising since spleen did not contain cells belonging to the enterochromaffin system (67, 279).

In some tissues, besides enteramine itself, a substance closely related to enteramine was found (65, 69, 70, 81). This substance, enteramine I (inactive enteramine), gives all the color reactions of enteramine, but is biologically inactive and is not attacked by amine oxidase. Enteramine I may be converted into enteramine (5-hydroxytryptamine) by simple alkaline treatment.

The posterior salivary glands of some octopods and the skin of certain amphibians contain enormous quantities of enteramine. The substance has been isolated from these materials in a pure state and its identification with 5-hydroxytryptamine been made highly probable (84, 88).

2) Rapport and his colleagues (201–204, 206) had quite a different aim: that of isolating and identifying the principle which is responsible for the vasoconstrictor and moderately hypertensive properties possessed by serum and defibrinated blood.

The serum vasoconstrictor has been well known for more than forty years under various denominations: "vaso-constrictines" (18), "adrenalinähnliche Substanz" (183, 258), "vasoconstrictor principle or substance" (30, 148, 154, 243, 244, 245), "Spätgift" (119, 120, 121), "thrombocytin" or "thrombotonin" (200, 224, 225).

To Rapport and his colleagues belongs the credit for having produced, through outstanding work, more and more active and purified preparations of beef serum, to the point of finally obtaining the active substance, called by them *serotonin*,

in a pure state, and for having subsequently suggested the identity of this substance with creatinine and 5-hydroxytryptamine sulfate.

The researches of the two groups of investigators met only after serotonin on the one hand and enteramine on the other had been isolated, and both substances identified with 5-hydroxytryptamine, and consequently with each other. The synthesis of 5-hydroxytryptamine (10, 20, 139, 141, 249, 250) has confirmed the above identification.

Bacq (13) suggests that the names enteramine and serotonin should now be dropped in favor of 5-hydroxytryptamine. The name enteramine, indeed, though exact from a chemical point of view and in indicating the main source of 5-hydroxytryptamine, cannot be correctly applied to all known localizations of the substance; the name serotonin, in its turn, is inexact both from the point of view of the origin of the substance and from that of its action. In the sense, at least, intended by Rapport and his colleagues, it is by no means a "tonin". Accepting Bacq's suggestion, the abbreviated form "5-HT" will be used throughout this paper to indicate 5-hydroxytryptamine.

II. OCCURRENCE AND DISTRIBUTION OF INDOLEALKYLAMINES

A. Indolealkylamines other than 5-HT

Gramine or *donaxine* (3-dimethylaminomethylindole). This substance was first isolated from alcohol extracts of chlorophyll-deficient barley mutants, and later from extracts of normal sprouting barley (106, 107, 176). The concentration of gramine in barley leaves increases from the base to the tip of the leaf and the total quantity, which remains constant for the first ten days of germination, disappears after one month (36).

Apart from barley, the substance has been obtained, under the name of donaxine, from the leaves of another graminaceous plant, *Arundo donax* (185, 186).

Tryptamine (3-(β-aminoethyl)-indole). This base originates, in small quantities, during the decomposition of casein and of tryptophan-containing cultural media by some putrefactive bacteria (108, 135). It has also been isolated, again in small quantities, from the urine of patients suffering from pellagra (253).

In the living vegetable organism, tryptamine has been detected, together with β-phenylethylamine, in the tops of *Acacia floribunda* (291); in the animal organism, in the skin of *Triton cristatus* and *Salamandra maculosa* (85).

5-Hydroxyindolealkylamines. These have been found in both the animal and vegetable kingdoms. The only animal tissue which contains 5-hydroxyindolealkylamines other than 5-HT is the skin of amphibians (54, 85, 137, 140, 156, 262, 292). Here we find, together with 5-HT, N-methylated, dehydrogenated and conjugated derivatives of this substance (N-methyl-5-HT; N,N-dimethyl-5-HT or *bufotenine*; (β-[5-hydroxy-indolyl-(3)]-ethyl)-trimethylammonium-hydroxide or *bufotenidine*; 3-(β-dimethyl-aminovinyl)-5-hydroxyindole or *dehydrobufotenine* and its sulfuric ester *bufothionine*) as well as hydroxyindolealkylamines of still unknown chemical constitution (*e.g.*, *bufoviridine*). Data regarding the distribution of these bases are given in Table II. For their biosynthetic relations see part V.

The occurrence of 5-hydroxyindolealkylamines in the vegetable kingdom has recently been demonstrated by Wieland and Motzel (293), who isolated bufotenine from *Amanita mappa*, and by Bowden *et al.* (30a), who found 5-HT in the trichomes from the pods of *Murcuna pruriens*, of which cowhage is composed. The concentration of 5-HT in this material, as determined by pharmacological assay, was of the order of 0.015 per cent.

5-Hydroxytryptophan. The immediate precursor of 5-HT so far has been found, in minute amounts, only in the cutaneous venom of *Bufo marinus* (262) and, apparently, in extracts of rabbit's gastric mucosa (266). The writer and I. Cortese (unpublished observations) did not succeed in detecting this amino acid on paper chromatograms of extracts of amphibian skin and of octopod posterior salivary glands.

B. 5-Hydroxytryptamine

Up to the present 5-HT has been found in all tissues containing cells belonging to the enterochromaffin system, in the blood, in the spleen and, apparently, in some central and peripheral nervous structures.

The identification and quantitative estimation of the substance in the various biological materials has been accomplished by means of its chemical isolation, through color reactions, and, above all, with the aid of paper chromatography and of pharmacological methods.

1. Tissues containing cells belonging to the enterochromaffin system. a. Gastrointestinal mucosa. Found for the first time in extracts of rabbit's gastric mucosa (58, 272), 5-HT has since been recognized in extracts of gastrointestinal mucosa of all the mammals, birds, reptiles and amphibians examined (58, 94, 97) and, furthermore, in gastrointestinal extracts of *Elasmobranchii* and *Chondrostei* (74, 97, 100) as well as in those of ascidians (74).

In the mucosa of the digestive tract of all these animal species 5-HT is localized, to a great extent at least, in the enterochromaffin cells which the writer and his collaborators consider to constitute a "diffuse endocrine organ" (115) designed for the production and storage of 5-HT. This view is corroborated by the observation that there is no 5-HT in extracts obtained from intestines lacking in enterochromaffin cells, such as those of *Teleostei* and *Cyclostomata*; by the fact that during embryonic development 5-HT makes its appearance in the intestinal wall contemporaneously with the first enterochromaffin cells (110); and by the very high content of 5-HT in metastases of carcinoids, which are tumors originating from the enterochromaffin cells (165, 166, 209). From these cells the substance is released into the circulatory stream, where most of it is taken up by the platelets. An external excretion of 5-HT into the intestinal lumen has not yet been proved.

The writer and his colleagues (94, 97, 110) have estimated the 5-HT content of the different intestinal sections in human beings and in 27 vertebrate species. The values obtained, ranging from 0.3 to 9.0 μg. of substance per gm. of fresh tissue (cf. Table I), agree with those reported by Feldberg and Toh (111) for the dog and the rabbit.

b. Posterior salivary glands of octopods. 5-HT is present in the salivary extracts

TABLE I

The content of 5-hydroxytryptamine in the gastro-intestinal tract of various animals

Animal species	5-HT content, as base (μg./gm. fresh tissue)			
	Stomach	Small intestine		Large intestine
		I	II	
Dog	5.20	3.70	4.30	2.80
Cat	0.45	0.88	0.53	1.19
Rabbit	4.90[a]–0.85[b]	3.30	3.70	2.70
Guinea-pig	1.40	5.00	3.40	0.70
Rat	1.40	1.20		3.90
Mouse	8.85	1.60		3.10
Bat		1.60		
Hedgehog	6.30	3.40		
Hen	—	4.90	4.50	4.10
Duck	—	3.10	4.10	3.60
Pigeon	—	1.10		
Tortoise		3.20		
Toad	2.20	1.60		
Bombinator pachypus	1.00	0.75		
Scylliorhinus canicula	0.60	2.60		
Scylliorhinus stellaris	0.30	2.30		
Torpedo marmorata	1.35	2.50		
Acipenser naccarii	0.30	0.34		
Acipenser sturio	—	0.38		
Ameiurus catus	0.30 (?)	<0.40 (?)		
Anguilla vulgaris	<0.30 (?)			
Tinca vulgaris	<0.20 (?)			
Petromyzon planeri	<0.20 (?)			

[a] Stomach body.

[b] Stomach pylorus.

I—proximal half of the small intestine. II—distal half of the small intestine.

of those species of octopods whose glands contain chromaffin cells, which suggests that in this case too these cells have to be considered as the site of formation and storage of 5-HT. The 5-HT content may be very high: *Octopus vulgaris* 420–510 μg./gm. of fresh tissue; *Eledone moschata* 760 μg./gm. (76, 97, 100). Following electric stimulation of the secretory nerve of the isolated posterior salivary glands of *Octopus vulgaris* and *Eledone moschata*, 5-HT passes into both the perfusate and the saliva, thus behaving at the same time as a hormone and as a product of external secretion (11, 13).

c. Hypobranchial body of Muricidae. 5-HT is present in large quantities in the hypobranchial body, median area, of *Murex trunculus* (80–290 μg./gm.), and in smaller amounts in the same part of *Murex brandaris* (75, 100). Presumably the substance has here the same significance as it has in the posterior salivary glands.

d. Skin (cutaneous glands) of amphibians. The positive results attained by researches on the indolealkylamines of the amphibian skin (80, 85, 100) are summarized in Table II.

In the following species of amphibians, none of the tabulated indole deriva-

TABLE II

The occurrence of indolealkylamines in the amphibian skin

Animal species	Tryptamine	5-HT	N-Methyl-5-HT	Bufotenine	Bufotenidine	Bufothionine	Dehydrobufotenine	Bufoviridine
Salamandra maculosa	+	+	−	−	−	−	−	−
Triton cristatus	+	−	−	−	−	−	−	−
Xenopus laevis	−	+	−	−	+++	−	−	−
Discoglossus pictus	−	+++	−	−	−	−	−	−
Bufo bufo bufo								
skin	−	+	+	++	+	− (?)	++	−
parotoids	−	+	?	+++	++	− (?)	++	−
Bufo viridis	−	+	?	+++	++	− (?)	+	++
Bufo calamita	−	+	+	++	+	−	+	++
Bufo americanus	−	++	+	++	++	− (?)	+	−
Bufo fowleri	−	++	+	++	++	− (?)	+	−
Bufo arenarum	−	+	−	++	?	[+]*	+++	−
Bufo mauretanicus	−	+++	−	−	−	−	?	−
Bufo gargarizans	−	++	+	−	++	−	?	−
Bufo marinus								
skin	−	+	+	−	−	− (?)	+	−
parotoids	−	?	+	−	−	− (?)	+++	−
Bufo paracnemis	−	?	−	+++	++	− (?)	+	−
Bufo regularis	−	+	−	−	−	−	−	−
Bufo bergei	−	+	−	−	−	−	−	−
Bufo kisoloensis	−	++	−	−	−	−	−	−
Bombinator pachypus	−	+++	−	−	−	−	−	−
Bombinator igneus	−	+	−	−	−	−	−	−
Hyla arborea	−	++	−	−	−	−	−	−
Hyla aurea	−	+	−	−	−	−	−	−
Acris crepitans	−	−	−	−	−	+	−	−
Rana esculenta	−	+	−	−	−	−	−	−
Rana pipiens	−	+	−	−	−	−	−	−
Rana clamitans	−	?	−	−	−	−	−	−
Rana palustris	−	+	−	−	−	−	−	−
Rana madagascariensis	−	+	−	−	−	−	−	−
Rana labrosa	−	+	−	−	−	−	−	−

* Jensen (156).

tives could be found in cutaneous extracts: *Amblystoma tigrinum* (Axolotl), *Pleurodeles waltlii*, *Euproctes rusconi*, *Pelobates fuscus*, *Leptodactylus ocellatus*, *Leptodactylus pentadactylus*, *Racophorus madagascariensis*, *Rana fuscigola*, *Chiromantis rufescens*, *Ptychadena mascariniensis*, *Leptopelis karissimbensis*, *Arthroleptis adolphi-friederici*, *Phyctimantis verrucosus*.

The indolealkylamine content of the skin may be extremely high, as shown by some values which have been established for 5-HT: *Discoglossus pictus*, 410 μg./gm. of fresh tissue (41–54 mgm./kgm. of body weight); *Bombinator pachypus*,

700–1000 μg./gm. (175–250 mgm./kgm.!) (97, 100). It seems likely that all the indolealkylamines of the skin must be considered as materials destined merely for excretion or external secretion, as are the adrenaline and noradrenaline found in skin extracts and in the cutaneous secretions of several species of tropical toad (127).

2. Blood. As stated in the introduction, the serum vasoconstrictor has been identified by Rapport (206) as 5-HT.

a. The source of 5-HT contained in serum. As early as 1912, O'Connor (183), as a result of his careful experimental observations, supposed that the serum vasoconstrictor did not pre-exist in the circulating plasma, but was released, during coagulation or defibrination of the blood, from the blood cells or, more probably, from the platelets. The hypothesis of the thrombocytic origin of the serum vasoconstrictor was later supported, with ever more convincing evidence, by Zucker and Stewart (301), Janeway *et al.* (154), Hirose (148), Freund (119, 120), Borgert and Keitel (30), Simon (243, 244), Zucker (302, 304, 305), Bracco and Curti (31, 32), and finally by Reid and Rand (199, 200, 224) and by Zucker *et al.* (306, 307). By fractional centrifugation of ox-blood rendered non-coagulable with sodium citrate, Reid and Rand succeeded in definitively establishing that the presence of platelets at the time of clotting was a necessary condition for the appearance of 5-HT in the serum. Zucker *et al.*, in their turn, found that the content of 5-HT in bovine platelets (1.7×10^{-9} μg./platelet) was more than sufficient to account for all the 5-HT present in bovine serum. No other indolic or phenolic substances were found in the platelet extracts.

It is evident that the thrombocytes contain substances or molecular groups capable of anchoring 5-HT with especial selectivity and tenacity. This anchoring is very important in that, amongst other things, it provides a defence for 5-HT against amine oxidase, to which the substance would otherwise inevitably be exposed. Experimental demonstration of the capacity of the platelets to absorb 5-HT from their suspending medium has been provided recently by Humphrey and Toh (152).

It is probable that there exists for every animal species a definite limit to the capacity of the thrombocytes to take up 5-HT. Apart from the results of Humphrey and Toh's researches, this view is supported by the conspicuous reduction in serum 5-HT observed in essential thrombocytopenia and, to a lesser degree, in cirrhosis of the liver, a reduction which could hardly be explained if the residual platelets were able to take up the available 5-HT in unlimited measure (100).

When thrombocytes disintegrate, in the spleen or in other tissues or in the blood itself, 5-HT is of necessity set free either into the plasma or inside the macrophages. The further fate of the substance can then be very different inasmuch as, according to the circumstances, it may either be taken up by other thrombocytes, or undergo enzymatic inactivation, or finally act on the effector cells to provoke its peculiar biological actions.

It seems rather improbable that cellular disintegration is essential for the liberation of 5-HT from the thrombocytes, but nothing is known about the con-

ditions which regulate the possible "physiological" release of the substance by the intact circulating thrombocytes.

According to Bracco and Curti (33) serum contains a thermolabile factor which seems to be necessary for the diffusion of 5-HT from platelets into the serum. This factor is supposed to disappear in the 5-HT release process. Unfortunately the credibility of this interesting observation is seriously impaired by the fact that the above investigators do not seem experienced enough in working with isolated organs (see fig. 5, reference 31!)

Having demonstrated that platelets act as carriers even of adrenaline and noradrenaline, Weil Malherbe and Bone (283a) consider the platelets as an authentical store-house of circulating pharmacologically active amines.

b. The origin of 5-HT in the thrombocytes. In this connection at least two possibilities must be taken into consideration: (a) 5-HT originates in the thrombocytes or in the megacaryocytes during their development in bone marrow or in other hemopoietic organs. If this is so the enterochromaffin cells should obviously play the part of a simple emunctory of thrombocytic 5-HT. The mature platelet seems not to be capable of forming 5-HT from its precursor 5-hydroxytryptophan (265), which, moreover, does not occur in significant amounts in the platelet (307).

(b) 5-HT produced in the enterochromaffin cells and thence released into the plasma, is absorbed by the thrombocytes either immediately before or, more probably, after their penetration into the circulation. In the latter case the 5-HT uptake might occur either indiscriminately all over the circulatory system, or more probably in particular vascular areas, which may be in the organs producing 5-HT (gastrointestinal tract) or, less probably, in those which destroy thrombocytes (spleen).

This hypothesis seems to be supported by the bulk of experimental evidence and of comparative histochemical and biochemical data (100). One point on which particular stress should be laid is that the rate of metabolism of endogenous 5-HT, estimated from the urinary output of 5-hydroxyindole acetic acid, is intense enough not to support the theory that the disintegrating platelet is the only source of plasma 5-HT and the site of platelet formation the only site of formation of 5-HT, as postulated by Udenfriend and Weissbach (265).

c. The problem of the existence of free 5-HT in circulating plasma. If we allow that thrombocytic 5-HT has its origin in the enterochromaffin cells of the gastrointestinal mucosa, then it must of necessity be admitted that a part of the 5-HT, though perhaps a very small part, does exist and circulate free in the plasma. In fact, the exchange of 5-HT between thrombocytes and enterochromaffin cells can take place only through the mediation of plasma, and the substance released following damage or lysis of the platelets must obviously penetrate, at least in part, into the plasma. Moreover, only plasma 5-HT can be attacked by amine oxidase in the tissues, thereby giving origin to the 5-hydroxyindole acetic acid which the writer (100, 101, 102) and Titus and Udenfriend (257) have found in normal urine and which is considered to be derived from oxidative deamination of 5-HT. The well known extreme fragility of thrombocytes accounts for the

TABLE III

The content of 5-hydroxytryptamine in serum, hemolymph and spleen of various animals

Animal species	5-HT content		Animal species	5-HT content	
	Serum (μg./ml.)	Spleen (μg./gm.)		Serum (μg./ml.)	Spleen (μg./gm.)
Man			Sea gull	0.69	—
newborn	0.07	—	Stork	0.03	—
20–23 years old	0.12	—			
60–90 years old	0.09	—	*Tropidonotus natrix*	0.41	0.16
Ox	1.48	7.80	*Testudo graeca*	0.01	0.01
Goat	2.18	4.80			
Sheep	0.85	3.80	*Rana esculenta*	0.18	0.08
Horse	0.41	1.76	*Bufo bufo bufo*	0.02	—
Ass	0.41	3.10			
Hog	0.26	1.23	*Scylliorhinus canicula*	<0.02	<0.06
Dog	0.21	1.40	*Scylliorhinus stellaris*	<0.04	<0.06
Cat	3.80	8.40	*Torpedo marmorata*	<0.02	<0.06
Rabbit	3.53	19.60			
Guinea-pig	0.21	1.06	*Acipenser naccarii*	0.01	0.03
Rat	0.97	2.80	*Acipenser sturio*	0.025	0.03
Mouse	1.48	1.80			
Hamster	0.37	—	*Anguilla vulgaris*	<0.05	<0.05
Hedgehog	1.84	1.80	*Tinca vulgaris*	<0.04	<0.05
Bat (*Rhinolophus ferrum equinum*)	3.56	18.90	*Ameiurus catus*	<0.05	—
Hen	2.75	12.54	*Petromyzon planeri*	<0.05	—
Guinea-hen	2.66	—	*Petromyzon marinus*	<0.03	—
Turkey	0.11	—			
Duck	1.16	4.10	*Octopus vulgaris*	<0.04	Hemolymph
Pigeon	0.33	—	*Octopus macropus*	<0.04	Hemolymph
			Eledone moschata	<0.04	Hemolymph

circumstance that unambiguous evidence of the occurrence of free 5-HT in the circulating plasma is not yet available. Bearing in mind the above hardly avoidable source of error, Humphrey and Jaques (151) indicate for dog, rabbit and human plasmas a possible 5-HT content of 0.002 to 0.006 μg./ml.

d. 5-HT content of serum and hemolymph. The writer and Faustini (93, 97, 100) have estimated the 5-HT concentration in the serum of 36 species of vertebrates and in the hemolymph of 3 species of octopods. The results are shown in Table III.

