PHARMACOLOGICALLY ACTIVE SUBSTANCES OF MAMMALIAN ORIGIN[1,2]

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The limited space available for dealing with a subject of such extent and interest as this makes it very difficult to give an account of it, even a concise and approximate one. The sole purpose of this review article, then, is to stimulate the reader to look elsewhere in order to widen his knowledge of this up-to-date and fascinating field of research.

The term "pharmacologically active substances" is necessarily ambiguous, and, consequently, the choice of substances to be included under that denomination will be largely arbitrary.

We shall confine ourselves to dealing essentially with those compounds which are active on smooth muscles and blood vessels and make up the group called "local hormones" or "tissue hormones." They are compounds which carry out their predominant actions in the immediate neighborhood of their site of production. We shall not consider the true hormones (e.g., the polypeptides of the neurophypophysis) nor the neurohumoral mediators or nervous transmitters. So the catecholamines will be discussed briefly, not as neurohormones, but as possible local hormones. Similarly, only active compounds present under normal conditions will be considered, leaving aside those liberated solely under pathological conditions.

The three groups of active compounds to be taken into consideration in succession are (*a*) amines, (*b*) polypeptides, and (*c*) lipid-soluble organic acids.

AMINES

Rapid improvement of purification and detection methods of amines in biological materials and, perhaps even more, routine use of monoamineoxidase (MAO) and diamineoxidase inhibitors have led in the last few years to a conspicuous increase in the number of known biogenic amines. It may be predicted that the list of these compounds is destined to lengthen in the near future.

In this paper we will include among the biogenic amines the classic compounds, which have powerful actions on smooth muscles and blood vessels, and their derivatives still bearing the amino group in the molecule. Many

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

[2] Abbreviations used in this chapter include: CNS (central nervous system); 5-HT (5-hydroxytryptamine); MAO (monoamineoxidase); PPS (pain-producing substance).

of these derivatives have been considered inactive metabolites of the authentic amines, but this opinion should be dropped because it is largely arbitrary. In fact, there is no valid reason for excluding the possibility that in certain sites or on effector structures other than smooth muscle, methylated, acetylated, or conjugated derivatives may be as active as, or even more active than their supposed precursors. Melatonin (139, 140) and psilocybin (206) are striking examples in favor of this assumption.

As already stated, this review is not intended to discuss in detail the amines involved in the transmission of nerve impulses. However, evidence is accumulating which suggests the possibility that these amines too (to be more precise, above all, the catecholamines) may also act as local hormones producing their effects independent of any direct nervous activity.

Biogenic amines are small, very agile molecules, which can be rapidly synthetized and rapidly destroyed. They have all the requirements for a quick, brief action, which may, of course, also represent the initiation of long-lasting, delayed chain reactions.

Phenylalkyl- and Phenylalkanolamines

Phenylethylamine.—In normal urine this is present in amounts less than 20 μg./day, but after MAO blockade and feeding extra phenylalanine (the precursor amino acid), this quantity increases up to 60 μg./day. Urine of phenylketonuric patients does not differ from urine of normal subjects in its phenylethylamine content, but treatment with MAO inhibitors produces an enormous increase of the amine content, up to 3000 μg./day, and more (117). Exogenous phenylethylamine possesses only moderate peripheral effects, but it is possible that these are considerably reinforced after MAO blockade and that, following administration of the precursor amino acid plus MAO inhibitors, central effects also make their appearanc

Tyramines.—All three tyramines, ortho, meta, and para, have been shown, quite recently, to be normal constituents of mammalian urine (117), and it is quite certain that in the near future they will be identified in mammalian tissues as well. This implies that the mammalian organism will also contain all three precursor tyrosines, i.e., that phenylalanine may be hydroxylated in ortho, meta, and para position (78). The enzyme capable of decarboxylating ortho- and meta-tyrosine was detected in the mammalian organism long before the detection of the tyrosines themselves (25).

The detection in mammals of tyrosines and tyramines, other than para isomers, was preceded by their demonstration in lower vertebrates and invertebrates, leptodactyline [(*m*-hydroxyphenylethyl)-trimethylammonium] in *Leptodactylus* skin (68), and *o*-tyrosine in acid hydrolysates of the larval cuticles of the insect, *Calliphora vomitoria* (56).

It may be of interest to remember that tyramine or a tyramine-like substance is a normal constituent of human saliva (175) and that N-acetyl-*p*-tyramine has been isolated from the silkworm (36). It is quite possible that N-acetyl-tyramines are also found in mammalian organisms, since they

are capable of N-acetylating catecholamines and 5-hydroxytryptamine.

Octopamine.—This amine, *p*-hydroxyphenylethanolamine, was first detected in extracts of the posterior salivary glands of octopoda (64). Now it has apparently been found in extracts of ganglia of octopoda (46) and, what is more important, in urine and several tissues of mammals treated with MAO inhibitors. In extracts of some rabbit tissues, octopamine became, after MAO blockade, the most prominent phenolic amine (119).

At present it cannot be decided whether octopamine originates from hydroxylation of *p*-tyramine or from decarboxylation of *p*-hydroxyphenylserine, an amino acid hitherto unknown in nature. Mammalian and invertebrate organisms contain enzyme systems capable of catalyzing both reactions.

Since both meta- and ortho-tyramine occur in invertebrates and mammals, it may be that even their hydroxylation derivatives, *m*-octopamine and *o*-octopamine, will be detected in the future in biological materials.

The above list of phenylalkyl- and phenylalkanolamines occurring in the mammalian organism should be considered provisional. At the Institute of Pharmacology, University of Parma, another hydroxyphenylalkylamine and another phenolic amino acid have been found on chromatograms of tissue extracts from lower vertebrates and invertebrates. Their identification is in progress.

Tyramines and octopamine do not produce particularly remarkable peripheral pharmacological effects, as they are 100 times less potent than epinephrine or norepinephrine. This statement, however, is of negligible importance because it is conceivable that on particular effector structures or enzyme systems they may be as active as, or even more active than catecholamines. Mitoma and co-workers (153), for example, found that in experimental animals, presumably after having been decarboxylated in brain tissue to the corresponding amines, intravenous ortho- or meta-tyrosine produced marked signs of central excitation and sympathetic stimulation resembling those caused by *d*-amphetamine.

Catecholamines.—It now seems highly probable that catecholamines may display in the mammalian organism functions other than those of nervous transmitters. In fact, it is possible that catecholamines are released in tissues independently of any direct nervous activity and that they act to produce, at the site of their liberation, discrete and localized responses, exactly as do authentic local hormones.

In favor of this opinion: (*a*) Nordenstam and co-workers (2, 156) have demonstrated in the connective tissue of the human skin the existence of small, eel-like chromaffin cells which are believed to store catecholamines. It is not known whether biosynthesis and release of catecholamines by these cells is controlled by the autonomic nervous system. If not, the amines would act as local hormones, possibly in the regulation of the caliber of blood vessels (35, 161b). It is of interest, in this regard, that calf skin, the vessels of which are subjected to greater hydrostatic pressure, contains two

to four times more chromaffin cells than abdominal skin.

(*b*) Falck and co-workers (23, 77) found particular chromaffin cells which were clearly distinguishable by histochemical methods from all other mammalian chromaffin cells in liver, lung, and gastrointestinal tract of cows, sheep, and goats. Since the distribution and number of these cells closely followed the dopamine content in various tissues, the Scandinavian investigators suggest that the chromaffin cells are storage structures for dopamine. The function of the huge accumulation of dopamine in ruminant tissues (up to 10 to 25 μg./gm.) is unknown, since it is unknown whether the dopamine is formed locally or is taken up from the blood (177). However, the fact that only ruminants possess dopamine-storing cells is in favor of the idea that the dopamine in the cells is a local hormone rather than a nervous transmitter.