The tabulated data agree satisfactorily with those reported by Rapport *et al.* (204) and Zucker *et al.* (307) for the ox, by Reid (226) for the cat, and by Humphrey and Jaques (151) for seven species of mammals. Quite recently Udenfriend and Weissbach (265) have obtained somewhat higher values, using a specific chemical method for 5-HT: human being 0.1 μg./ml. of whole blood, guinea-pig 0.15, rat 0.20, dog 0.25, ox 1.7, rabbit 4.0.

The following conclusions may be drawn from Table III: (a) 5-HT is present,

in detectable amounts, in the serum of all the animal species whose intestine contains typical enterochromaffin cells and, therefore, 5-HT itself. Apparent exceptions are the *Elasmobranchii* and the *Chondrostei* for which we may suppose the quantity of serum 5-HT is so small (scarcity or absence of thrombocytes ?) as not to be detectable by the usual test-objects.

(b) 5-HT is always lacking in serum, where typical enterochromaffin cells are not present in the gastrointestinal mucosa (*Teleostei*, *Cyclostomata*).

(c) The 5-HT content in serum varies conspicuously from one animal species to another; remarkable also are individual variations in the same species.

(d) Serum 5-HT shows no quantitative relation to that of gastrointestinal mucosa.

Blood samples collected by puncture of the jugular vein, the renal vein, the femoral vein and the femoral artery of the anesthetized dog showed no significant differences in their 5-HT content. The same is true for blood samples obtained in anesthetized human beings by puncture of the brachial vein and the femoral artery, as well as by catheterization of the jugular and the right renal vein (105).

In an acute experiment on a dog weighing 8 kgm., in which 50 ml. of blood were withdrawn from the femoral artery every hour until death, the last blood sample contained only 20–30 % less 5-HT than the first one (105).

In preliminary researches the writer has estimated the 5-HT content in the serum of 84 patients suffering from various diseases (100). The values obtained show, among other things, that the serum 5-HT level of hypertensive patients (24 cases) is by no means higher than normal, but rather lower. This statement provides additional evidence that 5-HT does not intervene directly either in provoking or in maintaining the high vascular tone which is the cause of the hypertensive disease. The 5-HT values were also subnormal in cirrhosis of the liver and extremely low in Werlhof's disease.

A similar investigation has been carried out by Bigelow (21). He found that serum 5-HT was diminished in five thrombocytopenic patients, two hemophiliacs with normal platelet count, two patients with vascular purpura, two instances of severe hypoprothrombinemia, as well as in four splenomegalic patients with marked thrombocytosis. The serum 5-HT level was not decreased in three subjects with pseudohemophilia. Remission of thrombocytopenia following splenectomy was associated with the restoration of serum 5-HT to normal.

3. Spleen. 5-HT was estimated in spleen extracts of 26 species of vertebrates (93, 100). It was found that the 5-HT content in the spleen is always strictly dependent upon that in the serum. In mammals and birds, 1 gm. of spleen tissue usually contains from 2 to 7 times more of this substance than 1 ml. of the corresponding serum. This statement leaves little doubt as to the origin of the 5-HT in the spleen: the disintegrating thrombocytes are certainly the source of the substance.

In different cases, splenic 5-HT may represent quite a modest part of the total 5-HT content of the organism or a more important part. In this connection the species differences in the size of the spleen are also to be kept in mind. Faustini

(110) has been able to establish that 5-HT appears in the spleen from the earliest stages of its embryonic development. In addition to normal human spleens, the 5-HT content has been studied in pathological spleens, from autopsy or operation. No particularly noteworthy results emerged (71).

4. Central and peripheral nervous tissue. According to some recent investigations, it seems probable that the central nervous system of mammals as well as certain ganglia and peripheral nerves of invertebrates contain detectable quantities of 5-HT.

Amin, Crawford and Gaddum (4, 5) first found that acetone extracts from various parts of the central nervous system of the dog stimulated the atropinized estrous uterus of the rat. The spasmogenic effect corresponded, per gm. of fresh tissue, to that provoked by 0.01–0.35 μg. of 5-HT. The area richest in 5-HT was the hypothalamus, followed by the area postrema, the mid-brain, the colliculi, the nuclei cuneatus and gracilis, the gray matter of the spinal cord, the medial part of the thalamus and finally by area 4 of the cortex. 5-HT was not found in the nucleus caudatus, the cerebellum, the white matter nor in other central or peripheral nervous structures.

Similar results were obtained by Twarog and Page (260) and by Zetler and Schlosser (300). The first found that the 5-HT content of the whole brain of the dog is 0.1–0.36 μg./gm. and of the rat, 0.24 μg./gm. The isolated heart of *Venus mercenaria* was used as a test-object. Zetler and Schlosser, similarly using a molluscan heart preparation, the isolated heart of *Helix pomatia*, estimated the 5-HT content of ala cinerea and trigonum hypoglossi of the ox brain as 0.07 μg./gm.

In the invertebrates Florey and Florey (117, 118) claim to have found a substance biologically indistinguishable from 5-HT in aqueous extracts of cuttlefish and crab ganglia (20–82 μg. per gm. of dry weight) as well as in extracts of the nerves of crab legs (30–66 μg./gm.).

Welsh (285, 287) has confirmed these data working with extracts of molluscan ganglia and heart. In ganglia of *Venus mercenaria* (pooled cerebro-pleural, visceral and pedal ganglia) the ratio of 5-HT (about 15 μg. per gm. wet weight of tissue) to acetylcholine was found to be 4:1, in ganglia of *Busycon canaliculatum* about 1:1. Since 5-HT stimulates the heart of *Venus* and the visceral ganglion of this mollusc, following block of the cholinergic inhibitor nerves, Welsh is of the opinion that "the addition of 5-HT to the list of chemically-known substances that are produced by nerve cells and that act on effectors or other neurons would appear proper".

The researches referred to above are obviously of the greatest interest. Nevertheless, in our opinion, no incontrovertable proof that the active substance in mammalian nervous tissue is identical with 5-HT has yet been provided. We believe that, especially in the case of the discovery of new unexpected 5-HT localizations, the biological assay, whatever it may be, ought to be substantiated by chemical assay, e.g., by the chemical identification of 5-HT on paper chromatograms run with various solvent systems. Moreover, as Zetler and Schlosser (300) rightly point out, we must not forget that nervous tissue is always richly vascu-

larized and that blood may show in some cases very high 5-HT levels. Accordingly the possibility exists that the supposed brain 5-HT is in reality blood 5-HT. It seems improbable, however, that this is the only source of 5-HT in the brain.

5. Other localizations of 5-HT. Woolley and Shaw (297) claim to have found 5-HT in macerated carotid arteries; Twarog (261) states that the substance occurs in extracts of the anterior byssus retractor muscle of *Mytilus edulis* (1.0 μg./gm.). The above reservations apply also to these observations.

Convincing evidence has been provided quite recently by Jaques and Schachter (155) for the presence of 5-HT in the venom of the common wasp. Pooled wasp venom showed a 5-HT content of approximately 0.32 mgm./gm., which represents for a single venom apparatus a content of about 0.06 μg. of substance. This localization of 5-HT is rather curious and further emphasizes the widespread occurrence of the substance in nature. Since the concentration of 5-HT in venom is greater than the pain producing threshold concentration of this product for human skin (7), it seems possible, according to Jaques and Schachter, that 5-HT may account for some features of the human skin reaction following a wasp sting.

Unlike wasp venom, bee venom was found to contain little or no 5-HT (232a). These observations were fully confirmed by Erspamer and Pavan (unpublished observations) who found that the bee venom apparatus contains at best 0.0005 μg. of 5-HT per apparatus (= 1.3 mgm.), whereas the venom apparatus of *Polistes gallica*, a wasp, contains 0.7–0.8 μg. of 5-HT per apparatus (= 2.5 mgm.).

The quantitative studies on 5-HT reported above allow us to present reliable information about the total content of 5-HT in various mammals, including all the common laboratory animals. This content is shown in Table IV, in which the minute quantities of the substance present in the cerebro-spinal axis are disregarded.

The data in Table IV, must not be lost sight of when attempting to interpret the biological significance of 5-HT. Indeed; it is obvious that the only actions which can be thought of as possibly "physiological" are those provoked in the intact animal by 5-HT doses lower than or, at the most, equal to, the total 5-HT content of the organism, while all other actions have to be considered as "pharmacological".

III. TOXICITY OF INDOLEALKYLAMINES

Data on the acute toxicity of the indolealkylamines are, with few exceptions, quite incomplete; data on chronic toxicity are virtually lacking.

Gramine. Mouse, LD50 by intravenous route: 44.6 ± 1.4 mgm./kgm. (197); 45.6 mgm./kgm. (2). Rat, LD50 by intravenous route: 62.9 ± 2.7 mgm./kgm. (197).

Gramine first stimulates the central nervous system (clonic convulsions, hyperpnea), then depresses it. Death seems to be due to a central respiratory failure (254).

3-Diethylaminomethyl-indole. Mouse, LD50 by intravenous route: 26.6 mgm./kgm. (2).

TABLE IV

The content of 5-hydroxytryptamine in serum, spleen and gastro-intestinal tract of various animals

	5-HT in μg. per kgm. of Body Weight			
	Serum	Spleen	Gastro-intestinal tract	Total
Man	3.5	—	—	—
Ox	51.8	—	—	—
Goat	76.3	—	—	—
Sheep	29.7	—	—	—
Horse	14.3	—	—	—
Ass	14.3	—	—	—
Hog	9.1	—	—	—
Dog	7.3 (4.7%)	8.6 (5.4%)	141.6 (89.9%)	157.5 (100%)
Cat	133.0 (70.7%)	16.6 (8.8%)	38.4 (20.5%)	188.0 (100%)
Rabbit	123.5 (33.2%)	5.8 (1.6%)	242.6 (65.2%)	371.9 (100%)
Guinea-pig	7.3 (4.6%)	1.6 (1.3%)	142.2 (94.1%)	151.1 (100%)
Rat	34.0 (27.1%)	11.0 (8.9%)	80.1 (64.0%)	125.1 (100%)
Mouse	51.8 (14.7%)	15.6 (4.4%)	285.6 (80.9%)	353.0 (100%)
Hamster	13.0	—	—	—
Bat	124.6 (53.3%)	32.0 (13.8%)	77.1 (32.9%)	233.7 (100%)
Hedgehog	64.4 (20.3%)	8.3 (2.6%)	244.4 (77.1%)	317.1 (100%)
Hen	—	18.8	192.9	—
Duck	—	1.9	104.4	—

2-Methylgramine. According to Supniewski and Serafin-Gajewska (255) this alkaloid is 3 to 4 times more toxic than gramine.

5-Methoxytryptamine HCl. Mouse, LD50 by intravenous route: 60 mgm./kgm.; by subcutaneous route: 750 mgm./kgm. Rat, LD50 by subcutaneous route: 600 mgm./kgm. (83).

5-Hydroxytryptamine base. Mouse, LD50 by intravenous route: 160 mgm./kgm.; by subcutaneous route: >868 mgm./kgm. Rat, LD50 by intravenous route: 30 mgm./kgm.; by subcutaneous route: approximately 117 mgm./kgm. (123).

Anesthesia greatly enhances the toxicity of 5-HT. The rat anesthetized with pentobarbital may die following intravenous doses of the substance as small as 0.1 mgm./kgm. (51). Similar observations have been made in rabbits, guinea pigs, hens (51), dogs (177), and cats (100). Death is due to respiratory failure. In the rat at post mortem examination visceral congestion is frequently found. The kidney is either normal or shows a mottled surface. In section ischemic cortical areas may alternate with congested areas (83). Rats treated over a period of 30 to 60 days with daily doses of 0.8 mgm./kgm. of 5-HT showed no changes in systemic blood pressure (50).

Bufotenine. Guinea-pigs withstood 20 mgm. of the base; mice died after 0.5 mgm. injected intraperitoneally, apparently from respiratory failure; frogs reacted only to doses of 1 mgm./gm. of body weight with severe toxic phenomena (miosis, cramps, respiratory arrest, nicotinic posture, central and peripheral paralysis) (140).

IV. PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIONS OF INDOLEALKYLAMINES

A. Action on the systemic blood pressure

1. Dog. The initial response of the normotensive dog under pentobarbital anesthesia to medium doses of 5-HT (10–30 μg./kgm.) is usually hypertensive. The rise in pressure, however, is always moderate and barely correlated with the dose (59, 83, 100, 147, 189, 190, 191, 234, 235).

Small amounts of 5-HT (2–8 μg./kgm.) can, in different cases, produce a slight rise or a slight fall of blood pressure or even biphasic reactions (a fall followed by a slight rise); larger amounts (35–150 μg./kgm.) are either distinctly hypertensive (the pressure rise, however, rarely exceeds 40–60 mm. Hg), or else provoke polyphasic reactions (100, 190, 191, 234). Initial bradycardia is regularly observed, occasionally followed by increase of the heart rate over control levels at the height of the pressure response (234).

These results are those obtained in the earliest stages of the experiment. As this is continued and subsequent injections of 5-HT or related substances are given, the pressure reaction becomes still more irregular and unpredictable. We can, therefore, conclude with Page (190) that "the protean character of the response seems to be the most characteristic feature of the action of the tryptamines on arterial pressure".

Exclusion of the carotid sinus (190) and ganglionic blockade with tetraethylammonium or methonium compounds (190, 194, 234) seem to intensify the hypertensive reaction to 5-HT. According to Page (190) the same result can be obtained by transection of the spinal cord at C_6, atropinization of the animal or cervical vagotomy. This has not, however, been confirmed (59, 100, 123, 234).

The hypertensive effect of 5-HT is frequently weakened by sympatholytic agents (piperoxane, priscoline, yohimbine, dibenamine, dibenziline, ergotamine, hydrogenated ergot alkaloids, regitine, SY-28), much more rarely it is abolished or reversed completely (83, 91, 123, 190, 194, 234). Procaine diminishes the blood pressure rise and abolishes in most experiments the initial fall (234). Bilateral adrenalectomy during an acute experiment does not seem to alter the cardiovascular and respiratory response to 5-HT. Therefore, a secondary adrenaline output is not believed to be responsible for the blood pressure effect in the dog 235). Bilateral nephrectomy augments selectively the pressor actions of 5-HT and tryptamine, though less regularly and less prominently than the actions of angiotonin and renin (178). 1-Hydrazinophthalazine has by no means been proved to be a constant and specific inhibitor of 5-HT (100). 2-Methyl-3-ethyl-5-aminoindole and 2-methyl-3-methyl-5-aminoindole (294, 297) antagonize the circulatory effects of 5-HT inconstantly and partially. If indeed they are occasionally able to attenuate or abolish the hypertensive phase in the reaction to the substance they never reduce, rather they sometimes seem to accentuate, the hypotensive phase (194). The anti-5-HT action of the nitroindoles given orally is doubtful (194). 2-Methyl-3-ethyl-5-dimethylaminoindole and 1-methyl-2-methyl-3-ethyl-5-dimethylaminoindole (241), given either orally or subcutaneously, are similarly incapable of protecting normal dogs from the pressor effects

of 5-HT (240). An unequivocal inhibition of these effects cannot be obtained even with our most potent drugs antagonistic to 5-HT, lysergic acid diethylamide and 2-methyl-5-chlorogramine (100, 103).

Intravenous injections of 5-HT repeated at brief intervals provoke pressor reactions of progressively decreasing intensity. Such tachyphylaxis, however, seems to be of short duration (5–15 minutes), and this would explain why it was not noted by Page (190). According to him and to Freyburger *et al.* (123) 5-HT is 10 to 30 times less potent than l-adrenaline and 15 to 16 times less potent than l-noradrenaline.

Heymans and Van den Heuvel-Heymans (147) and Page *et al.* (191, 192, 193, 194) have recently made important contributions to the interpretation of the mechanism of the effects on circulation and respiration provoked by 5-HT in the dog. Having ascertained that 5-HT has no stimulant action on the chemoreceptors of the carotid sinus and that its circulatory and respiratory effects do not depend on stimulation of afferent fibers in the carotid sinus nerves or in the cervical vago-aortic nerves, Heymans and Van den Heuvel-Heymans maintain that 5-HT hypertension must be primarily due to peripheral vasoconstriction and secondarily to direct stimulation of the vasomotor centers.

Page *et al.* (191, 193) consider the pressure response to 5-HT to be the result of various distinct factors: 1) intense direct vasoconstriction; 2) a reflex, possibly of the nature of the Bezold-Jarisch effect, that can be blocked by atropine; 3) transient autonomic ganglion blockade; 4) strong peripheral action that reduces or abolishes neurogenic vasoconstriction; 5) cardiac stimulation. Factors 1) and, to a lesser extent 5), are believed to be responsible for the hypertension; factors 2) and 3) for the initial transient pressure fall; factor 4) for the more persistent secondary hypotension. On the basis of experiments carried out on normotensive dogs pre-treated with ganglion-blocking drugs and in dogs with neurogenic hypertension, as well as in cats and rabbits, both normal and with ganglionic blockade, Page and McCubbin (191) are of the opinion that inhibition of neurogenic vasoconstrictor tone is a decidedly more important phenomenon than direct vasoconstriction. They believe consequently that the direction and intensity of the pressure response to 5-HT are to a great extent determined by the degree of normal resting vasoconstrictor tone.

According to Schneider *et al.* (235) the amount or kind of adrenal steroids present in the organism also influences the blood pressure response to 5-HT. In contrast to the intact dog, the adrenalectomized animal, maintained for several weeks on desoxycorticosterone trimethylacetate, was found to react to 5-HT with only a transient pressure rise of a few seconds duration followed by a fall below control levels.

Responses to 5-methoxytryptamine (100) and to 7-hydroxytryptamine (189, 190) are similar to those just described. Both substances are, however, 3 to 5 times less potent than 5-HT.

Tryptamine (0.1–1 mgm./kgm.) causes a rise in systemic arterial pressure which is occasionally preceded or followed by a fall (162, 190, 213, 223). These effects can be seen when the vagus nerves are intact or divided. The pressor

response is reduced but not completely abolished by yohimbine (2–4 mgm./kgm.) (223); it seems, on the contrary, definitely augmented after spinal cord transection, ganglionic blockade and pitressin administration. Piperoxane and priscoline have little or no effect (190).

Reid (223) ascribes the rise in systemic arterial pressure to direct action on the vascular smooth muscle and, subordinately, to some stimulant action on the heart, the initial pressure fall to pulmonary vasoconstriction and the secondary fall possibly to coronary vasoconstriction. The response to tryptamine in neurogenic hypertensive dogs was somewhat less than in normal ones (190).

Bufotenine and even more bufotenidine differ from the above indolealkylamines having a primary amino group in the lateral chain, in being more regularly and potently hypertensive. Bufotenine and bufotenidine injected intravenously (0.05–1 mgm./kgm.) provoke a marked rise in blood pressure, which is potentiated by cocaine and abolished or reversed by yohimbine, and still more by yohimbine plus harmalol (212, 215, 217, 218, 219, 220). Sparteine shows no effect (221). Bufotenine increases the reactivity of the circulatory system to successive injections of adrenaline, but it inhibits in part the pressure rise provoked by carotid occlusion (214). On the basis of the above results and of his observation that bufotenine and bufotenidine have only little constrictor effect when applied locally on the vessels (212, 215), Raymond-Hamet holds the opinion that both substances are to be listed among the nicotine-like drugs, acting on the vessels and on the blood pressure through a discharge of adrenal sympathin (216, 218).

Gramine injected in the dog under chloralose anesthesia evokes variable responses, depending upon the dose: with 1.25–2.5 mgm./kgm. a moderate hypertension is predominant; with 8–9 mgm./kgm. the transient initial pressure rise is followed by prolonged hypotension (210). Gramine is to be listed among the minor adrenolytic drugs (211).

2. Cat. In the cat under pentobarbital or chloralose anesthesia, pressure changes produced by intravenous 5-HT are generally moderate and still less predictable than in the dog. The most common reaction is hypotension, sometimes followed, or interrupted, by a slight pressure increase (46, 47, 83, 123, 133, 190, 191, 225, 226, 233, 234). If present, this is less intense than that provoked by doses of adrenaline or noradrenaline 60 to 100 times smaller (83, 123). Simultaneously with the fall in blood pressure an abrupt and marked drop in heart rate is seen, rarely followed by a slight increase in heart rate (234).

Cocaine potentiates, as a rule, the pressor response to 5-HT. Purely hypotensive reactions after this alkaloid are rare, hypertension is more sustained and with large doses of 5-HT (20–40 μg./kgm.) a considerable secondary pressure rise may appear. This has been attributed to stimulation of the suprarenal medulla (123). Procaine 10 mgm./kgm. and nupercaine 0.5–0.7 mgm./kgm. antagonize the fall of pressure and bradycardia provoked by large doses of 5-HT (233, 234). Piperoxane and dibenamine generally reduce, but do not suppress, 5-HT hypertension (123). Yohimbine 1–2 mgm./kgm., on the contrary, does sometimes abolish it completely (226, 194). Whereas it seems well established

that ganglionic blockade can abolish the normal depressor action of 5-HT and possibly reverse it to a pressor one (191, 234), there exists no agreement concerning the influence of atropine and vagotomy. The statement of some research workers (47, 190, 234) that the hypotensive effect of 5-HT is largely abolished after atropine or vagotomy, and pressor action increased, has not been confirmed by others (123, 133, 225, 226).

When given intravenously to the vagotomized cat under dial anesthesia, 5-HT (0.25–0.5 mgm./kgm.) reinforces and prolonges the pressor effect of successive doses of adrenaline (5–10 μg./kgm.). Lecomte (163) attributes this sensitization, which disappears after 30 minutes and cannot be obtained with tryptamine, to the presence of a phenolic hydroxy group in the 5-HT molecule, which retards the oxidation of the adrenaline injected.

In marked contrast to the cat with intact nervous system, the pithed and the spinal cat always respond to 5-HT with a clear hypertensive reaction which may be somewhat enhanced by cocaine, but which is always at least 25 to 50 times less than that produced by adrenaline (83, 123, 191, 233).

Whereas the pressure rise is generally attributed to direct peripheral vasoconstriction, no agreement exists in the interpretation of the hypotension caused by 5-HT. Reid (226) ascribes the short-lived pressure fall that occasionally precedes hypertension to a primary constriction of the pulmonary arterial bed, with ensuing reduction in the volume of blood returning to the left heart; he offers no explanation for the prolonged secondary hypotension. Comroe *et al.* (47) believe that the hypotension is largely due to the associated bradycardia. Page and McCubbin (191) hold that the explanation for pressure changes given for the dog applies equally to the cat. Schneider and Yonkman (234) consider it to be part of a complex cardio-pulmonary mechanism. Finally, Ginzel and Kottegoda (133) maintain that the mechanism of the primary pressure fall may differ depending upon the magnitude of the dose of 5-HT injected. Hypotension which follows the introduction of small doses of 5-HT seems to be due predominantly to baroreceptor stimulation and secondarily to central stimulation; pulmonary vasoconstriction would come into play only with higher doses of the substance. Ginzel and Kottegoda rightly emphasize the ability of 5-HT and tryptamine to produce temporary desensitization to their own actions. It is very likely that many contradictory findings with these two substances and with their antagonists can be explained by this self-antagonism.

Bufotenine given to the cat under urethane anesthesia in doses of 0.2–0.3 mgm./kgm. elicits a triphasic response consisting of a transient fall of pressure, a rise, and a secondary more sustained fall (140).

As distinct from the 5-hydroxytryptamines, tryptamine has the characteristics of a predominantly hypertensive drug even in the cat, thus giving pressure responses similar to those described in the dog (138, 162, 223). Cocaine does not potentiate tryptamine hypertension (223); yohimbine, ergotoxine, hydergin, and, to a lesser degree, nicotine as well as diphenhydramine (benadryl) and other antihistaminic agents reduce it somewhat (138, 223); ganglionic blockade, atropine and curare show no remarkable effect (138, 163).

Tested in the decerebrated or pithed cat, N-methyltryptamine is less hypertensive than tryptamine, and N,N-dimethyltryptamine still less (42, 100). The trimethylammonium derivative seems, on the contrary, more active than tryptamine, possessing about $\frac{1}{20}$ of the pressor action of adrenaline (42). Gramine in doses of 10 to 20 mgm./kgm. causes a rise of pressure, but after doses of 30–40 mgm./kgm. this rise is preceded by a fall (197).

3. Rabbit. The rabbit under urethane or pentobarbital anesthesia reacts to crude enteramine extracts and to 5-HT with a pure hypotensive response, which is easily repeatable and satisfactorily correlated with the dose (64, 65, 83, 191, 234). Transient slowing of the heart rate associated with cardiac irregularities is also present with uniformity (234). Atropine, bilateral section of the vagus nerves and sympatholytic doses of dibenamine show either no effect or only a slight one on the depressor action of 5-HT (65, 91, 191). Schneider and Yonkman (234) could not confirm the statement of Page and McCubbin (191) that ganglionic blockade reverses the action of 5-HT from depressor to pressor.

The rabbit is less sensitive to tryptamine than either the dog or the cat. Injections of 0.2–1.8 mgm./kgm. cause a small rise in systemic arterial pressure which may be preceded by a transient fall and followed by a further fall lasting for 2 or 3 minutes. There is little change in pressure in the right ventricle (223). The response to bufotenine is like that observed in cats (140). Gramine in high doses causes a moderate lowering of the blood pressure (137, 254).

4. Rat. The intact unanesthetized rat responds to 5-HT given subcutaneously in doses of 0.4 mgm./kgm. and above with a regular pressure fall, lasting 90 to 180 minutes, accompanied by intense dilatation of the cutaneous vessels. Doses of less than 0.4 mgm./kgm. are either ineffective or nearly so. 5-Methoxytryptamine is approximately 4 times less potent than 5-HT (49, 83, 89). The lowering of pressure is not antagonized by intraperitoneal dibenamine, in doses of 10 mgm./kgm. (49).

The response to intravenous 5-HT can differ depending on the initial pressure level. In normal rats hypotension is by far the most frequent reaction; in renal hypertensive rats under anesthesia hypertension is predominant (236). The pressure rise seems to be increased, rather than diminished, by 1-hydrazinophthalazine (236).

5. Guinea-pig. Intravenous injections of 0.1–0.2 mgm./kgm. of 5-HT in the animal anesthetized with pentobarbital usually elicit moderate hypertensive responses. With higher doses (0.4–0.8 mgm./kgm.) hypertension is preceded or followed by a hypotensive phase (83).

6. Man. Spies and Stone (251) found that intravenous injections of 0.5–5 mgm. of 5-HT constantly produced a rise in both systolic and diastolic pressure independent of the initial pressure level, i.e. in normal as well as in hypotensive and hypertensive patients. After 5 to 10 minutes, the pressure returned to normal. Subsequent injections at intervals of 2 to 3 hours gave identical results.

Page and McCubbin (191) obtained partly discordant results from continuous pressure recordings in 20 hypertensive patients. They believe that the most common response of hypertensive human beings to small intravenous doses of

5-HT (0.06–0.12 mgm.) is slight hypotension with a negligible hypertensive component. Larger doses (0.3–1.8 mgm.) produced the typical triphasic response observed in dogs. Intravenous injection of 5-HT in human beings is accompanied by unpleasant though fleeting side effects. The subcutaneous administration of 8 mgm. of 5-HT did not affect the systemic blood pressure nor cause systemic side effects (Correale, unpublished observations).

Woolley and Shaw's antimetabolites do not in themselves modify the blood pressure of hypertensive patients, nor do they antagonize the pressor action of subsequent doses of 5-HT (153, 194, 251).

7. *Summary.* From all the preceding data concerning 5-HT two conclusions may be drawn:

(a) 5-HT is neither a pure hypertensive nor a pure hypotensive agent. According to the dose, the route of administration, the anesthetic used, the neurogenic vasoconstrictor tone, the general conditions of the cardio-vascular apparatus and, above all, the animal species, 5-HT can elicit hypotensive, hypertensive or mixed responses.

(b) The action of 5-HT on the systemic blood pressure is, in every case, very moderate and, what is more important, of doubtful physiological significance. We have to keep in mind that even an abrupt release of all the 5-HT in the platelets could barely modify the systemic blood pressure, and for only a few minutes at that.

On the basis of these considerations the writer considers the systemic vascular action of 5-HT as a "pharmacological" action (100).

B. Action on special vascular areas

1. Pulmonary vessels. The intravenous injection of serum vasoconstrictor or of pure 5-HT (5–40 μg.) in the cat causes a rise of pressure in the right ventricle and in the pulmonary artery, accompanied by a fall in the pulmonary vein (46, 47, 224, 225, 226). This phenomenon is due to an increase in the pulmonary resistance and not to cardiac stimulation. It is not abolished by vagotomy nor by atropine, but may be in part antagonized by yohimbine (226) and, more efficaciously, by ergotamine and lysergic acid diethylamide (132). The pressor response in the lesser circulation is lacking if 5-HT is injected into the pulmonary vein.

MacCanon and Horvath (174) have introduced 5-HT (29–45 μg./kgm.) directly into the pulmonary artery of dogs anesthetized with pentobarbital. After a latency of 5–8 seconds a systemic hypertensive response was observed, lasting 2 to 5 minutes. No alterations in cardiac output and pulmonary blood volume, measured by the tracer dilution technique, were noted in the prehypertensive stage. At the peak of the hypertensive reaction variable responses were obtained.

The action of tryptamine in the dog, the cat and the rabbit is similar to that just described for 5-HT (223).

According to Ginzel and Kottegoda (132) 1 dose of 5-HT is equipotent, on the pulmonary vessels, to 2.4 doses of tryptamine and to 10–20 doses of adrenaline or noradrenaline.

2. Portal vein. The portal pressure in the dog is raised by the systemic intravenous injection of tryptamine. This is not due to constriction of the hepatic sphincter but appears to be a passive result of increased arterial pressure (223).

3. Spleen vessels. 0.5–2 mgm. of bufotenine injected intravenously provoke a contraction of the dog spleen, which lasts longer than the pressure rise (217).

4. Renal vessels. According to Reid and Rand (224), and Reid (226), the eviscerated adrenalectomized cat responds to the introduction into the renal artery of serum vasoconstrictor and of pure 5-HT (20–100 μg.) with a remarkable reduction in the outflow from the renal vein, as a consequence of an increase in the intrarenal resistance. Reid holds, however, that the substance acts less strongly on renal vessels than on other vascular districts. Ginzel and Kottegoda (132) affirm that even in doses of 200 μg., injected intravenously, 5-HT does not decrease the renal outflow more than 20 per cent, and that 20 to 300 μg. of 5-HT injected intraarterially produce reductions in outflow varying from 10 to 40 per cent.

The dog preparation seems to be somewhat more sensitive. In Page's experiments (190) the injection of 5-HT into the renal circulation regularly produced intense renal vasoconstriction, although not so great as that caused by noradrenaline in equipressor doses. Raymond-Hamet (215, 217, 218) has studied the action of bufotenine and bufotenidine on the circulation of the dog kidney by plethysmographic methods. It was shown that both substances, but especially bufotenidine, consistently reduced the volume of the organ, thus indicating an intrarenal vasoconstriction.

In contrast to the 5-hydroxyindolealkylamines, tryptamine (1–2 mgm./kgm.) has very little action on the renal vessels of the cat and the dog (132, 213, 223). The kidney volume after gramine injections passively follows the pressure changes (210).

The powerful constrictor effects of subcutaneous 5-HT on the afferent renal vessels of the intact unanesthetized rat and of fresh defibrinated blood on the heart-lung-kidney preparation of the dog will be discussed in detail later.

5. Rabbit's ear preparation. The vessels of the rabbit's ear, prepared and perfused as described by Page (187), are very sensitive to 5-HT and capable of giving reproducible results within 10 to 20 per cent in the same preparation. 0.15 μg. of 5-HT were found by Page (190) equivalent in their constrictor activity to about 0.025 μg. of adrenaline. Similar results were obtained by Ginzel and Kottegoda (132), who observed that the action of 5-HT on the ear vessels was usually even stronger than that of adrenaline, and that this action was not antagonized by hexamethonium or tolazoline.

Gaddum and Hameed (126) have used the ear preparation routinely in their studies on the drugs antagonistic to 5-HT. They found that piperoxane (400 μg./l.), atropine (100–1000 μg./l.) and cocaine (10 mgm./l.) did not significantly affect the response to 5-HT when introduced into the perfusion liquid. Dibenamine (100 μg./l.) showed a moderate antagonistic effect; gramine (10 mgm./l.) decreased the sensitivity of the ear preparation to 5-HT more than 2000-fold and that to adrenaline by about 15-fold. The most powerful antagonists to 5-HT,

however, were found to be lysergic acid diethylamide (10 μg./l.), dihydroergotamine (20 μg./l.) and ergotamine (40 μg./l.). These drugs always depressed the response to 5-HT more than that to adrenaline.

Gaddum and Hameed further demonstrated that the action of 5-HT on the rabbit's ear was greatly increased when ephedrine (10 mgm./l.) or choline-*p*-tolyl ether bromide (1 mgm./l.) was present in the perfusion liquid. They believe that this potentiation of the vasoconstrictor effect of 5-HT is due to the inhibition of amine oxidase.

6. Vessels of the perfused hind leg of the cat and the dog. The blood vessels of hind leg of the cat constrict moderately after the injection of 1–10 μg. of 5-HT into the femoral artery. The vasoconstriction, lasting over a 5 minute period, is frequently preceded by vasodilatation, and usually declines after repeated injections (225, 226). Tryptamine is 0.3–1 times as active as 5-HT; adrenaline is 10 to 500 times more active, but the duration of constrictor response is much greater for 5-HT than for adrenaline (132). Yohimbine and, more effectively, lysergic acid diethylamide antagonize 5-HT vasoconstriction (132, 226). The vessels of the denervated hind leg of the dog, perfused with their own blood, are similarly constricted both by tryptamine and 5-HT, the latter substance being about 10 times more potent than the former (190). When the leg maintains nervous connections with the body, and tryptamine or 5-HT is given intravenously, the pressure rise in the general circulation may be accompanied by vasodilatation and a fall in arterial pressure in the leg. It is not yet clear whether this vasodilatation is of reflex or of direct peripheral origin (191).

The injection of 0.1 mgm./kgm. of bufotenine into the femoral artery of a dog caused an 82 per cent reduction in the venous outflow; a subsequent injection of 5 mgm./kgm. of the base, a reduction of only 30 per cent. The substance was 100 to 500 times less vasoconstrictor than adrenaline. Raymond-Hamet (212) considers these results as indicative of a nicotine-like action of bufotenine on the vessels.

7. Hind legs of the frog and the toad. The constrictor action of 5-HT (100), bufotenine (140), and gramine (197) on the vessels of these preparations is extremely poor and variable, certainly at least 1000 times less than that possessed by adrenaline.

8. Vessels of the rat's mesoappendix. The intravenous injection of 0.1 mgm. of 5-HT or the local application of 2–3 drops of a 1 per thousand solution at 37°, provokes, within a few seconds, a constriction of the terminal arterioles and of the arterial segment of the capillaries, accompanied by a marked stasis in the remaining capillary bed (51).

9. Vessels of the rat's tail. Following intravenous (51) or subcutaneous (49) injection of adequate doses of 5-HT there appears, simultaneously with the changes in systemic blood pressure, a constriction of the tail artery, as evidenced by absence of hemorrhage or shortening of bleeding time when the tail is severed. The local application of 5-HT on the severed tail has no vasoconstrictor effect.

10. Blood vessels of the human skin. Intradermal injection of 5-HT in concen-

trations up to 1 in 2×10^5 produced no appreciable action in the skin over the front of the forearm. Concentrations greater than this frequently caused the appearance of a red reaction, lasting 15 to 45 minutes; there was neither itching nor wheal formation. In subjects with clearly visible and raised subcutaneous veins, doses of 5-HT as small as 1 μg. caused the total disappearance of the venous network and a sharp demarcation between the area with venoconstriction and surrounding areas (226). The intradermal injection of tryptamine in concentrations up to 1 in 200 caused no blanching, but a weak red reaction around the site of injection (223).