Epinephrine and norepinephrine have been considered for years as the only active catecholamines in the mammalian organism. However, dopamine is now being considered by an increasing number of investigators not only as a precursor amine, but as a compound having its own definite physiological functions (39, 178); some of the so-called metabolites of epinephrine and norepinephrine, in their turn, are reconsidered in view of the possibility that they are not simple inactivation products.

Axelrod (9) showed that a major metabolic pathway for epinephrine and its congeners is O-methylation at the 3 position, and Evarts and co-workers (72) claimed that this O-methylation is an effective means of inactivating catecholamines. This conclusion may be exact in regard to the direct circulatory effects of catecholamines, but it seems largely arbitrary from a general point of view. Bacq (12) and Charlier (45), for example, maintain that metanephrine and normetanephrine act, through inhibition of methyltransferase, as physiological regulators of tissue sensitivity towards adrenergic mediators.

Other metabolites of catecholamines have been detected in mammalian urine; 2,4,5,-trihydroxyphenylethylamine, a metabolite of dopamine (181), and *N*-acetylnormetanephrine, a metabolite of norepinephine (186), and certainly others will be found by the routine use of MAO inhibitors and of inhibitors of the catechol O-methyl transferase. It should be added that Senoh and co-workers (182) succeeded in demonstrating that catechol O-methyl transferase is capable of catalyzing not only the *m*-O-methylation, but also the *p*-O-methylation of phenolic compounds. O-methylation of catecholamines at the 4 position (55b), instead of at the 3 position, would lead to a new series of compounds of the greatest interest.

Histamine

In spite of numerous papers and continuous interest in histamine, our present knowledge of the role of this amine in normal and pathological physiology is still largely obscure. However, it may be that the basis for a more rapid progress in histamine investigation has been laid by Kahlson

and co-workers (123) who have approached in a more dynamic way the problem of the physiological significance of the substance. Everyone will now agree with Kahlson that for the solution of the above fundamental problems, studies on the rate of histamine formation and metabolism and routine employment of drugs or diets capable of interfering in the biosynthesis, storage, and breakdown of histamine are more promising than static measurements of the histamine content of tissues. This statement, of course, can be applied generally to all other biogenic amines and, probably, to all the other active substances dealt with in this paper.

Biosynthesis, storage, and metabolism.—All the histamine appearing in tissues and urine of rats kept on a histamine-free diet is of endogenous origin. Intestinal bacteria do not contribute to its formation (95). The puzzling observation that urinary histamine is mainly in conjugated form in male rats and in free form in female rats has not received as yet any satisfactory explanation (155).

Histamine is formed in the organism by decarboxylation of histidine. This enzymic reaction occurs in a number of parenchymatous tissues, including brain (214), but it is of different intensity in different tissues and different animal species. Enormous differences could also be demonstrated between prenatal and postnatal life. In the liver of rat fetuses, for example, the histidine decarboxylase activity was 3000 to 5000 times more intense than in the liver of adult rats (124).

Histidine decarboxylase is, similarly to other decarboxylases, a pyridoxal-dependent enzyme. The consequence is that a pyridoxine-deficient diet may consistently reduce biosynthesis of histamine. The same result, of course, could be reached by the use of histidine decarboxylase inhibitors. However, pyridoxine-deficiency would necessarily impair decarboxylation of a number of other amino acids, and available histidine-decarboxylase inhibitors (e.g., semicarbazide) are unsatisfactory both in regard to intensity and specificity of effect. This makes diets and inhibitors very useful in studying the rate of histamine formation, but much less so in investigating the physiological consequences of lack in histamine biosynthesis.

Once formed, histamine may be immediately metabolized or stored. Storage, which necessarily implies a more or less strong bond between histamine and cellular constituents, may be independent of the rate of histamine formation and seems to be more conspicuous and fast in adult than in fetal life (123). As with other amines, histamine may be stored in sites different from those of its biosynthesis (e.g., blood platelets).

Histamine storage is evidently intended to meet hypernormal local or systemic requirements for the amine, under particular physiological or pathological conditions. Impairment of storage capacity could reasonably be expected to bring into light some aspects of histamine deficiency and be of usefulness in prophylaxis of pathological manifestations, resulting from abrupt release of the amine from its body depots (123). Cortisone, for example, seems to hinder storage of histamine (174).

We already know several products capable of liberating histamine from its binding sites (compound 48/80, reserpine, polymyxin B, 5-hydroxytryptophan, etc.). Unfortunately, their histamine-releasing activity varies from one animal species to another, and in the same species from one tissue to another. Moreover, none of them is a specific liberator, as release of other amines, heparin, and, presumably, other active products regularly accompanies liberation of histamine. Useful as they may prove in the elucidation of biochemical problems, this is the fundamental reason for the poor contribution of histamine-releasers to our knowledge of histamine function.

Free histamine is attacked by various enzyme systems. Although diamineoxidase seems to be the enzyme which acts most intensely and promptly *in vitro,* it is questionable whether it is the enzyme mainly responsible for the inactivation of histamine in the living organism under normal conditions. It may be that MAO plays a major role.

We now possess a growing number of drugs which inhibit diamineoxidase (aminoguanidine), MAO, or both [iproniazid and related compounds containing a hydrazine moiety (48, 90)], and it has already been shown that under the influence of these drugs histamine may accumulate in concentrations larger than normal in tissues, including nervous tissue (21), and a larger portion of the formed histamine may be excreted in the urine (128). MAO and diamineoxidase inhibitors will certainly contribute to our knowledge of histamine metabolism, but, again, it is doubtful whether they will contribute to a better understanding of the physiological significance of the amine. The reason for this doubt lies in the lack of specificity of the inhibitors and in the increasing number of biogenic amines found to be substrates for MAO and diamineoxidase.

Physiological functions of the histamine occurring outside the mast cells.—(*a*) Histamine in pregnancy: In pregnancy there is a more or less evident rise in the urinary excretion of histamine, which starts some time before term and falls just before or immediately after parturition. Although this increase in histamine production and excretion during pregnancy has been well known for a long time, it has been studied with accuracy only in the last few years by Kahlson and co-workers (120, 121). These investigators demonstrated that in rats the excess urinary histamine found in late pregnancy is of fetal origin, the fetal liver being the organ mainly responsible for the increased biosynthesis of the amine. Fetal histamine formation is considerable in man also (124).

Maternal urinary excretion of histamine falls abruptly at the time of parturition, and this fall is accompanied in the organism of the newborn rat by a rapid regression of the histidine-decarboxylase activity from an unparalleled height to the low level found in adult animals. This evidently means that excess histamine produced by the fetuses during pregnancy may have something to do with parturition or with fetal development.

In the rat in which the uterine smooth muscle is inhibited by histamine, it may be reasonably presumed that this substance might contribute to the in-

hibition of uterine contraction before term. In other species, in which histamine does not inhibit uterine motility, the function of fetal histamine ought, of course, to be different. Kahlson and co-workers (120) are of the opinion that histamine could interfere, in a way unknown as yet, in fetal development. In fact, during pregnancy, inhibition of histidine-decarboxylase brought about by administration of semicarbazide to rats fed a pyridoxine-deficient diet produced arrest of growth and death of fetuses.