11. Vasa vasorum. 5-HT causes a marked constriction of the vasa vasorum of both the swine carotid and coronary arteries (247).

C. Action on isolated artery strips or rings

Isolated ring or spiral strips of ox, dog and especially sheep carotid were used for several decades in qualitative demonstration and in quantitative titration of the serum vasoconstrictor. The preparation has recently been re-evaluated by Reid and Rand (199, 200, 225, 226) and by Woolley and Shaw (240, 297) for the study of 5-HT and of 5-HT antagonists. The arterial strips respond to 5-HT with a contraction which is, within certain limits, satisfactorily dependent upon the amount of substance introduced into the bath. The threshold dose varies for 5-HT from about 0.05 to 0.01 μg./ml. of nutrient liquid; that for tryptamine is 17 times greater.

The contraction of the arterial strip caused by 5-HT is inhibited by yohimbine and ergotoxine (239) as well as by antimetabolites of the 5-aminoindole type (297), especially by 2-methyl-3-ethyl-5-dimethylaminoindole and by 1,2-dimethyl-3-ethyl-5-dimethylaminoindole which are, in the artery test, about 250 times more active than the parent, unalkylated amine (240); cocaine, on the contrary, acts to enhance this contraction (224).

Handovsky (140) states that bufotenine, too, causes contraction of the isolated ox carotid artery in concentrations of 1 in 10^5.

D. Cardiac actions

1. Dog. Freyburger *et al.* (123) found that 5-HT introduced into the circuit of a heart-lung preparation, not only lacked any stimulant action but produced a certain decompensation, as shown by the rise of pressure in the left auricle. The results obtained by Page (190) and by Schneider and Yonkman (234) under other experimental conditions were different. Working on a cord-transected dog, Page observed that after 5-HT stroke volume and minute output, measured from pressure-pulse tracings, were nearly doubled and calculated total peripheral resistance slightly decreased. Schneider and Yonkman, in their turn, found that high doses of 5-HT (30–300 μg.) injected into the perfusion fluid of an isolated heart produced a marked increase in the heart rate and a clear increase in the coronary flow, whereas the increase in amplitude of contractions was small.

According to McCawley *et al.* (177), 5-HT in doses of 2 μg./kgm. or larger evokes sinus tachycardia in anesthetized dogs. The phenomenon cannot be pre-

vented by adrenergic blockade or atropine. The substance (0.35–0.8 mg./kgm.) does not affect the auricular fibrillation established by vagal stimulation in the thyrotoxic dog, and fails to produce ventricular tachycardia in the dog under chloroform anesthesia or ventricular fibrillation under cyclopropane anesthesia. In doses of 2.2 mgm./kgm. 5-HT causes respiratory arrest in the anesthetized dog. When the animal is protected by artificial respiration the cardiac effects of this lethal dose consist merely in transitory ventricular extrasystoles originating from abnormal foci.

The intravenous injection of tryptamine (0.6 mgm./kgm.) provokes an initial increase in coronary flow, which may be followed by a decrease for some minutes (223).

2. Cat. Using the technique of isolated heart perfusion, both Reid (226) and Schneider and Yonkman (234) showed that 2–200 μg. of 5-HT in the cat heart cause an increase in heart rate, an increase in coronary flow and an increase in contractile force, which, in sensitive hearts, can result in persistent systolic contraction. These results suggest that bradycardia observed in the intact cat after 5-HT, is caused by vagal reflexes masking the direct stimulant action of the substance on the heart. Schneider and Yonkman succeeded in obtaining direct evidence of the influence of 5-HT on vagal afferent impulses. Since the increase in rate of these impulses observed after 5-HT is abolished by procaine, they conclude that in all probability 5-HT acts through a peripheral stimulation of sensitive vagal fibers.

3. Rabbit. Twenty μg. of 5-HT in the isolated rabbit heart cause a positive inotropic and chronotropic response, which is less intense and less prolonged than that provoked by 5 μg. of adrenaline. After a rest period there appears complete tachyphylaxis (123). The coronary flow is scarcely influenced (234).

The isolated rabbit auricle reacts to 45 μg. of 5-HT in 100 ml. of nutrient liquid with an increase in heart rate similar to that obtainable with 3 μg. of adrenaline or 1.5 μg. of noradrenaline (177). Sinha and West (246) confirm that 5-HT, in concentrations of 1 in 10^6, has a powerful stimulant action on the isolated auricle. Stimulation is however preceded by a slight inhibition in the amplitude of the contractions and followed by a more marked depression. Atropine (10^{-7}) and hexamethonium (10^{-5}) can abolish the initial inhibiting effect, but not subsequent one; procaine (5×10^{-7}) causes a 50 per cent reduction in 5-HT stimulation.

4. Frog. No experiments have been published on the isolated frog's heart with 5-HT. Fifty-six μg. of bufotenine had no effect on the preparation; 230 μg. caused, after a latency period, a decrease in heart rate and a subsequent arrest of the organ (140). Gramine 5×10^4 showed a similar negative inotropic and chronotropic action (197).

5. Molluscs. Crude enteramine extracts and pure 5-HT provoke a marked increase in the amplitude and frequency of systolic contractions of *Octopus* and *Helix* hearts, whether isolated or *in situ*. The tonus, too, rises conspicuously, and with larger doses of the substance a persistent systolic arrest may result (12, 82, 300). The stimulant action extends over the pulsatile arteries, e.g. those

of the kidney and gills, with reinforcement of their contractions or with renewal of their rhythmic activity if this had disappeared. The hearts of *Murex trunculus*, *Murex brandaris*, *Dolium galea* and *Aplysia limacina* are less sensitive than *Helix* or *Octopus* hearts. Tryptamine shows a similar action, which is, however, less prompt and 20 to 25 times less intense.

On the basis of their experimental results, Erspamer and Ghiretti (82) and Bacq *et al.* (12) hesitate to consider 5-HT a chemical transmitter or a cardiovascular hormone.

Welsh (286) has recently carried out a thorough study of the action of 5-HT and acetylcholine on the heart of several other mollusc species, both perfused *in situ* and isolated. The hearts of *Pecten marinus* and *Modiolus modiolus* showed a low sensitivity to 5-HT, whereas those of *Venus mercenaria*, *Cyprina islandica* and *Buccinum undatum* proved most satisfactory in the bioassay of 5-HT (threshold = 10^{-10} gm./ml.). Lysergic acid diethylamide and ergot alkaloids blocked the stimulant action of 5-HT; mytolon, on the contrary, was ineffective. Welsh holds the opinion that the molluscan heart has "an excitory innervation mediated by 5-HT".

6. Crustaceans. The *Carcinus* heart is stimulated by 5-HT in dilutions down to 1 in 10^9. Adrenaline is about 100 times less active (117).

E. Action on extravascular smooth muscles

1. Gastro-intestinal tract. Rat. The rat *duodenum* is a very sensitive test-object for 5-HT (0.001–0.005 μg./ml.), bufotenine and bufotenidine, to which it responds, even when atropine is present, with a short-lived increase of tonus frequently accompanied by a reinforcement of spontaneous movements (65, 72, 83). The duodenal smooth muscle, however, seldom shows that reproducibility of response which is indispensable for an acceptable quantitative titration. Tachyphylaxis is particularly evident with bufotenine and bufotenidine (72). The stimulant action of 5-HT is strongly and irreversibly reduced by dibenamine (91). A clear inhibition is also produced by bufotenine (72).

The rat *colon* has been used successfully by Feldberg and Toh (111) and by others (53, 151, 166) not only in the qualitative demonstration, but also in the quantitative estimation of 5-HT in crude tissue extracts. Though the sensitivity of the preparation is somewhat variable, good responses are usually to be obtained with 0.01–0.02 μg. of the substance per ml. of nutrient liquid, and sometimes with considerably smaller doses, too. The organ reacts to 5-HT even after introduction into the suspension bath of atropine or mepyramine in amounts sufficient to render it insensitive to acetylcholine and histamine. It has the further advantage of being only slightly sensitive to substance P (111, 166).

Mouse. The mouse jejunum is stimulated by 5-HT in dilutions down to 1 in 3×10^9 to 5×10^8 (83).

Guinea-pig. The small intestine responds to 5-HT, in dilutions down to 1 in 10^8–10^9, with a contraction resembling that produced by histamine. Even the most intense spasmogenic effects, however, e.g. those provoked by 2–4 μg./ml. of 5-HT, are transitory and the smooth muscle becomes refractory to further doses of the substance (123, 124, 126).

It seems now well established that 5-HT stimulation of the small intestine of the guinea-pig is diminished by atropine (10^{-6}–10^{-8}). There is, however, no agreement about the degree of the antagonism (111, 124, 126, 166, 208, 231). A partial explanation of the discrepancies existing in the literature is suggested by Robertson (229), who found that atropine (10^{-7}–10^{-8}) blocked the action of 5-HT 2×10^{-9}, but not that of 5-HT 2×10^{-8}.

The effect of antihistamine drugs is not uniform: triphelenamine and mepyramine are inactive or barely active (111, 123, 124, 208); diphenhydramine, on the contrary, is clearly antagonistic to 5-HT (208, 224). Procaine, cocaine and moderate doses of β-tubocurarine and nicotine depress or abolish the spasmogenic effect of 5-HT; but high doses of nicotine as well as of hexamethonium or decamethonium have no inhibitory effect (126, 230, 246). From these observations the provisional conclusion is drawn by Rocha e Silva *et al.* (231) that 5-HT acts upon the post-ganglionic cholinergic fibers of the intramural nervous system of the guinea-pig ileum. This view has not been entirely accepted by Gaddum and Hameed (126). They believe that since the action of 5-HT on the guinea-pig's ileum is inhibited by atropine or cocaine in the same way that the action of nicotine is inhibited, the ganglia in the guinea-pig's intestine may contain two types of receptors, one of which is stimulated by nicotine and the other by 5-HT.

The jejunum of the guinea-pig is relatively insensitive to tryptamine (223). The minimum active concentration of bufotenine is 1 in 10^6 to 1 in 2×10^7; that of bufotenidine 1 in 10^7. Successive doses of all these substances provoke decreasing responses (72).

Using this preparation as a test-object, Quadbeck and Röhm (198a) found that if the activity of 5-HT is taken as 100, that of other tryptamines is as follows: 5-methoxytryptamine 33; 4-fluorotryptamine 3; 5-chlorotryptamine 2.5; 5-bromotryptamine 1.5; 5-methyltryptamine 0.5; tryptamine 0.17. On the same preparation Quadbeck and Röhm studied the anti-5-HT activity of some gramine derivatives. In accordance with the results of Erspamer (103) on the rat uterus, they showed that the most active product was 5-chloro-2-methylgramine; this was followed by 5-bromogramine, 5-methylgramine, 5-methoxygramine, 5-fluoro-2-methylgramine and other derivatives.

It seems worth remembering that the guinea-pig intestine is suitable for the biological demonstration of "substance C", a substance related to 5-HT which has been described by Dalgliesh *et al.* (53) in extracts of dog intestinal mucosa. Substance C is said to cause, in comparison with 5-HT, a less prompt but more sustained contraction of the gut.

The large intestine of the guinea-pig is less sensitive to the indolealkylamines than the small intestine (83).

Rabbit. The small intestine reacts to 5-HT (minimum active concentration 1 in 5×10^9 to 1 in 10^9), as well as to bufotenine (1 in 10^8 to 1 in 4×10^8) and to bufotenidine (1 in 2×10^9 to 1 in 10^8) with a short-lived tonus increase and with an increase in the amplitude of the pendulum movements. The reproducibility of the response is, however, not fully satisfactory and so the preparation may be used only as a subsidiary test-object in the quantitative titration of the

5-hydroxyindolealkylamines (72, 83, 246). The 5-HT stimulation of the rabbit intestine is not inhibited by sympatholytic drugs, nor by hexamethonium 10^{-4} or atropine 10^{-6}; higher concentrations of this alkaloid may, however, exert a partial antagonism (116, 229). In sharp contrast to the above hydroxyindolealkylamines, gramine causes an inhibition of the tonus and movements of the rabbit intestine (197). The rabbit rectum is less sensitive than the small intestine (83).

In a concentration of 1 in 10^5 to 10^6 the inhibitor of true-cholinesterase 284C51 greatly enhances the stimulant action of 5-HT and of nicotine on the rabbit ileum. These results are interpreted by Robertson (229a) as evidence in favour of an "indirect" action of 5-HT on the intestinal smooth muscle.

Cat. The small intestine responds to 5-HT with a prompt but transient tonus increase. The minimum active concentration is 1 in 10^5 (83). Tryptamine and gramine, too, increase the tonus of this preparation (42, 254).

Dog, *pigeon* and *frog*. The dog and pigeon duodenum and the frog stomach are insensitive to bufotenine and bufotenidine in concentrations up to 1 in 10^5 (72). The *frog rectum* has been extensively used by Vogt in his researches on "Darmstoff" and "Substanz DS", a principle which has been recently identified as 5-HT (282, 283). The preparation seems to be highly sensitive to 5-HT (the minimum active concentration is less than 1 in 10^8), to which it responds with the appearance of rhythmic movements and increase in tone. Atropine 2×10^{-6} and dibenamine do not influence the spasmogenic effect of 5-HT; dihydroergotamine 4.5×10^{-6} produces a reversible blockade.

The stimulant action of 5-HT on the gut is appreciable not only *in vitro*, but also *in vivo*. An increased motility or intermittent spasms of the intestine were observed in Thiry-Vella dogs or in anesthetized dogs and rabbits with exposed intestine (123). 5-HT and 5-methoxytryptamine are also capable, in adequate doses, of causing evacuation of the bowel in intact rats, mice, dogs, guinea-pigs, and even frogs (83).

2. Uterus. Rat. The diestrous uterus is barely sensitive to 5-HT and related substances and shows marked tachyphylaxis. The estrous uterus and, less regularly, the uterus removed in advanced pregnancy or immediately after delivery is, on the contrary, highly sensitive (64, 65, 100, 126). Pretreatment with vitamin E seems to enhance further the sensitivity (Faustini, unpublished observations). The introduction into the bath of as little as 0.002 to 0.01 μg of 5-HT per ml. of nutrient liquid is enough to produce a noticeable response, i. e. appearance or reinforcement of movements. Higher doses not only cause an increased motility, but also provoke a tonus increase, which is, within ample limits, proportional to the dose. On washing out with fresh Tyrode solution, relaxation is immediate and the original reactivity of the preparation is promptly restored (65, 100).

The above is true also for 5-methoxytryptamine, 5-aminotryptamine, 6-methoxytryptamine and 5,6-dimethoxytryptamine, but not for the N-methylated indolealkylamines (*e.g.*, butotenine, N,N-dimethyltryptamine). Indeed, the latter substances seem to cling to their receptors in the smooth muscle much more

tenaciously than 5-HT since relaxation is not immediate after washing out, and the original reactivity is restored with difficulty, or only in part (86, 100).

When the uterus-stimulating action of 5-HT is taken as 100, the activity of other indolealkylamines is as follows: N-methyl-5-HT 30–35; bufotenine 10; 5-hydroxy-3-dibutylaminoethylindole <0.1; 5-methoxytryptamine 25–30; 5-aminotryptamine 10–15; 6-methoxytryptamine <0.1; 5,6-dimethoxytryptamine 2; tryptamine 1–1.5; 1-methyltryptamine 1–3; N-methyltryptamine <0.5; N,N-dimethyltryptamine <0.5.

Indole, skatole, 5-methoxyindole, indoleacetic acid, indolepropionic acid, indolebutyric acid, 5-methoxyindoleacetic acid, 5-hydroxyindoleacetic acid, tryptophan, tryptophanol, hypophorine, bufotenidine, bufothionine, dehydrobufotenine, α,α-dimethyltryptamine, gramine, 2-methylgramine, 2-methyl-5-chlorogramine, 2-methyl-5-aminogramine, 2-methyl-5-nitrogramine, 2-methyl-5-bromogramine and 5,6-dimethoxygramine have been proved quite inactive (100, 103).

Owing to its high sensitivity to 5-HT, the conspicuous specificity of its response to the substance, and the easy reproducibility of this response, the estrous uterus of the rat has become one of the most used test-objects in the qualitative and quantitative assay of 5-HT, and in the study of substances antagonistic to 5-HT (4, 5, 125, 126, 166, 240). The following results have been obtained: (a) Atropine, methonium salts and 1-hydrazinophthalazine have little or no effect on the stimulant action of the indolealkylamines (96, 126, 180a, 229b). The same is true for ephedrine, amphetamine, mescaline and eserine (126).

(b) The antihistaminic agents cause a more or less consistent inhibition of the 5-HT contraction of the uterus. The degree of this inhibition is not proportional to their specific antihistaminic activity, nor to their generic spasmolytic properties (87, 126).

(c) Among the sympatholytic drugs, some antagonize the uterus-stimulant action of 5-HT very strongly (dibenamine, dibenzyline, natural and hydrogenated ergot alkaloids of the polypeptide type), others are less efficacious (yohimbine), still others are quite ineffective (piperoxane, priscoline, prosympal) (91, 126).

(d) Although a remarkable anti-5-HT activity is shown also by some of the aminoindoles of Woolley and Shaw (2-methyl-3-ethyl-5-dimethylaminoindole, 1,2-dimethyl-3-ethyl-5-dimethylaminoindole) and, still more, by gramine and some gramine derivatives (5-chloro-2-methylgramine, 5-bromo-2-methylgramine) (95, 103, 240), there is little doubt that lysergic acid diethylamide (100, 125, 126) can be considered the most potent drug antagonistic to 5-HT hitherto described.

Correll *et al.* (51) injected some unanesthetized pregnant rats intravenously with 1 mgm. of 5-HT 24 to 36 hours before they were due to give birth. None of the animals delivered prematurely, but of 48 rats born at term, 37 were dead.

Mouse. The mouse uterus behaves in a manner exactly similar to that of the rat (65, 83).

Guinea-pig and *rabbit.* The non-pregnant isolated uteri of these animals respond only to high doses of 5-HT, with contractions which are irregular both in

appearance and amplitude. On washing out, relaxation is slow (123, 225). The stimulant action possessed by bufotenine, bufotenidine (72) and gramine (197, 254) is still weaker: the minimum active dose of gramine on the guinea-pig uterus is 10 mgm./liter of nutrient liquid, that of 3-diethylaminomethylindole 4 mgm./liter (2). Both the above preparations are unsatisfactory for assay purposes.

Cat. According to Reid and Rand (225), the *in situ* uterus of the non-gravid, adrenalectomized cat relaxes on intravenous injection of 24 μg. of 5-HT or on the introduction of 9 μg. of the substance into the low thoracic aorta. Tryptamine, too, relaxes the cat uterus *in situ*, but contracts the isolated organ (162).

Dog. 5-HT acts on the uterus of the conscious ovariectomized bitch and of the anesthetized bitch with intact ovaries, to cause a slight transient contraction, followed by a more prolonged relaxation. The smallest active dose is 0.45 to 1.0 μg./kgm. of body weight. The substance also acts on the isolated uterus. There seems to be no tachyphylaxis (196).

Hamster. The isolated uterus is stimulated by high doses of gramine (197).

3. Urinary bladder. Dog. The isolated dog urinary bladder responds to 5-HT and related indolealkylamines with a tonus increase, which is roughly proportional to the dose. At the beginning the sensitivity is 1 to 3 in 10^5, then it declines (83).

The bladder of the anesthetized dog *in situ* reacts to intravenous 5-HT with an increase of tonus and with the appearance or reinforcement of rhythmic motility. The minimum active dose of 5-HT is 2 to 4 μg./kgm. The percentage activity of other indolealkylamines, in comparison with 5-HT, is as follows: 5-methoxytryptamine 30–35; bufotenine 5–10; tryptamine 1.5; 1-methyltryptamine 1–1.5; N-methyltryptamine and N,N-dimethyltryptamine <0.5. Bufothionine, dehydrobufotenine and, possibly, bufotenidine are inactive (83, 86).

When administered subcutaneously to the anesthetized dog, 5-HT is practically devoid of any stimulant action, up to doses of 1 mgm./kgm. Intravenous doses of 5-HT greater than 50 μg./kgm. and subcutaneous doses greater than 200–400 μg./kgm. regularly provoke micturition and resistance to vesical catheterization (83, 100) in the intact conscious dog.

Dibenamine effectively antagonizes the stimulation of the isolated bladder by 5-HT. *In vivo* the drug is capable of diminishing and shortening the reaction to 5-HT, but not of abolishing it (91). The same is true for lysergic acid diethylamide and 2-methyl-5-chlorogramine (Erspamer, unpublished observations).

Other animals. The rat's urinary bladder and, still more, that of the guinea-pig is insensitive to 5-HT (83). The same may be said for the rabbit's urinary bladder, in contact with bufotenine or bufotenidine (72). The cat's bladder is weakly stimulated by tryptamine (162).

4. Bronchial smooth muscle. Reid and Rand (225) and Comroe *et al.* (47) have shown that intravenous 5-HT provokes a moderate bronchoconstrictor action in the cat. Freyburger *et al.* (123) and Sinha and West (246) have briefly described the spasmogenic action of high doses of 5-HT on the tracheal chain of the guinea-pig and the cat.

Herxheimer (145, 146) has studied in detail the bronchial actions of 5-HT, administered by inhalation, in guinea-pigs and in human beings, both healthy and diseased. Guinea-pigs exposed to a 1% aerosol of 5-HT developed severe dyspnea, followed by convulsions. The symptoms were the same as occur with aerosols of histamine or acetylcholine. Atropine 0.32 mgm./kgm., dihydroergotamine 2 mgm./kgm., and especially the diethylamide of lysergic acid 0.2 mgm./kgm., gave considerable but incomplete protection against 5-HT. Mepyramine 1 mgm./kgm., on the contrary, had no effect. In 4 normal human subjects inhalation of a 0.67% aerosol of 5-HT did not modify the vital capacity of the lung nor the respiratory rate. In 3 of 6 asthmatic patients examined, the same inhalation provoked a severe attack of bronchospasm, which regressed spontaneously or was abolished by isoprenaline. It is concluded that 5-HT is not an important cause of the symptoms of anaphylactic shock in the guinea-pig and that in human beings the substance possesses a bronchial action similar to that of histamine or acetylcholine.

5. Nictitating membrane of the cat. The preparation contracts on intravenous injection of 20 μg./kgm., or on intracarotid injection of 0.5–5 μg./kgm., of 5-HT (83, 84, 166, 225). The denervated membrane is more sensitive than the innervated; its response is similarly enhanced by cocainization of the animal (83, 100, 225). Tryptamine, N-methyltryptamine, N,N-dimethyltryptamine, bufotenine and bufotenidine act on the preparation like 5-HT but, with the exception of bufotenidine, more weakly (200, 223). The tryptamine contraction of the innervated membrane is potentiated by cocaine and diphenhydramine, but depressed by yohimbine (223).

6. Smooth muscle of the iris. Intravenous (100–500 μg./kgm.) or intracarotid (0.5–5 μg./kgm.) injection of 5-HT provokes, in the anesthetized cat, a conspicuous constriction of the pupil, sometimes preceded by a transient dilatation. The action of the substance is directly on the smooth muscle of the sphincter of the iris (190, 223, 224). Tryptamine has essentially the same effect, but in 10 times larger doses (162, 190, 223); bufotenine has no appreciable action (140); gramine causes a transient pupillary constriction only in very high doses (107). Introduced into the conjunctival sac of the cat or the rabbit, bufotenine requires a 10% concentration to give myosis (140).

Whereas the isolated iris of the cat constricts on addition of high doses of 5-HT to the nutrient bath (223), the enucleated frog or toad eye responds to 5-HT with a torpid mydriatic reaction, in dilutions down to 1 in 10^5 (83). Bufotenidine requires much higher concentrations (1 in 1000) to be mydriatic; bufotenine has practically no effect (280).

F. Action on respiration

It is commonly observed that the intravenous injection of adequate doses of 5-HT and other indolealkylamines provokes transient respiratory effects in the experimental animal.

1. Cat. Reid and Rand (224, 225) and Reid (226) first found that intravenous injection, as well as the direct introduction into the right heart, of crude serotonin

preparations and of pure 5-HT (10–200 μg.) caused in the cat under chloralose anesthesia and with intact vagi, a brief period of apnea, lasting up to 30 seconds. This was frequently followed by tachypnea and simultaneous broncho-constriction, as shown by remarkable fluctuations in intrapleural pressure, reduced amplitude of the respiratory movements, and increase in the chest volume. The observations of the Australian investigators have been regularly confirmed.

Schneider and Yonkman (233, 234) have analyzed the frequency of the vagal afferent impulses during the respiratory responses provoked by 5-HT (50–100 μg./kgm., intravenously) in the normal cat and in the cat treated with ganglion-blocking agents and local anesthetics. They conclude that 5-HT has a peripheral point of attack on autonomic sensory nerve endings in the cardio-pulmonary region, including the pulmonary stretch receptors. For the most part these conclusions have been accepted by Mott and Paintal (180) and Comroe et al. (47), who similarly believe that the reflex apnea caused by 5-HT (15–45 μg./kgm.) is due to stimulation of receptors situated in the circulation between the large veins and the left atrium (180) or the ascending aorta (47). From these receptors (pulmonary pain receptors?) originate afferent vagal fibers having a conduction velocity of 5–6 m./sec.

According to Mott and Paintal there is no evidence that 5-HT stimulates or sensitizes pulmonary stretch endings, nor right or left atrial or depressor nerve endings. Comroe *et al.* hold the opinion that the receptors sensitive to 5-HT are probably different from veratridine-sensitive receptors and find that they are blocked by Woolley and Shaw's serotonin antimetabolites.

Ginzel and Kottegoda (133) have investigated more closely the action of 5-HT on aortic and carotid sinus receptors. They observed that small amounts of 5-HT (2–10 μg.) injected into the carotid sinus region caused a fall of blood pressure and a stimulation of respiration usually preceded by a short period of apnea. After section of the sinus nerves only the apneic response remained. The same stimulant action, but no apnea, was noted following introduction of 5-HT into the aortic body region. The effect was abolished after section of the vagus nerves, but remained unaffected after hexamethonium and atropine. Both 5-HT and tryptamine produced temporary desensitization to their own actions. Ginzel and Kottegoda believe that the excitation of respiration is due to chemoreceptor stimulation, and that the apnea, a phenomenon quite independent of the preceding, is probably of central origin.

Tryptamine (133, 190, 223), bufotenine and bufotenidine (218, 220) act on respiration and on the bronchial smooth muscle like 5-HT. Tryptamine is however about 20 times less active than 5-HT (133).

In discussing the results of their experiments Comroe *et al.* (46, 47) suggest the possibility that some of the cardiovascular disturbances seen in patients with pulmonary embolism or coronary thrombosis might result from local or reflex effects of 5-HT liberated from platelets in the process of blood coagulation. The insignificant cardiopulmonary effects seen in human beings after intravenous 5-HT (191, 251) and the extremely low 5-HT content of human platelets do not support Comroe's suggestion.

2. Dog. Concordant observations (58, 59, 147, 190, 234) demonstrate that in

the normal dog the intravenous injection of 5-HT (2–100 μg./kgm.) regularly provokes a transitory stimulation of respiration, followed in about 50% of the animals by a period of apnea (234). There is however no agreement in regard to the explanation of this phenomenon.

Douglas and Toh (58, 59) believe that 5-HT can exert its respiratory effects in several ways, which include stimulation of afferent fibers in the vagus and sinus nerves. A direct stimulation of the respiratory center is not completely excluded, but it is considered of secondary importance. This view has not been entirely accepted by Heymans and Van den Heuvel-Heymans (147), who failed to confirm that denervation of the carotid sinus and section of the nerves caused a reduction in the respiratory response to 5-HT. Accordingly, they consider 5-HT hyperpnea as predominantly due to central stimulation. Another opinion is held by Schneider and Yonkman (234), who maintain that the initial respiratory stimulation in the dog is primarily due to a cardio-pulmonary reflex, since it is greatly diminished or abolished after bilateral vagotomy and only a short period of respiratory depression is observed when the spinal cord is sectioned at C_6.

What has been said for 5-HT, applies also to 7-HT and to tryptamine, administered in higher doses. Initial hyperpnea is always the most prominent response, sometimes followed or interrupted by periods of apnea or reduced respiration (190, 223). Little is known about gramine, which seems however to increase depth and rate of respiratory movements and to cause bronchoconstriction (137, 254).

In marked contrast to the normal dog, but like the cat, the dog with neurogenic hypertension responds to 5-HT and tryptamine with a period of apnea, followed by deep, forced breathing (190).

3. Rabbit. The injection of 5-HT (50–100 μg./kgm.) into the anesthetized animal causes a marked immediate stimulation, resulting in respiratory arrest in inspiratory position in 50 per cent of the animals. A diminution of the respiratory response is evident after spinal cord section (234).

4. Rat. In the intact, non-anesthetized rat, the intraperitoneal injection of 1–8 mgm./kgm. of 5-HT causes a moderate reduction in respiratory rate (207).

For the respiratory failure provoked by high doses of indolealkylamines see Part III.

G. Action on crustacean striated muscle

The injection of 1 μg. of 5-HT into the musculature of the pincers of *Potamoebius*, through the tip of the propodite causes, after a short latency, an abrupt powerful closing of the pincers which lasts for several minutes. An exactly similar result is produced by extracts of nervous tissue of cephalopods and decapod crustaceans, extracts which like 5-HT, are also active on the heart (117, 118).

H. Action on impulse transmission in ganglia

5-HT, when injected into the carotid artery of a dog under light pentobarbital anesthesia in doses of 65 μg./kgm. causes an almost complete, though transient

(20 to 25 seconds) blockade of nerve impulse transmission through the ciliary ganglion. The substance has approximately half the ganglion-blocking action of adrenaline (Marrazzi and Hart, see reference 191).

I. Action on the central nervous system

The actions of exogenous 5-HT on the central nervous system are still insufficiently known. Although it can be assumed that they are of a purely pharmacological interest, it is probable that they will attract more attention following the recent observations (4, 5, 260, 300) on the occurrence of 5-HT in various parts of the cerebro-spinal axis.

The most studied of the central effects of 5-HT is that on the respiratory center, either stimulant or depressant according to the animal species and the dose. As pointed out previously, there is no agreement, so far, concerning the mechanism, direct or reflex, of this effect. Similarly, it has not yet been established whether the vomiting, which frequently occurs in the conscious dog after large doses of 5-HT, is of central or peripheral origin. All those who have administered 5-HT to human beings by the intravenous route describe the frequent appearance of dizziness and of peculiar sensations in the head (191, 251). It is not as yet possible to say whether these phenomena can be related to those seen by Feldberg and Sherwood (112, 114) in the cat after introduction of 5-HT into the lateral right ventricle through a permanent cannula. A dose of 10 μg. of the drug had no detectable effect; 100–200 μg. after a latency of about 6 to 8 minutes, produced a pronounced tendency to sit or lie down. The cat was not sleepy or drowsy, the eyelids were wide open and blinking movements were rare. Besides muscular weakness, tachypnea and bursts of profuse salivation were observed.

According to Wooley and Shaw (240, 298, 299), who believe that normal functioning of the brain depends on 5-HT, this substance is unable to penetrate into the central nervous tissue. This might possibly explain its ineffectiveness in preventing the epileptiform syndrome produced in mice by high doses (5–10 mgm./mouse, intraperitoneally) of an analog of 5-HT, the drug "medmain" (2-methyl-3-ethyl-5-dimethylaminoindole).

The intravenous injection of 30–40 mgm./kgm. of tryptamine in the cat provoked negativism and catalepsy besides salivation and myosis (181).

Evarts (105a) has made a recent, very interesting contribution to our knowledge of the psychopathological effects of 5-hydroxyindolealkylamines. He found that the administration of bufotenine to the monkey did not affect normal muscle power (muscle tone, deep tendon reflexes), while consistently altering the sensorium and the general behavior. The reactions to visual stimuli and to pain (corneal reflex) as well as the placing reaction were absent; the pupillary light reflex was intact. The experimental animal showed marked ataxia, swaying, wide-based gait and circling. It grasped the floor of the cage and ran blindly about the room, bumping into objects. No reaction was observed to being picked up and petted, and no aggressive behavior in response to abuse.

Evarts points out the similarity between the psychopathological effects of bufotenine and those of lysergic acid diethylamide.

J. Production of cutaneous pain

Armstrong *et al.* (7) found that 5-HT applied to the exposed base of a blister raised by cantharidin on the human forearm produced a pain reaction having special characteristics. The reaction appeared after a latent period of 10 to 45 seconds and usually lasted, with fluctuations, for several minutes. After a first application of 5-HT the area became refractory to successive applications of this substance, but remained sensitive to other pain-producing chemicals (8). 5-HT was active in concentrations down to 1 in 10^8; tryptamine was about 20 times less potent; tryptophan caused no pain even in a concentration of 1 in 1000.

Since the application of fresh blister fluid from cantharidin blisters or from thermal burns, as well as the application of human serum (6) provoked a similar reaction, the problem arose of the identification of the active agents in blister fluid and in serum. Armstrong and her colleagues succeeded in demonstrating that whereas the agent in serum was indistinguishable from 5-HT, the pain-producing substance in blister fluid certainly differed from 5-HT (9), behaving like bradykinin (159). It is evident from these findings that induced cutaneous pain could not be used for the quantitative demonstration of 5-HT in biological materials.

K. Metabolic effects

The influence of 5-HT on water metabolism will be fully discussed when dealing with the renal actions of the substance and with its action on the passage of water through the skin of amphibians.

1. Action on blood sugar. Correll *et al.* (51) found that the intravenous injection of 3 mgm. of 5-HT into non-anesthetized rats weighing 300 gm. produced an increase of plasma glucose level from 157 to 269 mgm. per cent, and that the injection of 10 mg. of the same substance into rabbits weighing 4 kgm. brought the level from 120 to 193 mgm. per cent. The presence of the adrenal medulla was not essential for the hyperglycemic response. The doses used by Correll *et al.* are enormous, certainly unphysiological, and therefore their results are of purely pharmacological interest.

In amounts of 5 to 20 mgm./kgm. gramine has no effect on the plasma glucose level of fasting rabbits (197).

2. Action on oxygen consumption. Rapport and Virno (207) have shown that 5-HT reduced the rate of oxygen consumption of the rat when injected intraperitoneally in doses of at least 1 mgm./kgm. The reduction varied in intensity between 24 and 61%, and in duration between 12 and 120 minutes. A similar effect was obtained with tryptamine and histamine at much higher dose levels. The effect was not observed in other experimental animals, even with enormous amounts of 5-HT. The phenomenon is attributed, at least to a great extent, to an action of the drug on the central nervous system or on the arterial vessels of the brain.

L. Action on the adrenal medulla

The partially purified serum vasoconstrictor or 5-HT injected into the cut stump of the superior mesenteric artery of the eviscerated cat causes a discharge

of adrenaline from the suprarenal medulla. There is a rise in blood pressure, an increase in heart rate, dilatation of the pupil and contraction of the nictitating membrane. The last two responses are more marked after the removal of the superior cervical ganglion (224, 225). The capacity of 5-HT to provoke an adrenaline discharge is not shared by tryptamine (223) but, according to Raymond-Hamet (216, 219), it is possessed to a very conspicuous degree by bufotenine and, still more, by bufotenidine.

M. Histamine-releasing activity

Tryptamine and 5-HT, according to Feldberg and Smith (113), are to be listed among the drugs able to release histamine. This property of the indolealkylamines was shown experimentally on perfused isolated skin flaps and gastrocnemius muscles of the cat and the dog after arterial injections of 1 to 5 mgm. of the substance (estimation of histamine in the venous effluent), as well as on rat tissues after subcutaneous or intraperitoneal injections of 2 mgm. of 5-HT or 13 mgm. of tryptamine (estimation of tissue histamine). The activity of the indolealkylamines was somewhat greater than that of propamidine, but about 100 times less than that of product 48/80. Feldberg and Smith do not exclude the possibility that histamine release is at the root of certain phenomena resembling effects of histamine which are seen in the intact animal after injection of tryptamine compounds.

N. Participation in the mechanism of hemostasis

As early as 1912–1913 O'Connor (183) and Zucker and Stewart (301) advanced the hypothesis that the serum vasoconstrictor, released by the platelets at the moment of blood coagulation, might intervene in the process of hemostasis in consequence of its direct spasmogenic action on the vascular smooth muscle around the clot occluding the injured vessel. This hypothesis has been re-evaluated recently, following the identification of the vasoconstrictor with 5-HT, and has been subjected to further experimental control.

Correll *et al.* (51) found that the injection of 0.01 to 1 mgm. of 5-HT into the saphenous vein of the rat shortened significantly, though only transiently, the bleeding time following the severing of the tip of the tail. When applied topically, 5-HT had no effect. Similar hemostatic effects were observed in mice, guinea-pigs, hens, and, to a lesser degree, in rabbits (0.25–1.5 mgm./kgm.). The behavior of the systemic blood pressure was not taken into consideration. Since 5-HT had no influence *in vitro* on the blood clotting system, Correll *et al.* conclude that the role of the substance in hemostasis is exclusively that of a humoral vasoconstrictor agent.

The above results have been confirmed by Correale (49) and by Lecomte *et al.* (164). Correale further stated that the hemostatic effect appearing in the rat after subcutaneous doses of 5-HT paralleled, in both intensity and duration, the hypotensive effect caused by the substance. There was no direct interdependence, however, between these two phenomena, as would be expected if the shortening of the bleeding time was caused by hypotension. Lecomte and

his collaborators found that the action of 5-HT (in doses of 10 μg./kgm. and larger, intravenously) on the bleeding time in the rabbit was at least 10 times less intense than that possessed by adrenaline or adrenochrome, and more fugacious.