(*b*) Histamine and growth processes: Kahlson & Rosengren (122) found that in the rat liver the rate of histamine formation due to a faster decarboxylation of histidine was considerably increased during the phase of regeneration following partial hepatectomy. When the regenerative growth was complete, histamine biosynthesis subsided. Furthermore, Kahlson (123) observed that the tensile strength of healing skin incisions in the rat was increased when the formation of histamine was accelerated and decreased when histamine formation was inhibited. From the above experiments, the investigators conclude that there is a strict connection between histamine formation and constructive metabolism in regenerating liver and healing wounds.

It is not necessary to emphasize the importance of the observations of the Kahlson group. Obviously, as stated by Kahlson himself, the final proof of the active participation of histamine in growth processes will have to await the findings of specific, nontoxic inhibitors of histidine-decarboxylase or of drugs capable of specifically inhibiting the coupling of histamine to its binding sites.

(*c*) Histamine as a chemical mediator in the central and peripheral nervous system: Histamine is found in very large amounts in the hypophysis (especially in the posterior lobe and in the stalk) and in the pineal gland (55b); in considerable amounts in the hypothalamus, corpus mammillare, supraoptic region, and area postrema; and in very small amounts in other parts of the brain (1, 53, 103). No histamine is present in dog cerebrospinal fluid (1). Whereas histamine extractable from the hypophysis may be, in part, of mastcell origin, this is not the case for brain histamine (1, 103). The hypothalamus is capable of forming histamine at a much faster rate than other parts of the brain (214).

Considerable quantities of histamine were also detected in optic nerves, dorsal roots (208), and postganglionic sympathetic fibers of mammals [up to 100 μg./gm. (75)] as well as in ganglia of octopods [2 to 4 μg/gm. (21)].

Although, in vertebrate brain, histamine is said to be insensible to reserpine (205), this is by no means true for the optic ganglia of octopoda. Histamine behaved in these ganglia exactly as did catecholamines and 5-hydroxytryptamine. In fact, reserpine produced a 90 per cent depletion of the histamine content, and β-phenylisopropylhydrazine, a powerful promptly acting MAO inhibitor, a 100 per cent increase of the amine level. A 70 to 100 per cent rise of the histamine concentration was observed within 30

min. after administration of β-phenylisopropylhydrazine. This means that the half-life of histamine in optic ganglia of octopoda is of 15 to 20 min., a figure of the same magnitude as that found in invertebrate ganglia and mammalian brain tissue for 5-HT (21, 200).

From a physiological point of view, it is remarkable that low doses of histamine were found to stimulate the anterior pituitary gland to secrete ACTH (83), to enhance the electrical activity of the cerebellum (53), to stimulate the hypothalamus and the superior cervical ganglion (197), and to inhibit, as intensely as 5-hydroxytryptamine, transcallosally evoked potentials in the optic cortex (91).

Histamine is now being reconsidered and re-evaluated as a possible chemical mediator of the cerebral control of the pituitary gland as well as a possible mediator in the transmission of nervous impulses within and outside the central nervous systems (CNS). Crossland (53) suggests that even the sedating action of antihistamine drugs may be related to the central effects of histamine.

Histamine in the CNS has been ignored for a long time by several investigators as a second-class amine. But this amine, however annoying the fact may be, has the same citizenship rights in the CNS as catecholamines and 5-HT, whose function in the CNS is approximately as obscure as that of histamine.

Shore and co-workers (182b) have recently proposed and employed a fluorometric method for the assay of histamine in tissues. Having found with their method that brain tissue contained very low levels of histamine (why are levels of 0.2 to 0.4 μg./gm. considered very low?) and that gross dissection of brain showed no marked localization of brain histamine, the above investigators conclude that the uniform distribution of the amine suggests that it may be associated with nonnervous, perhaps vascular, tissue. The findings of Shore and co-workers are at complete variance, in almost every respect, with those of investigators who have used biological and colorimetric assay procedures for histamine. It is evident that either fluorometric assay or bioassay are incorrect. In the writer's opinion, biological activity is something more than fluorescence, especially when this activity is determined with the critical accuracy employed by Adam (1).

Physiological functions of mast cell histamine.—Histamine has for a long time been involved in the pathogenesis of the generalized and local anaphylactic reaction, as well as in the pathogenesis of the so-called anaphylactoid reactions produced by the systemic or local application of ovoalbumin, dextran, and other high-molecular weight substances. In addition, the amine has often been considered as one of the factors responsible for the early vascular reactions to injury produced by physical and chemical agents.

The histamine implicated in these reactions is generally considered to be produced in, and released from, the mast cells. This opinion has developed since Riley (164) and other investigators (28) demonstrated that

in a number of tissues of adult mammals there was a close relationship between the number of mast cells and histamine content, the histamine concentration in different tissues of the guinea pig being 0.025 to 0.034 μg. per mast cell.

However, whereas it remains definitely established that in the guinea pig histamine has a prominent role in the acute phenomena of both systemic anaphylactic reaction and Schultz-Dale reaction, results obtained in other animal species were considerably more complicated or largely divergent, and other known and unknown substances have put forth their candidature as mediators in anaphylaxis and, still more, in processes reactive to local injury.

5-Hydroxytryptamine, for example, has been claimed to perform an important role in anaphylaxis in mice (79, 196) and, possibly, in rabbits (204), but neither histamine nor 5-hydroxytryptamine seems to play a decisive part in anaphylaxis in the rat (30).

In regard to early vascular reactions to injury, the intervention or, better, the importance of histamine is still more questionable. Spector & Willoughby (188, 189), for example, think that in the rat 5-hydroxytryptamine may be of greater importance, or as important as histamine as a mediator of increased capillary permeability in the earliest phases of acute inflammation produced by thermal injury applied on the skin or by turpentine injected into the pleura, and that neither amine interferes in the main sequence of inflammatory events. Similarly, Wilhelm (215) suggests that histamine appears to mediate, following mild thermal injury, only an immediate permeability response which is minor in the guinea pig and minimal in the rat, and that the histamine permeability effect has no necessary function as an initiation of the major delayed inflammatory responses. Finally, Smith & Miles (187) claim that both histamine and 5-hydroxytryptamine play an inessential role in early inflammation of the rat peritoneal cavity produced by bacteria and bacterial toxins.

Anaphylaxis and inflammation are complicated phenomena which cannot be explained only in terms of histamine, 5-hydroxytryptamine, or other amines. That these compounds may intervene in the above processes is possible, but their relative importance and their relationship to other shock-producing or inflammatory agents of lipidic (slow-reacting substance A) or polypeptide and protein nature [bradykinin (28c), anaphylatoxin (97), leucotaxine and exudin (151), globulins affecting capiliary permeability (152)] remains to be established.

It has been recently shown that an imidazole N-methyltransferase present in mouse liver is capable of N-methylating histamine (32), and at the same time N,N-dimethylhistamine has been detected by Bertaccini (unpublished observations) in extracts of posterior salivary glands of an octopod, *Eledone moschata*. Thus, N-methylhistamines also ought to be listed among the naturally occurring amines of possible biological interest.

5-Hydroxytryptamine (5-HT) and Related Indolealkylamines

5-Hydroxytryptamine (5-HT, enteramine, serotonin) has been the object during the last few years of intense and extensive research reported in an enormous number of publications. It is impossible to give here even a concise account of these studies [see review articles by Page (158), Lewis (143, 143b), Levy (142), Erspamer (65, 71), etc.], and as a consequence, attention will be focussed only on a limited number of topics of the 5-HT investigation, above all on the problem of the biological significance of 5-HT in the mammalian organism.

Occurrence and distribution of the enterochromaffin cell system and 5-HT in mammals.—The enterochromaffin cell system, and hence 5-HT, is present in the gastrointestinal mucosa of all the examined vertebrate species, including all classes of fishes, as well as in the gastrointestinal tract of the ascidians and of *Amphyoxus lanceolatus* (202b). The recent finding that even the intestinal mucosa of *Teleostei* may contain, at least in restricted areas, a limited number of enterochromaffin cells [Erspamer (unpublished observations)] has filled a hitherto incomprehensible gap and points to a general biological significance of the 5-HT produced by the enterochromaffin cells.

In addition to the gastrointestinal mucosa, 5-HT is also present in considerable amounts in blood platelets and in organs which capture blood platelets (spleen, occasionally lung) as well as in the central nervous system. The substance has, in addition, been found in mast cells and, consequently, in all tissues of rats and mice containing mast cells (49, 66).

Two other indolealkylamines have been shown in the last few years to be normal constituents of the mammalian organism: tryptamine, present in very low concentrations in urine and in several parenchymatous tissues, including brain (107), and melatonin (5-methoxy-N-acetyltryptamine), present, again in very minute amounts, in the pineal gland and in peripheral nerves of mammals (139, 140, 141b).

Biosynthesis and metabolism of indolealkylamines.—It is generally accepted that the enterochromaffin cells of the gastrointestinal mucosa are the main site of production and storage of 5-HT in mammals (55, 65, 66). These cells contain both the enzyme systems which catalyze the two-step passage leading from dietary L-tryptophan to 5-HT. Evidence for this assumption is given, on one hand, by the enormous increase in 5-HT, and eventually 5-hydroxytrytophan production in cases of malignant argentaffinoma (195) (i.e., in cases of neoplastic proliferation of the 5-HT-secreting cells) and, on the other hand, by the conspicuous decrease in 5-HT metabolism following removal of large segments of human (19, 105, 106), dog (69, 169), and rat intestine (20), i.e., following reduction of the 5-HT-secreting tissue. Blood and spleen 5-HT originates, at least to its greatest extent, from the gastrointestinal mucosa.

As for mast cell 5-HT, it now seems certain that the substance may be synthetized entirely within the mast cells themselves. This has been shown

by Schindler and co-workers (176), who found that neoplastic mast cells obtained from a mouse mastocytoma maintained, even after many generations in culture, their high intracellular level of 5-HT. However, it appears once again necessary to emphasize the fact that only in rats and mice do normal or pathological mast cells contain 5-HT. No detectable amounts of 5-HT could be found in mast cells from other mammals (159). The consequence is that, from a general point of view, 5-HT in mast cells of mice and rats is little more than a biochemical rarity, exactly as is the occurrence of 5-HT in the hypobranchial gland of some prosobranchiate molluscs or in the body of some coelenterates (65, 149).

The last extremely important and constant example of 5-HT in the mammalian organism is brain 5-HT. The problem of the origin of cerebral 5-HT is not completely solved. In fact, even if it is certain that brain tissue can decarboxylate 5-hydroxytrytophan, there is no direct evidence that this tissue is also capable of carrying out the oxidation of L-tryptophan into 5-hydroxytrytophan. Indirect evidence, however, seems to strongly support the view that even brain 5-HT may originate locally from L-tryptophan. We refer to the occurrence of considerable amounts of 5-HT in ganglia and peripheral nerves of molluscs and crustaceans apparently lacking the amine in all other tissues (66) and to the presence of 5-HT in the brain of rats deprived operatively of the whole gastrointestinal tract (20).

There is no doubt that the limiting step in the 5-HT biosynthesis is the hypdroxylation of tryptophan to 5-hydroxytrytophan, catalyzed by a tryptophan hydroxylase which apparently has now been traced to the mammalian gastrointestinal mucosa. Decarboxylation of 5-hydroxytrytophan is carried out in a number of tissues by a widespread, poorly specific decarboxylase, which is indistinguishable from dopadecarboxylase (22, 169b, 209), and from the enzyme decarboxylating 4-hydroxytryptophan (70).

Tissue tryptamine is formed by decarboxylation of L-tryptophan, possibly catalyzed by the same enzyme which decarboxylates 5-hydroxytrytophan (201); melatonin derives from O-methylation (provoked by an indole O-methyltransferase) of N-acetyl-5-HT, which may be considered a normal metabolite of 5-HT (150). It has been claimed that the indole O-methyl transferase is a specific enzyme, strictly localized in the pineal gland (10).

Once formed, 5-HT may be stored in the cells in which it has been synthetized or may be released in a free form into the interstitial liquids or into the plasma. The free 5-HT, which is the active form of the amine, is promptly subjected to attack by several enzyme systems acting both on the lateral chain and on the indole ring. Monoamineoxidase causes the oxidative deamination of 5-HT with formation of 5-hydroxyindoleacetic acid; ceruloplasmin attacks the phenolic hydroxy group with possible formation of a *p*-quinone imine derivative (163); other enzyme systems produce acetylation of the amino group in the lateral chain, or catalyze O-conjugation with glucuronic acid (207) or, to a lesser extent, with sulphuric acid (41, 150). N-methylated derivatives of 5-HT, as found in amphibian skin, are not known in the mammalian organism.

Following administration of radioactive 5-HT to rats, approximately 40 to 90 per cent of the radioactivity was found in urine in the form of seven radioactive compounds giving a positive *p*-dimethylaminobenzaldehyde reaction (127, 150).

Monoamineoxidase seems to be the enzyme system mainly responsible for the inactivation of tryptamine, but, besides oxidative deamination, hydroxylation of the indole ring at the 6 position should also be considered as an alternate metabolic pathway for the amine (116). The principal pathway of metabolism of melatonin is 6-hydroxylation, followed by O-conjugation of the N-acetyl-5-methoxy-6-hydroxytryptamine with glucuronic and with sulphuric acids (131).

An enormous contribution has been afforded in the study of the biosynthesis and metabolism of indolealkylamines by the routine use of MAO inhibitors. They have been of decisive importance, for example, in the detection in tissues or urines of tryptamine and several 5-HT metabolites, as well as in the determination of the turnover rate of 5-HT and tryptamine in the central nervous system. It is to be expected that dopadecarboxylase inhibitors will also help to solve many problems connected with the biosynthesis of tryptamine and 5-HT.

The limiting step in the biosynthesis of 5-HT is, as already stated, the hydroxylation of L-tryptophan at the 5 position. The tremendous interest afforded by a better knowledge of the apparently specific enzyme system involved in the reaction is obvious, as it is obvious that the eventual discovery of specific inhibitors of the L-tryptophan hydroxylase would probably offer the key to the understanding of the function of 5-HT within and outside the cental nervous system.

Physiological significance of 5-HT.—(*a*) 5-HT of enterochromaffin origin: Under normal conditions, a quantity of 5-HT corresponding to that contained in the gastrointestinal mucosa is synthesized by the enterochromaffin cells in 8 to 20 hr. (69, 200). From the enterochromaffin cells 5-HT is released into the interstitial liquids and then into the plasma where it is largely absorbed by the platelets. It it highly probable that very minute amounts of 5-HT circulate free in the plasma and that platelets can liberate an aliquot of their 5-HT without necessarily being disrupted or altered.