O. Action on capillary resistance

According to Bracco *et al.* (34), 5-HT given intravenously in doses of 1 mgm./kgm. of body weight produces in guinea-pigs and rats a marked increase in capillary resistance. The effect appears after 10 minutes and lasts for about three hours. With daily injections of 5-HT the capillary resistance remains high throughout the treatment period. The capillary crisis following surgical stress is similarly prevented or removed by intraperitoneal injections of 2.5 mgm./kgm. of 5-HT. Under daily administration of the same dose of 5-HT, no fall of the capillary resistance is observed during treatment. From the results of their experiments Bracco and his colleagues conclude that 5-HT possesses not only a local vasoconstrictor effect but also a protective action on capillaries.

No attention whatever has been paid by the above investigators to the behavior of the systemic blood pressure. Yet it is well known that the enormous doses of 5-HT they have used provoke in guinea-pigs and especially in rats a conspicuous and prolonged fall in blood pressure, accompanied by important changes in the calibre of the cutaneous vessels. The possibility that the observed increase in capillary resistance is, at least in part, a direct or indirect consequence of these systemic circulatory changes cannot be excluded *a priori*. At any rate, the above observations are of purely pharmacological interest. They throw no light on the problem of the physiological significance of 5-HT, since the doses of this substance which have been used are absolutely unphysiological, being 6–7 times (guinea-pig) or 20 times (rat) higher than the total 5-HT content of the entire experimental animal.

P. Action on the circulating eosinophils

According to Halberg (138a), 5-HT, cinobufotenine (a mixture consisting of about 90% bufotenidine and 10% 5-HT + N-methyl-5-HT + bufotenine) and tryptamine in mice inhibit the ascending phase of the daily eosinophil rhythm when administered intraperitoneally in high doses (about 10–25 mgm./kgm., in terms of the free bases). 5-Hydroxykynurenine has no action.

5-HT and cortisone act reciprocally to potentiate their eosinopenic effects. Indeed, the combined administration of non-eosinopenic doses of cortisone reinforces the eosinopenic action of 5-HT in doses of 0.125, 1.25 and 12.5 mgm./kgm., and conversely the effects upon number of eosinophils induced by cortisone in 0.3 and 3 mgm./kgm. doses is strengthened by a non-eosinopenic dose of 5-HT (21.5 μg./kgm.). Halberg considers the eosinopenic effect of high doses of tryptamines as a pure pharmacological effect, but believes that the synergism between 5-HT and cortisone, whose mechanism remains to be investigated, may have physiological significance and may be listed among the factors interfering with the production of "eosinophil responses".

The suggestion of Halberg seems theoretically acceptable, since the amount of 5-HT which potentiates the eosinopenic action of cortisone is lower than that present in mouse blood (52 μg./kgm.), and very much lower, of course, than that contained in the entire mouse (363 μg./kgm.). Halberg's results have been confirmed by Bertelli *et al.* (20a) who found that 5-HT given intravenously or injected into the cisterna magna of the dog in doses of 20–40 μg./kgm. of body weight significantly reduced the number of circulating eosinophils, and injected intraperitoneally into the rat in doses of 0.2–0.8 mgm./kgm. moderately reduced the ascorbic acid content of the adrenal glands. The above effects are ascribed to a stimulation of the anterior pituitary with ensuing release of ACTH.

Q. Action on the circulation and function of the kidney

The writer and his coworkers (78, 79, 83, 84, 88, 89, 90, 91, 97, 100, 101) regard this as the physiological action of 5-HT and therefore consider the substance as a hormone regulating the circulation and function of the kidney. 5-HT shows antidiuretic action in all the mammals studied (human beings, dogs, rats, rabbits, guinea-pigs); in the various species, however, it is possible that this action is accomplished by somewhat different mechanisms. In only two species of mammals, the rat and the dog, have the actions of 5-HT upon the kidney been investigated with sufficient accuracy.

1. Rat. The following conclusions may be drawn from the writer's experiments (89, 91, 95, 100) carried out on about 3500 groups of 4 rats each. The antidiuretic action of 5-HT appears under most varied conditions of hydration, but is especially evident after a single dose of 5 ml. of water per 100 gm. of body weight (83, 89). The substance inhibits not only water diuresis but diuresis produced by osmotic diuretics and xanthine derivatives (96). When 5-HT and posterior pituitary extracts are administered simultaneously the antidiuretic effects of the two hormones are simply additive (96).

The antidiuretic action of 5-HT in the rat has been indirectly confirmed by Heller and his coworkers (56, 130, 131, 142). It has been shown, in fact, that "stable antidiuretic substance" found by them in rat serum may be identified with 5-HT (56a, 98).

Among the few species studied, the rat is the most sensitive to 5-HT. It is sufficient to inject subcutaneously as little as 4 μg. of 5-HT base per kgm. to produce a significant reduction in the volume of urine. This dose is at least 50–100 times less than the minimum required to lower the systemic blood pressure (49, 83) and at least 20000 times less than the median lethal dose by the subcutaneous route (83, 123). It corresponds to about $\frac{1}{30}$ of the 5-HT content in the organism, per kgm. of body weight (97, 100), and is certainly less than the quantity of endogenous 5-HT which is destroyed by amine oxidase every hour, as estimated by the urinary output in 5-hydroxyindoleacetic acid (3–4 μg./kgm./hour) (101).

Higher doses cause a very conspicuous reduction in diuresis or even a virtual suppression of urine flow for several hours. The final result is that even 7 to 9 hours after the water load the animals treated with 5-HT excrete 10 to 20% less water than the control animals.

The reduction in urine volume is accompanied by a simultaneous reduction in chloride excretion (104).

The most appropriate and effective way of administering 5-HT is subcutaneously, as shown by the intensity, and still more by the duration of the antidiuretic effect. After intraperitoneal or intravenous injection the substance is several times less active; when given by mouth it is practically inactive (89, 98). This statement explains the negative results of Ames and Van Dyke (3).

Through the simultaneous evaluation of urine flow and renal excretion of test substances (thiosulfate, creatinine, PAH) in large groups of animals, it was demonstrated that 5-HT antidiuresis in the rat is due primarily and essentially to a reduction in the glomerular filtration rate. This reduction, which is accompanied by a simultaneous diminution in the blood flow through the peritubular capillary network, must be ascribed, at least in great part, to a constriction of the afferent glomerular vessels leading to a fall in intraglomerular hydrostatic pressure. The point of attack of 5-HT in the rat kidney is therefore the smooth muscle, or analogous contractile structures of the afferent glomerular system. Systemic hypotension and increase in the endocapsular pressure should really be listed among the factors causing a reduction in the filtration pressure, but neither of them can interfere when the dose of 5-HT is less than 0.4 mgm./kgm.

After 5-HT the percentage reduction in the urine volume is greater than that in the glomerular filtration rate and still more than that in the renal plasma flow, as measured by the PAH excretion. A higher percentage of the glomerular filtrate has therefore been reabsorbed by the tubules of the treated animals than by those of the controls. Even without excluding the possibility of some direct or indirect stimulant effect of 5-HT on the tubular epithelium, the writer believes that the phenomenon may be explained on the theory that, when the glomerular filtration rate is decreased, the reduced glomerular load is more completely reabsorbed during the slower passage down the nephron and less is excreted (237).

Another possibility has to be kept in mind if we accept the suggestion of Trueta *et al.* (254, pp. 120–122) that the vasa recta are concerned with the reabsorption of water: it is the possibility that 5-HT causes, as a consequence of afferent vasospasm, a partial diversion of the blood from the cortex to the medulla with ensuing increase in the passive reabsorption of water into the blood of the vasa recta through the thin walls of the thin segment of the loop of Henle. It is at any rate quite improbable that 5-HT acts indirectly on the tubules through the posterior pituitary.

After the administration of high, unphysiological doses of 5-HT (2–10 mgm./kgm.) the afferent vasoconstriction induced by the substance becomes visible even macroscopically through the mottled aspect of the kidney surface, due to the presence of ischemic cortical areas, more or less extensive, interposed between congested areas or areas with an apparently normal blood supply. If the injury is particularly intense or repeated, ischemia results in degeneration and necrosis (83, and unpublished observations). Apart from the above direct proofs

there is also indirect evidence of the vascular point of attack of 5-HT in the rat kidney (92).

Among the indolealkylamines, 5-HT is by far the most active on diuresis. The percentage activity of other 5-HT-like substances is as follows: N-methyl-5-HT 30–40, bufotenine 6–7, bufotenidine 0.3, tryptamine 0.3–0.5, methyltryptamine 0.1–0.3, N-methyltryptamine and N,N-dimethyltryptamine 0.3, 5-methoxytryptamine 25–40. No antidiuretic activity is shown by bufothionine, dehydrobufotenine, 6-methoxytryptamine, gramine, 2-methylgramine, 2-methyl-5-aminogramine, 2-methyl-5-nitrogramine, 2-methyl-5-chlorogramine, and by indole derivatives not bearing an amino group in the lateral chain (86, 100).

5-HT antidiuresis (0.1–0.5 mgm. of 5-HT/kgm. subcutaneously) is prevented, but not suppressed once it has begun, by dibenamine, by the natural or hydrogenated ergot alkaloids of the ergotamine and ergotoxine groups and by lysergic acid diethylamide (91, 100). Gramine and gramine derivatives (95, 103), the N-methyltryptamines, the aminoindoles of Woolley and Shaw, including "medmain" and "1-methylmedmain" (95, 100) as well as some antihistaminic agents have a less consistent action (87). The antidiuretic effect of 5-HT does not appear to be significantly affected by ganglion-blocking drugs, atropine, papaverine, nitrites, ethyl alcohol, 1-hydrazinophthalazine, histamine, adenosine or theophylline (96).

The writer wishes to lay particular emphasis on two of the experimental results obtained in the rat: the fact that 5-HT and serum extracts are much more active when injected subcutaneously than when given intraperitoneally or intravenously and the fact that the majority of the drugs antagonistic to 5-HT are capable of preventing 5-HT antidiuresis but not of interrupting it.

Without excluding alternative interpretations, in our opinion these facts may be explained by assuming that 5-HT occupies its receptors within the walls of the afferent renal vessels slowly and gradually, but very tenaciously. The gradual occupation would account for the necessity of a prolonged supply of small quantities of substance, as experimentally ensured by subcutaneous injection; the tenacity of the occupation explains satisfactorily on the one hand the conspicuous duration of the antidiuretic effect and, on the other, the inability of some of the most powerful antimetabolites to remove 5-HT from its renal receptors. We may add that a certain slowness in the uptake and release processes of 5-HT by the receptors in the kidney is well suited to the function we tend to attribute to the substance in the control of renal hemodynamics and consequent control of the water metabolism of the organism. A lasting and gradual release of 5-HT into the plasma could be "physiologically" ensured by the platelets, which are, as repeatedly stated, to be considered as circulating reservoirs of the substance, drawing upon the richer deposits represented by the enterochromaffin cells of the gastrointestinal mucosa, and capable of protecting 5-HT from attack by amine oxidase.

In sharp contrast to the writer's observations and conclusions, Corcoran *et al.* (48) claim that 5-HT has no effect on water diuresis in the rat when given in subcutaneous doses of about 130 μg. per kgm. of body weight. Only larger

doses were found to inhibit transiently diuresis due to oral water loads. They conclude that "the inhibitory effect of small doses of 5-HT on water diuresis in rats and dogs presumably results from the posterior-pituitary-stimulating effect of pain and the reputed relationship between the kidney and the action of serotonin in subjectively tolerable dosage is indirect and in some degree artefactual". These experiments of Corcoran and his colleagues have been carried out on 9 groups of 6 rats each, using a procedure absolutely different from that employed by the writer and his collaborators in their experiments on 3500 groups of 4 rats each. Further work has been carried out quite recently by the writer and Correale (104) on 1100 rats. The conclusions and the views expressed in this chapter have been fully confirmed and the objections of Corcoran *et al.* decidedly rejected.

2. Dog. 5-HT antidiuresis always seems to occur in the rat through a predominantly vascular mechanism, whatever the dose of substance introduced into the organism may be. In the dog the mechanism appears to be more complex, in spite of the fact that in preliminary experiments of the writer and Ottolenghi (79), in which very large doses of crude enteramine extracts were used, no fundamental difference was found between the dog and the rat.

In clearance experiments carried out in a series of 30 trained colpotomized bitches, Sala and Castegnaro (232) were able to show that the subcutaneous injection of 0.04 to 0.8 mgm./kgm. of 5-HT base constantly produced a more or less conspicuous reduction in urine volume. The glomerular filtration rate remained unaltered for lower doses of 5-HT, while frequently showing a slight reduction for higher doses. The behavior of the renal plasma flow was rather surprising: it remained unchanged or slightly diminished at first and increased, in the majority of cases, 20 to 30 minutes after the injection of the drug. Sala and Castegnaro conclude that in the dog, too, large doses of 5-HT may constrict the afferent glomerular vessels. They attach little importance to this afferent vasospasm in the mechanism of the antidiuresis induced by lower, more nearly physiological doses, and much more to the increased reabsorption of water from the renal tubule.

The preceding observations have been confirmed by Barac (14) and by Pickford (196). Barac performed his experiments on the dog under chloralose anesthesia, intravenously infused with saline. Under these conditions the intravenous injection of 0.8–0.9 mgm. of 5-HT/kgm. (a dose 5–6 times greater than the total content of substance in the entire dog!) produced a conspicuous reduction in the urine volume (85–100%), reaching its maximum after 5–20 minutes and generally coinciding with an increase in the systemic blood pressure. Antidiuresis was also evoked after adrenalectomy, after hypophysectomy as well as after section of the renal nerves, so that the phenomenon was the consequence of a direct renal action of 5-HT. The intimate mechanism of the renal action of the substance is not clear to Barac and to Nizet *et al.* (182), who exclude, however, the possibility of 5-HT provoking intrarenal vasoconstriction.

From a physiological point of view, Pickford's observations are more important. Having demonstrated that 5-HT is a potent antidiuretic substance

(minimum active dose by intravenous route: 4 to 20 μg./kgm. according to the individual animal; time of maximum urinary inhibition: 1½ to 10 minutes after injection), she believes that this effect is largely due to a diminution in filtration rate, as measured by creatinine clearance. The reason for the fall in intraglomerular pressure induced by 5-HT is however by no means clear since the irregular behavior of the diodone clearance is evidence against an afferent vasospasm. Pickford agrees with Barac that there appears to be no central element in the antidiuretic action.

The only discordant voice is again that of Corcoran *et al.* (48), who failed to confirm the antidiuretic action of 5-HT in the dog, but the experimental procedure employed by the Cleveland observers was substantially different from that used by Sala and Castegnaro (232).

The antidiuretic action of 5-HT may now be considered proven. The problem of its mechanism, however, remains unsettled. The somewhat different results obtained in the rat and the dog suggest the possibility that this mechanism may differ in the various animal species. It will be the aim of future researches to establish whether these differences are substantial (vascular action in the rat and tubular action in the dog) or more apparent than real.

On the basis of a series of considerations and of the uncontroverted fact that physiological doses of 5-HT may cause a striking renal vasoconstrictor effect also in the dog (see renal actions of the "Spätgift"), the writer tends to emphasize the predominant importance of the vascular point of attack of 5-HT in the kidney, whatever the species (100).

It should, however, be stressed here that the acceptance of this view does not necessarily imply that the substance acts on the same section of the intrarenal vascular system in all species of animals, nor that it causes vasoconstriction on all sections of the intrarenal vascular bed and in every case. Some experimental observations (83) support the view that in the rat afferent vasoconstriction is accompanied by dilatation of the glomerular and peritubular capillaries. In the dog, too, the observations of Sala and Castegnaro (232), as well as those of Corcoran *et al.* (48), could be explained by postulating a glomerular or postglomerular vasodilatation, or both, or a dilatation of the medullary pathway, as described by Trueta *et al.* (259).

The conspicuous differences which are known to exist in the development and structure of those formations which go by the name of "Polkissen" or "juxtaglomerular apparatus", as well as the differences in intrarenal vasomotor tone and in the intensity and type of reaction of the renal vessels to vasoactive drugs and to tourniquet applications, seem to afford the clearest evidence that intrarenal circulation is not necessarily controlled by exactly the same mechanism in every animal species.

R. Effect of 5-HT on the regulation of water exchange through the skin of amphibians

The problem of the possible effect of 5-HT on the passage of water through the skin of amphibians (141b) is clearly posed by the statement that only in amphibians amongst the vertebrates, does the skin play a highly important

role in regulating water metabolism, and that only in amphibians does the skin contain 5-HT or similar substances, which are often present in enormous amounts. The writer's experiments on the frog (99) have however given entirely negative results. Even large doses of 5-HT, sometimes larger than the animal's total 5-HT content, failed to affect significantly the passage of water through the skin. It is, therefore, doubtful whether the substance plays any part in the cutaneous regulation of water metabolism.

S. Miscellaneous effects

1. *Protective effect against x-radiation.* The survival rate of rats, exposed to lethal x-ray dosage, was found by Gray *et al.* (134a) to be significantly increased after intraperitoneal pretreatment with 5-HT (9 mgm./kgm.) or para-amino-propiophenone (16–32 mgm./kgm.). The protective effect of these agents is assumed to be due to their property of producing a temporary tissue anoxia, *i.e.*, of causing vasoconstriction.

2. *Inhibitory effect on cholinesterase.* 5-Hydroxytryptamine 10^{-3} inhibits the cholinesterase of human serum (50%) as well as that of human erythrocytes and of rat and guinea pig liver and brain (35%). The inhibitory effect of 5-HT 2×10^{-4} is inconstant. Tryptamine seems to be equally active (162a). Bufotenine was recognized as a strong inhibitor of serum cholinesterase in 1937 by Sobotka and Antopol (248a), who called attention to the structural analogy between this indolealkylamine and eserine.

V. BIOSYNTHESIS AND FATE OF INDOLEALKYLAMINES

A. Biosynthesis

Nothing certain is known about the origin of gramine which, however, does not seem to be a secondary breakdown product of tryptophan (137). This amino acid must, on the contrary, be considered the primary precursor of all the biogenic indolealkylamines containing two carbon atoms on the aliphatic chain (137, 263). Tryptamine originates directly from tryptophan through decarboxylation catalyzed by tryptophan decarboxylase (288).

For the conversion of tryptophan into 5-HT two pathways may be postulated as shown in Figure 1. The pathway which passes through 5-hydroxytryptophan seems to be the more likely. Indeed, Udenfriend *et al.* (45a, 263, 264, 266) have recognized in animal tissues the existence of two enzyme systems which catalyze respectively the oxidation of L-tryptophan to 5-hydroxytryptophan (tryptophan oxidase) and the decarboxylation of 5-hydroxytryptophan to 5-HT (5-hydroxy-tryptophan decarboxylase). The other route can be traced with certainty only up to tryptamine; there is as yet no proof of the existence in animal tissues of a tryptamine oxidase. As far as we know at present, only in the skin of amphibians and in the tissues of some fungi do those processes of methylation, dehydrogenation and condensation take place which lead to the N-methylated, dehydrogenated and sulfoconjugated 5-hydroxyindolealkylamines.