It is evident that 5-HT may display its biological actions either immediately after its release while still in the interstitial liquid surrounding the enterochromaffin cells or after penetration into the circulatory stream. In the first case, 5-HT should be regarded as a local hormone, in the second as a true hormone.

Smith and co-wokers (185) suggest the possibility that 5-HT "has no normal role as a circulating hormone," and this opinion is largely shared by Lembeck (137), who similarly believes that 5-HT displays mainly local actions, because the other pharmacological effects of the substance become apparent only after injections of exogenous 5-HT or following hyperproduction of 5-HT by malignant argentaffinomas.

The hypothesis of a local function of 5-HT within the gastrointestinal mucosa soon after its release from the enterochromaffin cells is at first sight very attractive, and it has been recently substantiated by important results obtained following introduction of very low doses of 5-HT and 5-hydroxytrytophan into the lumen of the isolated guinea-pig ileum. According to Bülbring and her co-workers (33, 34) and to Lembeck (137), 5-HT could stimulate intestinal motility by sensitizing the sensory receptors in the intestinal mucosa which trigger the peristaltic reflex, and, as a consequence, the formation of 5-HT by the enterochromaffin cells might be a part of the physiological mechanism required for peristalsis.

However, once again extreme caution seems to be necessary in generalizing results obtained in one animal species or in transferring experimental data achieved on isolated intestines (or even on intestinal loops *in situ*) to the gastrointestinal tract of the intact animal. In fact, if we admit that the 5-HT which enhances the peristaltic reflex has a local origin, then it would be rather difficult to give an acceptable explanation of the conspicuous differences in the distribution of the enterochromaffin cell system and of 5-HT along the gastrointestinal tract of the different vertebrate species. What can be the meaning of the enormous accumulation of enterochromaffin cells and 5-HT in the intramural portion of the pancreatic duct of the rabbit? What is the reason for the predominant accumulation of 5-HT in the stomach of mice, hedgehogs, dogs, in the small intestine of guinea pigs and chickens, and in the large intestine of rats, horses, and cows? What is the significance of the enterochromaffin cells found in the mammalian pancreatic islets and in the thymus of some birds (65, 66)?

Moreover, Smith and co-workers (185) were unable to find "any evidence for alimentary control of the release of 5-HT from argentaffinoma and from the normal argentaffin cells," and Johnsen and co-workers (118) failed to observe any relationship between meals (i.e., motor and secretory activity of the bowels) and the peak in the diurnal variation in 5-HT metabolism, as inferred from the diurnal variation in the urinary excretion of 5-hydroxyindoles.

Finally, although 5-HT introduced into the lumen of an isolated guinea-pig intestine produced a stimulation of peristalisis in very low concentrations, a very high dosage was required to produce short bursts of peristalsis when introduced into a loop of the guinea-pig intestine *in situ* (34), and no intestinal or systemic response was elicited when it was given intrajejunally to normal volunteers in doses up to 10 to 15 mg. (47). This suggests that the mucosa of the isolated intestine had undergone some alterations and that results obtained under such unphysiological conditions cannot be extended to the intestines of the intact animal.

Of course, 5-HT could act on the gastroinestinal tract also via the blood. But, frankly, it would be rather surprising if the 5-HT released from the intestines had to return to the same intestines, after having been exposed to severe enzyme attacks and to large losses, to display there its main physiological action.

At any rate, even if there is as yet no proof that physiological doses of 5-HT may influence the gastrointestinal secretory activity, there are experimental results which demonstrate that stimulation of intestinal motility may be produced by physiological amounts of 5-HT injected intravenously or intraarterially (34, 184b). These positive findings are certainly of great importance, but it is evident that they must be reproduced on *in situ* intestines of numerous animal species, including lower vertebrates, before they can be taken as convincing evidence that 5-HT has a general significance as a hormone participating in the control of intestinal motility.

Besides that just now discussed, several other theories have been advanced in an attempt to elucidate the biological significance of extracerebral 5-HT. All these theories, which see in 5-HT a factor influencing hemostasis, or consider that it controls vascular tone and, therefore, the systemic blood pressure, or, finally, regard the substance as a hormone participating in the regulation of renal function, have been critically reviewed in preceding papers (65, 67, 158). Here we wish only to call attention to a few more recent data.

The belief that 5-HT is involved in the process of blood coagulation, in the vasoconstriction subsequent to vascular injury, or, in capillary resistance has been further impaired (*a*) by the observation that capillary resistance and clotting time remained unchanged in reserpine pretreated animals (219) or humans (104), the blood of which contained as little as 2 to 5 per cent of normal 5-HT, as well as in pyridoxine-deficient chickens, the blood of which contained similarly less than 10 per cent of normal 5-HT (199); and (*b*) by the circumstance that there was no correlation whatever in human hemorrhagic diseases between tendency to spontaneous hemorrhage, on the one hand, and platelet or blood 5-HT levels and urinary excretion of 5-hydroxyindoleacetic acid, on the other (190).

The theory that 5-HT controls vascular tone is reinvigorated from time to time by new experimental and clinical data but, unfortunately, results and conclusions reached by the different research workers are often exactly opposite. In accordance with Page (158), Gordon and co-workers (92) suggest that a "biological role of serotonin lies in its negative interaction with adrenalines," and that 5-HT may decrease small vessel tone "through its capacity to antagonize the vasoconstrictor action of noradrenaline on small vessels." Woolley & Shaw (217), on the contrary, continue to maintain their original view that 5-HT is a hypertensive agent, possibly involved in human hypertensive disease, and consider as strong evidence for this assumption the fact that some of their 5-HT antimetabolites reduce or abolish not only the increase of blood pressure produced by exogenous 5-HT in dogs, but also spontaneous hypertension in human beings (216).

Actually, it is by no means possible to exclude that 5-HT may act physiologically on the vascular smooth muscle, at least in some areas, but we are at present unaware of the importance, type, and direction of this action,

and there is no evidence whatever that 5-HT may be involved directly in the pathogenesis of human hypertensive disease.

There is nothing new concerning the theory that 5-HT is a hormonal product participating in the regulation of the intrarenal circulation and the function of the kidney. In spite of the fact that the existence of an antidiuretic effect of 5-HT has been generally confirmed in all mammals studied, we must admit that so far only for rats and mice do the renal actions of 5-HT fulfill the main conditions necessary for being considered "physiological." In this respect, some experimental results of Bertaccini (unpublished observations) may be of interest. This investigator succeeded in demonstrating that oral administration of L-tryptophan produced in rats significant antidiuretic effect, probably attributable to excess production and release of 5-HT by the enterochromaffin cells.

(*b*) Mast cell 5-HT: It has already been pointed out that only mast cells of rats and mice contain detectable amounts of 5-HT. It is, of course, quite possible that mast cell 5-HT may participate in these species (together with histamine, polypeptides, proteins, and lipid-soluble organic acids) in the local regulation of caliber, permeability, and resistance of blood vessels and, hence, in the pathogenesis of the anaphylactic and anaphylactoid reaction (171, 213), and also, possibly, in the pathogenesis of the early manifestations of inflammatory reaction (188, 189). Nobody, however, succeeded in demonstrating that 5-HT can display the same local actions in other species lacking 5-HT in their mast cells. Consequently, mast cell 5-HT remains for the present a characteristic peculiar to some rodents, with no general biological significance.

(*c*) 5-HT in the central nervous system: This is the localization of 5-HT in the mammalian organism which presently raises the greatest interest. So far, more than 350 papers directly or indirectly pertaining to this subject have been published, and it is often extremely difficult to evaluate exactly the reliability and accuracy of experimental results and the consistency and solidity of interpretations and hypotheses.

Our opinion is that 5-HT must certainly possess important and, perhaps essential functions within the central nervous system. In this we agree with Gaddum (5, 85), Woolley & Shaw (218), and all other research workers who have investigated the problem of brain 5-HT. However, things appear rather complicated when trying to give a solid experimental basis to the above opinion and, still more, when attempting exactly to define and delimit the central functions of 5-HT.

The pieces of evidence which have been brought in favor of the hypothesis which sees in 5-HT a neurohumoral transmitter are as follows:

(*1*) Occurrence of 5-HT within the central nervous system is a fundamental observation. 5-HT seems to be present not only in the brain of all vertebrates examined, but also in ganglia and peripheral nerves of molluscs and crustaceans, and it is highly probable that neural 5-HT is of autochtonal origin. Another important circumstance is that, at least in mammals,

5-HT is preferentially contained in peculiar areas of the brain (brain-stem structures, rhinencephalic structures, neostriatum), some of which are part of, or are functionally connected with the autonomic system or with the reticular activating system.

Several investigators have laid emphasis on the occurrence, in the same central areas containing 5-HT, of the enzyme systems which synthetize or inactivate 5-HT. Unfortunately, the demonstration that neural tissue is capable of converting L-tryptophan to 5-hydroxytrytophan is as yet lacking. The presence in the brain of amineoxidase and of dopadecarboxylase is apparently of much less importance, because of the widespread and irregular distribution of these enzyme systems in parenchymatous tissues.

(*2*) Turnover rate of 5-HT in the central nervous system is another basic observation. With the aid of powerful and promptly acting amineoxidase inhibitors it was demonstrated that the half life of cerebral 5-HT is not longer than 10 to 30 min. (200). Similar figures were obtained for the 5-HT present in the optic ganglia of octopoda (*21*). This high rate of turnover fits very well the assumption that 5-HT is a neurohumor.

(*3*) Another set of experimental results strongly suggesting that 5-HT plays a role in controlling nervous processes has been afforded by the use of 5-hydroxytrytophan, the immediate precursor of 5-HT. It is certain that 5-hydroxytrytophan is capable, in contrast to the deriving amine, of penetrating into the central nervous system, and it has been demonstrated that 5-hydroxytrytophan is there decarboxylated to 5-HT. In fact, brain 5-HT may be increased several times after injection of large doses of 5-hydroxytrytophan, and a further increase occurs when MAO inhibitors are first administered.

These facts are now firmly established, but, surprisingly enough, there are still substantial discrepancies not only in the interpretation, but even in the description of the effects elicited by the huge increase in brain 5-HT obtained in this way. Some investigators observed a marked central stimulation (*27*), others an evident central depression (143b, 147). It is clear that discrepancies, while stimulating further studies, tend at present to obscure the problem of the central actions of 5-HT.

In spite of these reservations, it appears that the theory that 5-HT is involved in the control of activity of the central nervous system has a solid basis in the three groups of observations above—only in these three, however, since many other experimental results, at first sight very brilliant and unequivocal in their interpretation, have revealed their real inconsistency and ambiguity on further investigation. Examples of this are the results obtained with reserpine and the MAO inhibitors. In fact, reserpine liberates not only 5-HT and related indoleaklylamines, but also catecholamines, histamine, ATP (130), and perhaps other active products; amineoxidase inhibitors, in their turn, block the oxidative deamination of a number of biogenic amines which are substrates for MAO. In addition, it may be that

these drugs act on other enzyme systems. All this, of course, makes it very difficult to ascribe the effects of reserpine to the lack of one amine rather than to that of the other and, similarly, to attribute the actions of MAO inhibitors to an excess of one amine rather than to that of another. It may be seen that everyone interprets his results as he likes.

So far, no light can be thrown on the problem in discussion by the use of 5-HT-antagonists. Thus, it seems probable that the central effects of *N,N*-diethyl-D-lysergamide, to quote the major representative of 5-HT antagonists, have nothing to do with the anti-5-HT action of this drug (170).

5-HT has often been introduced, alone or with other drugs, into the lateral ventricles of the brain of experimental animals and humans; similarly, it has been injected intravenously or into the carotid or vertebral artery in order to study its effects on spontaneous and evoked electrical activity of different areas of the brain, on synaptic transmission in the lateral geniculate nucleus, on transcallosal transmission, on mono- and polysynaptic spinal reflexes, and on somatic and visceral behavior (71).

Unfortunately, results of these experiments were often contradictory. On the whole, inhibition and depression seemed predominant, but even facilitation and stimulation were frequently observed. It should be added (*a*) that the administered doses of 5-HT were often quite unphysiological; (*b*) that, with a few exceptions, the possibility could not be ruled out that 5-HT might act indirectly through local changes in caliber or permeability of cerebral blood vessels, or through discharges evoked from peripheral receptor organs or central chemoreceptors possibly lying outside the blood brain barrier (31); (*c*) that there is no concordance of opinion in regard to the problem of the relationship of 5-HT to the other neurohumors.

It is obvious that our limited knowledge of the physiological significance of 5-HT makes it very difficult to understand its possible role in psychiatric disorders (129). It may well be that 5-HT intervenes, alone or, much more likely, together with catecholamines and other substances, in the production of mental diseases. However, in this regard we have to consider the possibility that qualitative aberrations in the biosynthesis of 5-HT (e.g., formation of N-methylated derivatives or of derivatives bearing the phenolic hydroxy group in 4, 6, or 7 position instead of in 5) may play a more important part than hyperproduction or deficiency of 5-HT.

Physiological significance of tryptamine and melatonin.—The significance of tryptamine in the organism is completely obscure. It may be, that similarly to 5-HT, it plays a role in the central nervous system.

Melatonin is known to possess a tremendously powerful lightening action on the melanocytes of the skin of *Rana pipiens* (threshold dose 10^{-6} to 10^{-7} μg. per ml.) and to reverse the action of the melanocyte-stimulating hormone. Lerner and co-workers (140, 141) suggest that melatonin may be a neurohormone interfering with the transmission of nerve impulses and of significance in the pathogenesis of vitiligo and malignant melanomas.

POLYPEPTIDES

Angiotensin

Angiotensin (hypertensin, angiotonin) is a vasoconstrictor polypeptide produced by the action of renin, an enzyme, upon a substrate contained in the α-2 globulin fraction of the plasma.

Origin and release of renin.—Cook & Pickering (50, 51) and Nairn and co-workers (154) succeeded in demonstrating, by ingenious techniques, that in the rabbit kidney renin is located in or very close to the glomeruli, more precisely in a structure at the vascular pole of the glomerulus, possibly the juxtaglomerular apparatus of Goormaghtigh. The outer glomeruli always contained three to six times more renin than the inner glomeruli.

Nothing is known about the release of renin from the kidney under normal conditions. It has been reported that blood of normal dogs contains approximately 0.02 to 0.05 Goldblatt units of pressor substance (equalling 0.004 to 0.01 μg. angiotensin II) per liter, and blood of normal human beings 0.015 units per liter (183), but these findings could not be confirmed by other research workers (160).