5-Hydroxytryptophan shows a remarkable stability to the kynurenine-form-

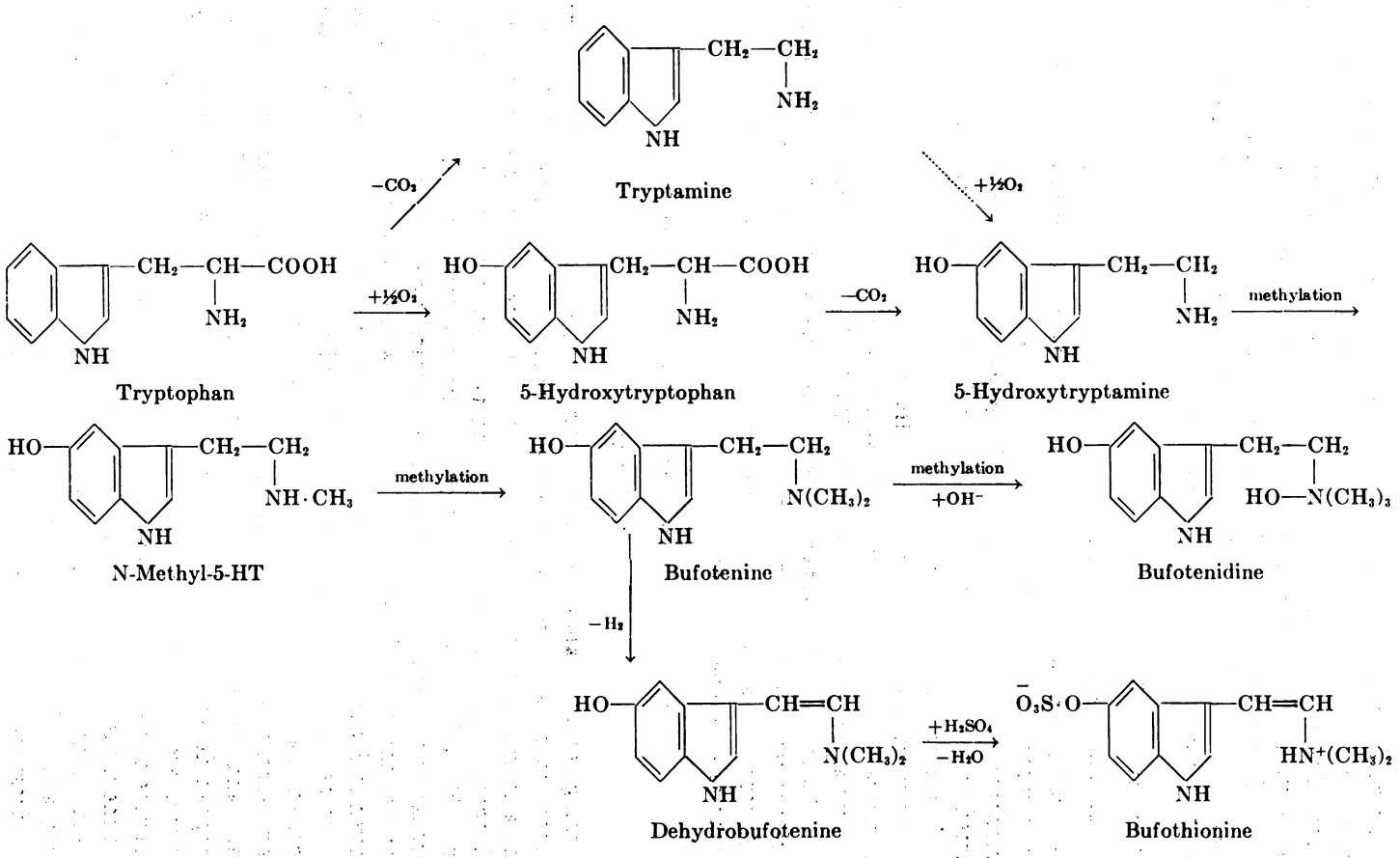

FIG. 1. The conversion of tryptophan into 5-hydroxytryptamine and related substances

ing enzyme, and this emphasizes, according to Ek and Witkop (60), the distinct nature of the two possible pathways in the degradation of tryptophan.

5-Hydroxytryptophan decarboxylase may be inhibited by several substances, particularly by carbonyl reagents and chelating compounds. Beiler and Martin (19), who made this observation, believe that a metal is involved in the enzyme and that pyridoxal phosphate may display a coenzyme function.

B. Fate

It is commonly observed that the pharmacodynamic effects of even large doses of indolealkylamines introduced into the organism wear off more or less rapidly. The active molecule must, therefore, have been removed from the plasma in some way, and consequently rendered unavailable to the tissue receptors. This removal may come about in various ways: through selective absorption of the substance by the circulating thrombocytes, through its enzymic inactivation, and finally through its elimination from the organism unchanged.

1. Uptake of indolealkylamines by the thrombocytes. If we regard the thrombocytes merely as carriers of 5-HT we must suppose that they can absorb endogenous 5-HT from the plasma, into which the substance has been released by the enterochromaffin cells of the gastrointestinal mucosa. The consequence of this absorption would be that, at least temporarily, some of the endogenous 5-HT would be unavailable for its receptors and would escape enzyme inactivation. We do not know whether, and to what extent, the thrombocytes take up and transport exogenous 5-HT and other indolealkylamines.

2. Enzymatic inactivation of indolealkylamines. This appears to be by far the most important way of removal of exogenous and endogenous indolealkylamines. Even the 5-HT which is taken up by the thrombocytes must, sooner or later, be attacked by enzymes.

Taking the most important of them, 5-HT, as a pattern for all the indolealkylamines, we have to consider the following possible pathways for its inactivation as illustrated in Figure 2.

α) The possibility of an oxidative deamination of tryptamine was first demonstrated by Ewins and Laidlaw (109) and by Guggenheim and Löffler (136). Indoleacetic acid and small quantities of indolyl-(3)-ethylalcohol (tryptophol) were, in fact, isolated as breakdown products of tryptamine, after adding this substance to the perfusion liquid of the rabbit's liver, and indoleaceturic acid was found as a metabolic end-product of tryptamine in dog's urine.

Subsequent experiments *in vitro* confirmed the above *in vivo* observations and showed that not only tryptamine, but also N-methyltryptamine, 5-HT, N-methyl-5-HT, and bufotenine are substrates of amine oxidase (23–29, 35, 68, 70, 72, 100, 123, 134, 175, 198). Tryptamine is usually oxidized more rapidly than 5-HT, bufotenidine about 8 to 25 times more slowly (26, 134). 5-Hydroxyindoleacetic acid was recently detected, by paper chromatography, in 5-HT solutions incubated with homogenates of mammalian intestine, liver and kidney and of octopod hepatopancreas (102, 257), as well as in extracts of posterior

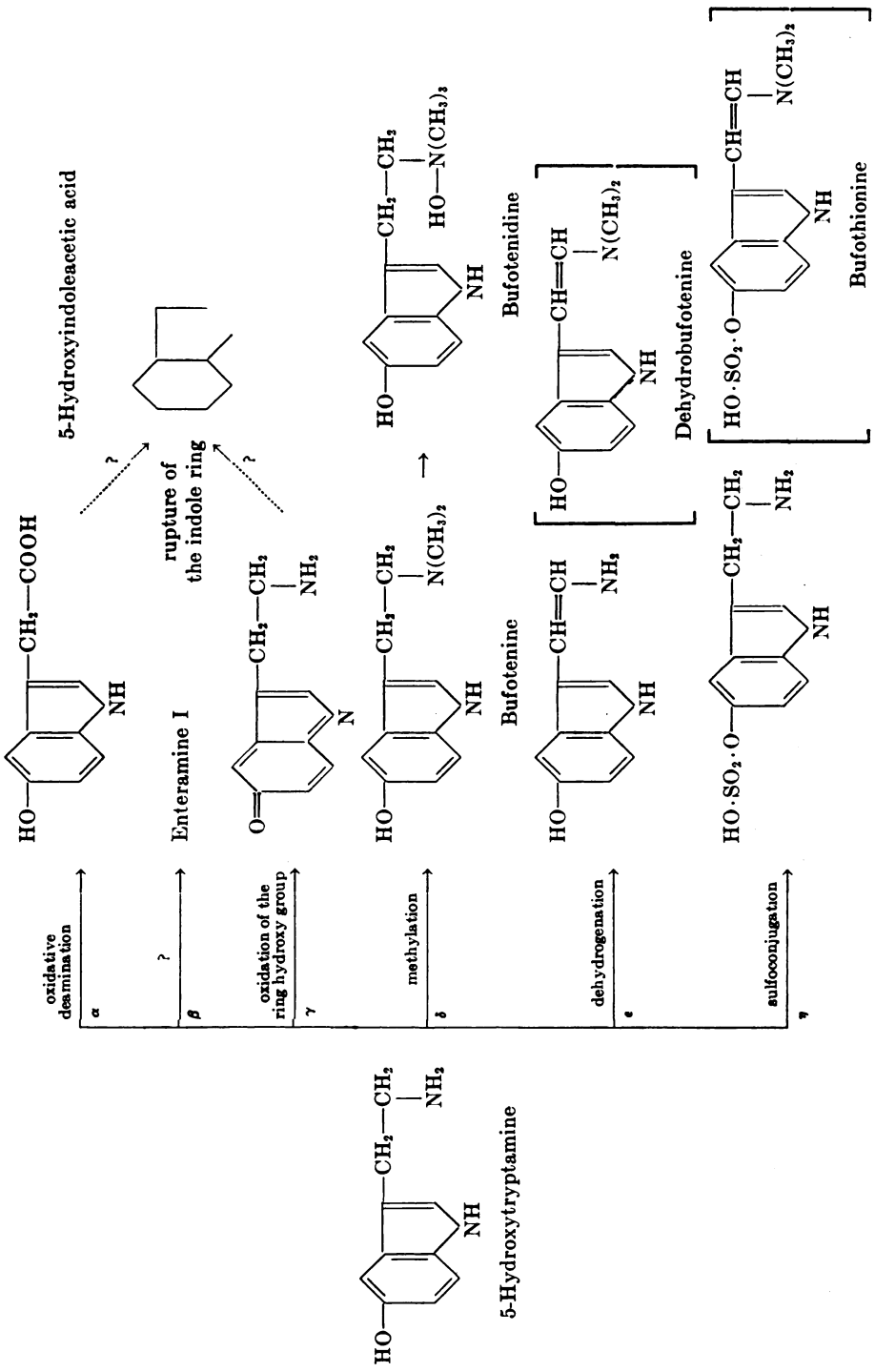

FIG. 2. Possible pathways of inactivation of 5-hydroxytryptamine

salivary glands of *Eledone*, which is a tissue rich in both 5-HT and amine oxidase (81, 102).

Recently the writer (100, 101, 102) succeeded in demonstrating that the urine of normal human beings, dogs, rats, hogs, calves, kids and lambs contains a hydroxyindole derivative which is chromatographically indistinguishable from 5-hydroxyindoleacetic acid. The urine content of this derivative, ranging from 1 to 4 μg./ml. became up to 50 times greater after the oral or parenteral administration of 5-HT. Similar results were obtained simultaneously by Titus and Udenfriend (257).

In rats 5.5 per cent and 28–33 per cent of the administered 5-HT (6 mgm./kgm.) was recovered from urine as 5-hydroxyindoleacetic acid, the lower value having been obtained after oral administration, the higher after subcutaneous or intraperitoneal administration. In a dog 25–26 per cent of the subcutaneously injected 5-HT (1.2 mgm./kgm.) appeared in the urine as 5-hydroxyindoleacetic acid. In 8 normal human beings the recovery of intramuscularly injected 5-HT (0.13 mgm./kgm.) was 20 per cent; in a patient suffering from diabetes insipidus 52 per cent.

These observations demonstrate on the one hand that the removal of 5-HT may really be considered a normal function of amine oxidase in the tissues, as suggested by Blaschko (25) and by Blaschko and Philpot (26), and on the other hand that the metabolism of endogenous 5-HT must be very intense.

In human beings the daily urinary output of 5-hydroxyindoleacetic acid is about 3 to 6 mgm. according to the writer (102), and 10 mgm. according to Titus and Udenfriend (257). When we keep in mind that to produce 3–6 mgm. of 5-hydroxyindoleacetic acid at least the same quantity of 5-HT must have been oxidized, and that the 5-HT content of human blood is approximately 0.05 μg./ml., we must conclude that an amount of substance corresponding to the entire blood 5-HT has to be metabolized at least every two or three hours. The quantitative estimation of 5-hydroxyindoleacetic acid in human urine is suggested as a possible index of the intensity of 5-HT metabolism in normal and pathological conditions. However, these conclusions and suggestions are obviously true only if 5-HT is the sole precursor of the urinary 5-hydroxyindoleacetic acid.

In contrast to the above animal species, the urine of adult rabbits, guinea-pigs, horses and oxen contains no detectable 5-hydroxyindoleacetic acid, or at most quantities less than 0.2–0.3 μg./ml. After the subcutaneous administration of 5-HT in rabbits (2.5 mgm./kgm.) and guinea-pigs (8 mgm./kgm.) virtually no 5-hydroxyindoleacetic acid was recovered from the urine. It may be supposed that in adult herbivores the metabolic pathway for 5-HT is different from that existing in suckling herbivores and in omnivorous or carnivorous animals or, more probably, that oxidative deamination is only the first step of a more radical breakdown process, which may even involve the rupture of the indole ring.

5-Hydroxyindoleacetic acid has been found in rat's urine even after the administration of bufotenine. This affords evidence that the N,N-dimethylalkylamines are also attacked by amine oxidase *in vivo*.

The fate of 5-methoxytryptamine and of the tryptamines not bearing hydroxy groups on the indole ring is similar to that just described. 5-Methoxytryptamine yields very large amounts of 5-methoxyindoleacetic acid (41 to 71 per cent of recovery, according to the administration route and the dose); tryptamine yields moderate amounts of indoleacetic acid and large amounts of indoleaceturic acid (26 per cent and 59 per cent respectively after 20 mgm./kgm., subcutaneously); N,N-dimethyltryptamine yields small amounts of indoleacetic acid and moderate amounts of indoleaceturic acid (2.7 per cent and 14.7 per cent respectively after 20 mgm./kgm., subcutaneously).

Indoleacetic acid has been described as a constituent of normal urine (57, 161, 293a). It may be asked whether this product is derived from the oxidative deamination of endogenous tryptamine. The above breakdown products of exogenous indolalkylamines can be demonstrated not only in urine, but also in the blood of the rat (102).

5-Hydroxyindoleacetic acid and 7-hydroxyindoleacetic acid show only 6 and 3 per cent respectively of the activity of indoleacetic acid (heteroauxin) in the pea slit-internode test (60).

β) As we do not know the chemical structure of enteramine I (69, 81, 100), that is to say, the nature of the change undergone by 5-HT in its side-chain, we are completely in the dark not only with regard to the possibility of the occurrence of this change in the living organism, but also with regard to the necessity of the intervention of enzymes in the reaction enteramine $\rightleftarrows$ enteramine I. The existence of a form I has not so far been recognized for other indolealkylamines.

γ) The possibility of the oxidation of the hydroxyindolealkylamines in the indole ring instead of in the side chain has been suggested by Blaschko and Philpot (26) and by Govier *et al.* (134). The existence of this metabolic pathway may perhaps account for the rapid inactivation *in vivo* of bufotenidine, which is not attacked by amine oxidase (72).

δ, ϵ, η) Methylation, sulfoconjugation and dehydrogenation of 5-HT and similar 5-hydroxyindolealkylamines have so far been observed only in the skin of amphibians, and possibly in the tissues of some fungi (293). All these processes cause a weakening or even the complete abolition of the specific effects of 5-HT on the diuresis of hydrated rats and on the estrous-uterus of the rat (86, 100).

3. Excretion of indolealkylamines. The remaining mode of removal of the indolealkylamines from the organism consists in their elimination in unchanged form. There are two possible elimination routes: through the cells of the enterochromaffin system, and by renal excretion.

The external secretion of indolealkylamines through the cutaneous glands of amphibians is a well established fact, and so is that of 5-HT by the salivary glands of *Octopus vulgaris* and *Eledone moschata* (11). There is, on the other hand, no available evidence that the enterochromaffin cells secrete 5-HT into the intestinal lumen (100).

According to Twarog and Page (260) both endogenous and exogenous 5-HT are excreted unchanged in the urine. They found, indeed, that the substance is

present in small, though not negligible amounts (0.045 to 0.45 μg./ml.) in the normal urine of human beings and dogs, and that approximately 3 per cent of 5-HT infused intravenously can be recovered from dog's urine.

The writer (100) has not yet been able to produce unambiguous evidence to confirm the occurrence of 5-HT in normal urine of human beings and rats; he has found, however, that rat's urine contains small quantities of 5-HT or other indolealkylamines after the intraperitoneal injection of large doses (4–10 mgm./kgm.) of these substances.

VI. PHYSIOLOGICAL SIGNIFICANCE OF THE INDOLEALKYLAMINES

The discussion will be limited to 5-HT. Three hypotheses, each one virtually independent of the other, have been proposed. The first sees in 5-HT a factor influencing hemostasis; the second considers that it controls vascular tone and therefore the systemic blood pressure; and the third regards the substance as a hormone participating in the regulation of the function of the kidney.

1. 5-HT as a factor participating in hemostasis. This is the oldest of the hypotheses if we agree, as we must, that the serum vasoconstrictor which appears on destruction of the platelets is 5-HT. Here is what O'Connor (183) actually wrote on the subject in 1912: "Der Nachweis, dass bei der Gerinnung des Blutes eine gefässverengernde Substanz aus den zerfallenden Zellen frei wird, erscheint mir auch von teleologischer Bedeutung für das Verständnis der Blutstillung zu sein: es ist zweckmässig, dass bei der Gerinnung entstehende Substanzen lokal die Gefässe zu verengern vermögen, aus denen die Blutung erfolgt".

In 1913 Zucker and Stewart (301) insisted: "Aus diesen Versuchen ergibt sich, dass beim Blutplättchenzerfall eine in anderen Geweben wohl nicht vorkommende, mehr oder weniger an Blutgefässe angepasste Pressorsubstanz frei wird. Es drängt sich hierbei der Gedanke auf, dass möglicherweise bei Blutstillung nicht nur Gerinnselbildung, sondern auch ein durch diese frei gewordene Substanz hervorgerufenes Zusammenziehen der verletzten Gefässe eine Rolle spielt".

These clearly expressed views have been accepted by several modern research workers (31, 34, 37, 38, 39, 42a, 46, 47, 51, 123, 224, 226, 302, 304, 305), but unfortunately they are based on data obtained exclusively from mammals and have necessarily been formulated without taking into account essential quantitative data.