Origin and fate of angiotensin.—Renin produced by the kidney cortex acts upon a substrate contained in the α-2 globulin fraction of the plasma (angiotensinogen, hypertensinogen, angiotonin precursor). Skeggs and co-workers (183, 184) succeeded in demonstrating that, unlike all other known enzymes, renin acts between two leucine residues in the middle of a very long polypeptide chain. This achievement was preceded by the demonstration that trypsin releases from the protein substrate of renin a tetradecapeptide (which soon after was also prepared by synthesis) capable of yielding the decapeptide angiotensin I on treatment with renin.

The result of the action of renin on its protein substrate from horse or ox plamsa is an inactive decapeptide called angiotensin I. This is then converted to an active octapeptide, angiotensin II, by the action of a proteolytic enzyme contained in the blood plasma having the unique property of absolutely requiring a monovalent anion for its activation (183).

In vivo angiotensin I is approximately as active as angiotensin II because its conversion to angiotensin II by the plasmatic angiotensin-converting enzyme is very rapid. Once formed, angiotensin is rapidly destroyed in blood and tissues by one or more proteolytic enzymes (angiotensinase, hypertensinase). Destruction may be avoided, and this is very important both in the bioassay and in the large scale preparation of angiotensin by acid treatment of the plasma before addition of renin, or by partial purification of the substrate or by carrying out the incubation of plasma with renin in the presence of charcoal which absorbs angiotensin (61).

Chemical constitution of the angiotensins.—Isolation, purification, and elucidation of the amino acid composition and amino acid sequence of ox and horse angiotensins were accomplished independently in 1956–57 by two groups of research workers (60, 183). The proposed structure of the

polypeptides was soon confirmed by synthesis (179, 180).

It was shown that both horse and ox angiotensins I were decapeptides differing from each other only in the amino acid residue situated in the fifth position from the N-terminal residue. This amino acid is valine for ox angiotensin (val^5-angiotensin) and isoleucine for horse angiotensin (ileu^5-angiotensin).

The amino acid sequences for the three natural angiotensins which have been isolated so far are as follows:

Ox hypertensin I: Asp. Arg. Val. Tyr. Val. His. Pro. Phe. His. Leu.

Horse hypertensin I: Asp. Arg. Val. Tyr. Ileu. His. Pro. Phe. His. Leu.

Horse hypertensin II: Asp. Arg. Val. Tyr. Ileu. His. Pro. Phe.

The plasma enzyme converting hypertensin I to hypertensin II removes the two terminal amino acids, histidine and leucine, as one dipeptide and not individually. Several synthetic analogues of hypertensin II have been prepared by Schwyzer (180).

The pharmacological actions of angiotensin have now been thoroughly re-examined using pure synthetic angiotensin preparations.

Extravascular smooth muscles.—Like various other polypeptides, synthetic angiotensin strongly stimulates the isolated intestines and uteri of several mammalian species. For the guinea pig intestine a linear dose-response relationship was observed in the range of 0.001 to 0.01 μg/ml. Sometimes the sensitivity of the muscle preparation sharply decreased after a maximum stimulus, simulating tachyphylaxis (94).

Blood pressure.—The characteristic effect of angiotensin injected intravenously is a sharp rise of the blood pressure. The shape of the pressure curve is similar to that produced by epinephrine, but shows no secondary fall below the initial level. The pressor action of angiotensin is demonstrable in all examined species, the rat being the most suitable laboratory animal for the quantitative evaluation of this activity. An appreciable rise of blood pressure may be obtained in dogs and rats with doses of angiotensin as low as 0.01 to 0.03 μg./kg., the polypeptide being about 20 times more potent than norepinephrine (94). In human beings the minimum active dose of angiotensin was found to be of the order of 0.5 μg. (183). Continuous infusion of angiotensin in rats and dogs resulted in an elevation of blood pressure during the infusion and a rapid return to the preceding level after its cessation.

Different vascular areas.—Coronary blood flow of cats was either unaffected or increased concomitantly with the pressure rise produced by doses of angiotensin between 0.01 to 1 μg./kg. (94); similarly, there was no reduction of flow in the dog pulmonary vessels (38), in the cat femoral artery (94), nor in the human forearm (160). In contrast to these vascular areas, skin vessels (37, 160), renal arteries, and, still more, mesenteric arteries were constricted by angiotensin (94). It is mainly because of its effects on the vessels in the splanchnic areas that angiotensin produces systemic hypertension.

Isolated heart.—The isolated heart of rabbits and cats responded to hypertensin with a diminution of coronary flow and a slight increase of the amplitude of beat (94).

Diuresis.—In a dosage range in which hypertensin increased blood pressure (infusion of 0.1 to 1 μg./kg./min.), the polypeptide produced a definite antidiuretic effect in hydrated animals. With low doses of angiotensin, sodium and potassium excretion were diminished, especially at the beginning of infusion; with high doses sodium excretion rose markedly during the infusion, the excretion of potassium being only slightly elevated or remaining unchanged (94).

In human beings doses of angiotensin as low as 0.01 μg./kg./min. were found to produce antidiuresis accompanied by a decrease in *p*-aminohippuric acid and insulin clearances, pointing to a vasoconstriction in the renal arterioles (26). With larger doses of angiotensin (0.1 μg./kg./min.) it was almost possible to stop urine flow. In contrast with the increased sodium excretion observed in dogs, there was in every case a marked reduction of electrolyte excretion (160). From the available data it cannot be decided whether angiotensin has an antidiuretic action independent of its vasoconstrictor effect.

Numerous angiotensin-like polypeptides have already been synthetized and studied pharmacologically in comparison with the natural products. An analysis of the relationship between their structure and their activity has been carried out by Elliott (61) and by Gross & Turrian (94). On the whole, it has been found that even rather large variations in the structure of the polypeptides have little or no influence on their specific action (94) and that amino acid sequence is not necessarily as important as size and shape of the molecule in the determination of biological activity (61). Considering more closely the naturally occurring polypeptides only, it has been demonstrated that the octapeptides are about two to four times as active as the corresponding decapeptides.

Physiological and physiopathological significance of the renin-angiotensin system.—The possible role of the renin-angiotensin pressor system under physiological conditions is largely obscure. Skeggs (183, 184) suggests that this system, by virtue of its ability to produce constriction of the arteriolar bed of the glomerulus, may have the normal function of controlling glomerular blood pressure and filtration rate. However, as Gross & Turrian (94) rightly point out "the fact that it is possible to provoke by renin the appearance in plasma of a pressor polypeptide does not yet prove that this polypeptide has any physiological significance." It has already been noted that the occurrence of angiotensin in normal plasma, even in that returning from the kidney, has been questioned (160).

The renin-angiotensin system has been considered by several investigators as the cause of the raised blood pressure in experimental renal hypertension resulting from the constriction of the main renal arteries of animals as well as the cause, or one of the more important causes, of raised blood

pressure in patients with malignant hypertension. However, the renin theory of malignant hypertension was decidedly rejected by other authoritative research workers.

Concerning experimental renal hypertension, the pieces of evidence put forward by the sustainers of the renin theory are briefly as follows: (*a*) Hypertension following the constriction of the renal arteries is caused by a chemical agent released into the blood stream, as shown by the fact that removal of the ischemic kidney produces a drop in blood pressure. (*b*) This agent ought to be renin because ischemic kidneys are loaded with renin, because there is no other pressor material known which will maintain hypertension, and because (and this seems to be the most important point) blood of renal hypertensive animals contains a conspicuously increased amount of angiotensin (0.1 to 1.6 units per liter instead of the normal 0.02 to 0.05 units). Such an amount would be quite sufficient to explain the elevation in blood pressure observed in this condition (183).