The following objections might be raised. (a) It is very doubtful whether the quantities of 5-HT released at the moment of blood coagulation are ever sufficient, in any animal species, to constrict the injured vessels. To give one example: the vessels of the perfused hind leg of the toad do not react to even 200–400 μg. of 5-HT, a quantity of substance contained in as much as five to ten litres of the animal's blood!

(b) The fact that various vascular areas in the same animal show widely different sensitivities to 5-HT, is not in accordance with the general and ubiquitous character which the hemostatic action of 5-HT ought to have. The most important experiments in this field are those of Brun (37, 38, 39) on the rat.

Having shown that while certain vascular areas respond intensely to the serum vasoconstrictor others are practically insensitive to it, he concludes that the intervention of such a vasoconstrictor in hemostasis cannot be considered general. Other observations serve to underline those of Brun (see 100).

(c) It is possible to increase the intensity of the post-traumatic vasoconstriction of the mesenteric and ear vessels of the rabbit and of the vessels of the frog's interdigital membrane, while inhibiting with heparin the formation of the hemostatic plug, or even the accumulation of platelets at the wound edges. Moreover, post-traumatic spasm of the rabbit's ear vessels may occur despite the spontaneous absence of any intravascular thrombosis or platelet agglutination (149).

(d) *Elasmobranchii* and *Chondrostei*, among the fishes, possess typical enterochromaffin cells and 5-HT; *Cyclostomata* and *Teleostei* are without them. No experimental data are available to justify the conclusion that the process of hemostasis comes about in different ways in the various groups of fishes.

(e) A normal enterochromaffin system is present in the gastrointestinal mucosa of the ascidians, but they have no thrombocytes nor there is any evidence of a coagulation of the hemolymph. How could 5-HT intervene in the mechanism of hemostasis?

(f) A quantity of 5-HT corresponding to the total 5-HT content of the blood is metabolized in human beings, dogs and rats at least every 1–3 hours. This large turnover of 5-HT seems excessive for hemostasis.

In a recent paper, Page and McCubbin (191) attribute to 5-HT the main function of depressing, through a peripheral mechanism, the neurogenic vascular tone, thus provoking a predominant vasodilatation. This would make 5-HT antihemostatic.

2. 5-HT as a factor controlling arteriolar tone. The hypothesis of a possible "physiological" intervention of 5-HT in the control of arteriolar tone, and therefore of the systemic blood pressure (179), would not be worth special attention had it not been taken up in the last few months by Page and McCubbin (191), who formulate it thus: "Serotonin is a naturally occurring compound and the possibility that it has a physiological regulatory function on neurogenic vasoconstriction should be kept in mind".

The Cleveland observers postulate two opposite actions of 5-HT in the control of arteriolar tone: one positive, spasmogenic, due to direct stimulation of the vascular smooth muscle, the other negative, spasmolytic, which may be attributed to inhibition of the neurogenic vasoconstrictor tone, as a consequence of a possible prevention of the release of adrenergic mediators at sympathetic nerve endings. The "physiological" action of 5-HT is presumed to be the second, since it appears even after small doses of the substance; the direct vasoconstrictor action would, on the contrary, become prominent only with large amounts of 5-HT, or under conditions of depressed neurogenic vasoconstriction. To sum up: 5-HT would be in reality much more a "serohypotonin" than a "serotonin".

Page and McCubbin are the first to acknowledge the incompleteness and provisional nature of their hypothesis. Even when we consider it as an attempt

to interpret only the "pharmacological" effects of 5-HT, there is a whole series of experimental data which seem irreconciliable with it. For example, the differences in the pressure responses elicited by 5-HT in the various animal species are not satisfactorily accounted for, and there is no explanation for the failure of the sympatholytic drugs to abolish 5-HT hypotension.

When we come to attribute to 5-HT a definite significance in the "physiological" regulation of the arteriolar tone, the objections to Page and McCubbin's hypothesis are much more serious. In the formulation of their hypothesis the American observers have in fact neglected the available quantitative and comparative biochemical data. We must not forget that to have an insignificant, short-lived effect on arterial pressure in human beings at least 60 to 120 μg. of 5-HT injected intravenously are required (191, 251), that is to say an amount of substance equivalent to that contained in 1200 to 2400 ml. of blood; that the sudden, massive release into the plasma of the entire platelet 5-HT of a dog (7.3 μg./kgm.) would cause nothing but an insignificant and brief pressure change; and that the subcutaneous injection, in both the dog and the rat, of quantities of 5-HT equivalent to the total content of the substance in the organism has no effect whatever on the blood pressure. Moreover, it remains to be explained why some groups of fishes contain 5-HT, and others do not.

3. 5-HT as a factor regulating renal function and hemodynamics. According to the writer and his colleagues (84, 88, 89, 91, 97, 100, 101, 102, 104),5-HT is a hormone participating in the control of the hemodynamics and function of the kidney. The renal action of 5-HT has been studied with sufficient accuracy in only a few animal species, but in spite of this undeniable gap in the evidence, the hypothesis under discussion seems to be supported by a certain number of impressive experimental and comparative anatomical data:

(a) The renal action of 5-HT has been observed for definitely physiological doses of the substance. To have an antidiuretic action in the rat, due mainly to afferent vasoconstriction, it is enough to inject 4 μg./kgm. of 5-HT subcutaneously, that is $\frac{1}{8}$ of the blood 5-HT, and $\frac{1}{30}$ of that contained in the entire organism. This dose is wholly without effect on other functions. The urinary output of 5-hydroxyindoleacetic acid demonstrates that at least 9–10 μg. of 5-HT/hour/kgm. of body weight are released into the plasma of the rat, and this quantity is probably sufficient to influence the urine flow appreciably.

To provoke a powerful constriction of the vessels of the dog kidney a dose of 5-HT lower than the small quantity of substance in the animal's blood (0.04–0.28 μg./ml.) is certainly sufficient. In describing their perfusion experiments on the dog kidney with fresh defibrinated blood, Starling and Verney (252) state: "As soon as the defibrinated blood reaches the kidney, the vessels constrict firmly, so that it may be impossible to force a drop through them". And Herrick and Markowitz (144) add: "It is almost impossible to force more than a few cubic centimeters of blood a minute through the kidney in a heart-lung-kidney perfusion except when the blood has previously been circulated through the lungs".

We know that the vasoconstrictor principle of fresh defibrinated blood is

5-HT. In the dog 5 per cent of the total 5-HT is present in the circulation, another 5 per cent is contained in the spleen and 90 per cent in the gastrointestinal mucosa; it may be calculated that a dose of less than $\frac{1}{20}$ of the total amount in the dog's body would cause marked renal vasoconstriction. Moreover, from the urinary output of 5-hydroxyindoleacetic acid (100, 102, 257), we know that a quantity of 5-HT corresponding to the total content of this substance in the dog's blood is metabolized at least every two hours.

(b) Dicker and Ginsburg (56), Heller (142), and Ginsburg and Heller (130, 131), have found recently that subcutaneous administration of rat serum causes in the rat an evident antidiuretic action. The active principle, called "stable antidiuretic substance", has now been identified as 5-HT (56a, 98). This represents a further confirmation of the effective antidiuretic action of 5-HT (discovered by the Bristol group in researches completely independent of those on enteramine) as well as of the possibly physiological nature of this action, since an aliquot of the circulating 5-HT (1 ml. serum per 100 gm. rat) is enough to provoke it.

(c) The hypothesis we advance is in agreement with all the available data on the distribution of the enterochromaffin cell system throughout the entire animal series.

Typical enterochromaffin cells and 5-HT are lacking in only two groups of vertebrates, the *Cyclostomata* and *Teleostei*, and it is just in the extremely important group of *Teleostei* that, owing to the particular environmental osmotic gradient and the consequent necessity for reducing glomerular activity, the glomerular apparatus has undergone marked reduction in size and capillarity, and in many species has disappeared entirely, leading to the formation of the aglomerular kidney (248).

In sharp contrast with that of *Teleostei*, the kidney of *Elasmobranchii* shows large and well developed glomeruli and, at the same time, the gastrointestinal mucosa contains typical enterochromaffin cells and abundant 5-HT.

* * *

After this paper had been submitted for publication, a brief communication of Woolley and Shaw on the biological significance of 5-HT appeared in the Proceedings of the National Academy of Sciences, New York (299) followed by a more comprehensive article in the British Medical Journal (299a). The thesis of these articles is that some pharmacological findings indicate that 5-HT "has an important role to play in mental processes and that the suppression of its action results in a mental disorder".

Woolley and Shaw's new hypothesis is based essentially on the observations (a) that brain and other nervous structures contain 5-HT and (b) that several drugs antagonistic to 5-HT produce in man and animals mental and nervous disturbances (schizophrenia, hallucinations and euphoria, epileptiform convulsions) which may be interpreted as being due to the inhibition of the action of 5-HT in the central nervous system.

At present the highly stimulating hypothesis of Woolley and Shaw seems to be rather fragile, certainly much more so than the hypothesis they have appar-

ently abandoned on the participation of 5-HT in the maintenance of the systemic blood pressure. Among other things, insufficient consideration has been given by them to the widespread distribution of 5-HT in the animal kingdom, and to the fact that available data on the occurrence of 5-HT in the central nervous system of vertebrates are as yet extremely scanty, being virtually limited to the dog (4, 5) and the ox (300).

It may be of interest, in connection with Woolley and Shaw's hypothesis, to recall the results of two recent investigations. Lewis and McIlwain (168a) found that 5-HT, 10^{-4} to 10^{-5} M, had little effect on normal respiration of separated guinea pig cerebral cortex in glucose-saline media or on its respiratory response to electrical pulses, and did not decrease but tended to increase the inhibiting effect of lysergic acid diethylamide, 3×10^{-5} to 8×10^{-6} M, on respiration and glycolysis. Hoffer, Osmond and Smythies (148a), in their turn, have demonstrated that the injection of adrenochrome into human beings is followed by the appearance of hallucinations. They point out that, if it is true that adrenochrome is a normal breakdown product of adrenaline, then it is the first naturally occurring substance to be shown to cause hallucinations. Since the formula of adrenochrome suggests that it might act as an inhibitor of 5-HT (162b), the compound was tried by Erspamer (unpublished observations) on the rat uterus preparation and in the 5-HT antidiuresis test. There was no antagonistic action to 5-HT.

VII. SUMMARY

1. The gastrointestinal mucosa of all vertebrates, with the exception of *Teleostei* and *Cyclostomata*, and of ascidians contains a peculiar system of cells, known as the "enterochromaffin cell system," which is furnished with specific granules characterized by several well-defined and distinctive histochemical and physicochemical features.

The gastrointestinal enterochromaffin system, or enterochromaffin system in the strict sense, is a part of a broader system, which includes also the chromaffin cells of the posterior salivary glands of *Octopoda* and of the hypobranchial body of *Muricidae*, as well as the granular chromaffin cells of the cutaneous glands of amphibians.

The specific secretion or storage product of the enterochromaffin system is 5-hydroxytryptamine (enteramine, serotonin), possibly together with its immediate precursor 5-hydroxytryptophan and, in the case of amphibian skin, with closely related indolealkylamines.

The enterochromaffin cells of the gastrointestinal mucosa are believed to release their secretion product only into the blood or lymph; for other cells of the enterochromaffin system an external secretion must be admitted, alone or together with an internal secretion. 5-HT released into the blood seems to be selectively absorbed by the thrombocytes, which represent authentic circulating reservoirs of the substance. The conditions which regulate the physiological liberation of 5-HT from the intact thrombocytes are obscure. Of course, when these cells disintegrate, 5-HT diffuses into their suspending medium or temporarily accumulates in the organs in which thrombocyte destruction is particularly intense (spleen).

The occurrence of 5-HT and related indolealkylamines in a histochemically undetectable form or concentration has been demonstrated, besides in platelets and in spleen, in other animal tissues, as well as in vegetable tissues. All these findings emphasize the widespread distribution of the substance in the living organism.

Data are now available on the 5-HT content in blood, spleen, gastrointestinal mucosa, and hence in the entire organism of numerous vertebrate species.

2. The pharmacology of the indolealkylamines must be considered separately for each member of this group of substances. Slight modifications in the indole ring (displacement or suppression of the phenolic hydroxy group) or in the side chain may produce, in certain instances, insignificant changes in the pharmacological effects, or, on the contrary, profound alterations. Moreover, it seems quite arbitrary to generalize to all animal species the results obtained in a single species.

The most characteristic and important pharmacological property of the indolealkylamines is certainly that of being smooth muscle stimulants. With few exceptions all extravascular smooth muscles are brought into contraction by these substances, both *in vitro* and *in vivo*. Some muscular preparations are particularly sensitive and may be used in the biological assay of 5-HT and other indolealkylamines (estrous uterus of the rat, rat's colon).

The isolated vascular smooth muscle is predominantly constricted by the indolealkylamines, but this does not necessarily imply that the same occurs for the vessels *in situ* in the intact animal. Indeed, the changes in the tone of the most important vascular areas of the organism, and consequently in the systemic blood pressure, induced by these substances are the result of manifold, direct and reflex cardiovascular actions. The importance of the various factors which participate in determining the pressure response to the indolealkylamines varies not only according to the substance administered but, for the same substance, according to the dose, the route of administration, the anesthetic used, the neurogenic vasoconstrictor tone and, above all, the animal species.

The effect on the systemic blood pressure is particularly erratic and unpredictable in the case of 5-HT. Tryptamine and closely related substances, without hydroxy groups on the indole ring, are predominantly hypertensive. The same is true for bufotenidine. Other indolealkylamines behave in a manner similar to 5-HT; still others are entirely inactive (Table V).

Some vascular areas seem to possess an exceedingly high sensitivity to 5-HT. Among them the renal one is worthy of particular attention since the constriction of the afferent vascular bed is believed to be the most important cause of antidiuresis observed in rats after small, "physiological" doses of 5-HT.

5-HT has no influence whatever on the blood clotting system. The ability of the substance to shorten the bleeding time, on which much emphasis has been laid by several investigators, is a pure consequence of its vasoconstrictor action.

The action of 5-HT and tryptamine on the mammalian heart is stimulant, but generally rather moderate. Very sensitive to 5-HT are the hearts of some molluscs.

TABLE V

Effect on blood pressure and on respiration of indolealkylamines administered intravenously to different animal species

Effect on	5-HT	Bufotenine	Bufotenidine	Tryptamine	Gramine	Observations
Blood pressure						+ indicates increase in blood pressure, − pressure fall; in parentheses are occasional effects
Man:						
normal.....	+					
hypertensive..	(−)+−					
Dog:						
normal.....	(−)+−	+	+	(−)+(−)	+(−)	
hypertensive..	(+)−					
Cat:						
normal.....	(+)−(+)	(−)+(−)		+	(−)+	
spinal.....	+	+	+	+		
Rabbit.....	−	(−)+(−)		(−)+(−)		
Rat.....	(+)−					
Guinea-pig.....	(−)+−					
Respiration						+ indicates respiratory stimulation, − reduced respiratory rate or apnea; in parentheses are occasional effects
Dog:						
normal.....	+(−)			+(−)	+	
hypertensive..	−+			−+		
Cat.....	−+	−+	−+	−+		
Rabbit.....	+−					

Respiration is influenced by the indolealkylamines through different mechanisms. The final result varies according to the dose, the experimental conditions and the animal species. Stimulation seems on the whole predominant, but it is often preceded, followed or interrupted by periods of apnea or reduced respiratory rate.

Several other effects have been described, mainly for 5-HT: on the central nervous system and intraganglionar nerve impulse transmission, on the permeability of capillaries, on the production of cutaneous pain, on the adrenal medulla, on the release of histamine, on the eosinophils, and on the blood sugar. The importance of these effects is, with some possible exceptions, purely pharmacological.

3. The primary precursor of all indolealkylamines containing two carbon atoms on the lateral chain is L-tryptophan. L-Tryptophan oxidase catalyses the oxidation of this product to 5-hydroxytryptophan which, in its turn, is transformed into the corresponding amine, 5-HT, by 5-hydroxytryptophan decarboxylase. It is probable that all other natural 5-hydroxyindolealkylamines originate from 5-HT through methylation, dehydrogenation and condensation processes.

Amine oxidase is of fundamental importance in the inactivation of 5-HT and related indolealkylamines in the living organism. Substrates of amine oxidase are not only amines with a primary amino group in the lateral chain, but also

those with a secondary and even with a tertiary amino group. The quaternary ammonium bases, *e.g.*, bufotenidine, seem, on the contrary, not to be attacked by the enzyme.

The main breakdown product resulting from the attack of amine oxidase on 5-HT is 5-hydroxyindoleacetic acid, which is a normal constituent of the urine of carnivorous and omnivorous mammals, and probably of other groups of vertebrates.

Administration of 5-HT provokes a very remarkable increase in the urinary output of 5-hydroxyindoleacetic acid, but this only in those animal species whose urine contains the acid as a normal constituent. Five to 60 per cent of the 5-HT administered may be recovered from urine as 5-hydroxyindoleacetic acid. Administration of 5-methoxytryptamine is followed by the appearance in urine of great amounts of 5-methoxyindoleacetic acid, that of tryptamine and N-methyl tryptamines by the appearance of varying amounts of indoleacetic acid, both free and conjugated with glycine (indoleaceturic acid).

From the study of the excretion of 5-hydroxyindoleacetic acid in normal urine and in urine of animals given 5-HT it is apparent that the metabolism of endogenous 5-HT is very intense. It is, therefore, to be expected that the biosynthesis of 5-HT also occurs very rapidly starting from the widely distributed L-tryptophan, and that the storage of 5-HT in the enterochromaffin cells can be easily and promptly renewed, when exhausted.

4. The biological significance of the indolealkylamines other than 5-HT is entirely unknown. It may be that most of them simply represent metabolic end-products destined to be eliminated from the organism.

For 5-HT four principal hypotheses, each one virtually independent of the other, have been advanced: one sees in 5-HT a factor influencing hemostasis; another considers that it controls vascular tone and therefore the systemic blood pressure; a third regards the substance as a hormone participating in the regulation of the function of the kidney; the last attributes to 5-HT an important role in maintaining normal mental processes, and considers the lack of 5-HT in the central nervous system as a possible cause of some mental disorders.

None of these hypotheses is fully satisfactory, and against more than one of them the fundamental objection may be raised that it does not take into necessary account the distribution of 5-HT in the animal kingdom, nor the essential data on the 5-HT content and on the rate of metabolism of the substance in the organism of the experimental animal. Indeed, when trying to explain the function of 5-HT, the wide distribution of this product in vertebrates and invertebrates should be always kept in mind. Moreover, it is quite obvious that amongst the many biological actions possessed by 5-HT, the only ones which are to be taken as possibly "physiological" are those provoked by doses smaller than the total 5-HT content of the entire organism of the experimental animal.

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