However, the most important of the above positive findings could not be confirmed in careful control experiments by Peart (160). Samples of blood were collected by catheter directly from the renal vein of rabbits with hypertension caused by renal artery clipping. At no time was it possible to show the presence of renin or angiotensin in the renal vein blood samples. Similarly, Blaquier and co-workers (24b) succeeded in demonstrating, in cross-circulation experiments in rats, that whereas immediate hypertension which follows release of clamps that had occluded one or both renal pedicles for four hours might be humorally mediated, the maintenance of the elevated blood pressure in chronic renal hypertension was not dependent on circulating pressor material.

In regard to human malignant hypertension, the claims to have demonstrated an increased amount of angiotensin in the circulating blood of hypertensive patients are again more or less balanced by negative findings. Whereas Skeggs and co-workers (125, 183) affirm that the average content of angiotensin in patients with benign essential hypertension is twice, and in patients with malignant essential hypertension 20 times that of normotensive controls, Peart and co-workers (160) were unable to detect any angiotensin in the plasma of blood collected directly from the renal vein of a wide range of patients with severe hypertension, including those with malignant hypertension and unilateral renal disease. In addition, no differences could be found between normal and hypertensive patients in the rate of formation of angiotensin following addition of renin to plasma.

Other pieces of indirect evidence unfavorable to the hypothesis that angiotensin is the agent responsible for human renal hypertension consist of the facts that although intravenous infusion of angiotensin lowers the skin flow, the hand blood flow in hypertensive patients is quite normal and that, in sharp contrast to the effect produced in normal subjects, angiotensin produces in patients with severe renal hypertension a rise in urine flow and electrolyte excretion (160).

It is evident that the above sharply contrasting experimental results make practically untenable any theory concerned with the physiological and physiopathological meaning of the renin-angiotensin system. However, negative findings, although sometimes disconcerting, are opposed by an impressive number of positive results which cannot be ignored or underevaluated. That the renin-angiotensin system is the only factor responsible for experimental and human renal hypertension seems very doubtful, but it is quite possible that it interferes with the mechanism involved in the establishment and maintenance of hypertension. It may be hoped that the present large availability of pure angiotensin will help to elucidate this extremely fascinating problem.

Kallikreins

Kallikreins are high-molecular weight, undializable, thermolabile proteins which may be obtained from precursors (kallikreinogens) by the action of trypsin or other proteolytic enzymes.

The kallikreinogens contained in different tissues or fluids of the body are not identical to each other because they give origin, by incubation with trypsin, to different kallikreins. These may be distinguished from each other not only on the basis of their susceptibility to inactivators (e.g., soybean-trypsin inactivator) and to reactivating procedures, but also through their ability to release kallidin from blood plasma. Bird kallikrein, for example, cannot liberate kallidin from the serum of mammals, and guinea pig salivary kallikrein fails to release kallidin from its own plasma (82, 173, 210).

The main sources of kallikreinogens in the mammalian organism are blood plasma, pancreas, submaxillary glands, intestinal tract (especially large intestine), and, possibly, brain. The kallikreinogen of blood plasma seems to be very similar or identical to intestinal kallikreinogen, but different from pancreatic and salivary kallikreinogens. Normal urine contains only active kallikrein which derives from plasma kallikreinogen activated by the renal tubules. Following renal tubular injury in rats or nephrosis in human patients, kallikreinogen appears and active kallikrein is reduced in urine (211). The existence of different kallikreinogen and kallikrein systems is also demonstrated by the fact that various diets had different influences on the kallikrein content of different organs (80).

The concentration of kallikreinogen in plasma and intestine of dogs and rats was not altered either after pancreatectomy (210) or after total atrophy of the pancreas produced by ligation of the pancreatic duct (80). This demonstrates that the pancreas cannot be the source, or the main source, of the kallikreinogen of the plasma or of the gut, and hence of urinary kallikrein, and excludes at the same time the possibility of an endocrine secretion of pancreatic kallikreinogen (80). Similarly, the extirpation of the submaxillary glands of mice and dogs had practically no influence on the daily kallikrein output in the urine (212). On the other hand, in a series of experiments the external secretion of pancreatic kallikrein

was shown to parallel the secretion of other pancreatic digestive enzymes, more precisely that of proteolytic enzymes (80).

It appears from the above that blood kallikreinogen has its origin in other parts of the body than in the pancreas or salivary glands. Because of the strict resemblance or identity between plasma and intestinal kallikreinogens, it would seem advisable to check the intestine as possible source of the blood kallikreinogen.

When put into contact with blood, kallikrein acts both as an enzyme releasing from the pseudoglobulin fraction kallidin (a dializable, powerful smooth muscle stimulant principle) and as substrate for proteolytic blood enzymes (the so-called kallikrein-inactivators) which provoke its inactivation. It is clear that the yield in kallidin by kallikrein is much higher when the kallikrein-inactivators have been previously destroyed (82, 166).

Kallikreins alone are inactive on isolated smooth muscle preparations; they were, however, found to be extremely effective in increasing capillary permeability to a circulating dye in the guinea pig, and to possess a powerful hypotensive action when injected into the animal, especially dog (24, 82). It is a matter of controversy whether these actions are also, at least in part, attributable to the molecule of kallikrein itself or only to the kallidin derived from it (11).

The biological significance of the kallikreins is completely obscure, and it may be that it is different for the different kallikreins. Schachter (173) suggests that pancreatic and salivary kallikreins, which are in many respects analogous to other digestive exocrine secretions, may display their physiological role "inside" rather than "outside" the gastrointestinal tract. The opinion that kallikrein has some significance in shock is untenable, because kallikrein was not capable of producing in dogs the main features of the shock syndrome (205b).

Bradykinin-Like Popypeptides

Active polypeptides of low molecular weight, producing hypotension and stimulation of extravascular smooth muscles, are released by the action of different enzymes on precursors existing in blood plasma. These active compounds are the bradykinin-like polypeptides. Because there are still considerable discrepancies of opinion in regard to the unity or multiplicity of these polypeptides, it seems advisable to first list them.

Origin of the bradykinin-like polypeptides.—The polypeptide bradykinin is released from an inactive precursor, bradykininogen, present in the α_2-globulin fraction of the plasma, by incubation with trypsin or the venom of *Bothrops jararaca* (166, 167), as well as by activation of the plasminogen-plasmin system of the plasma by simple application of heat and changing the pH (165). According to Hamberg & Rocha e Silva (98) the release of bradykinin is related to the esterase activity of trypsin and of the venom of *B. jararaca* rather than to the proteolytic activity of these materials.

Kallidin is liberated from an inactive precursor occurring in plasma,

kallidinogen, by kallikrein. However, it should be stressed that not every kallikrein is capable of acting on every kallidinogen, and that it may happen that a kallikrein is inactive even on the blood kallidinogen of its own species. It seems that kallikrein, too, is not a proteolytic enzyme but rather an esterolytic enzyme of the type found in snake venom (166).

A kallidin-like substance is released from plasma or serum of some mammals by simple dilution with distilled water. Dilution probably activates serum kallikreinogen to kallikrein which then releases kallidin (172, 173).

Plasma-kinin is produced from the pseudoglobulin fraction of plasma by the action of plasmin, a proteolytic enzyme. Plasmin is present in the blood in an inactive form, plasminogen, which may be transformed into the active enzyme by different activators, such as urokinase, streptokinase, and kallikrein (11, 113, 144).

Pain-producing substance (PPS) develops when fresh human plasma is brought into contact with glass. Its name originated from the fact that, besides contracting smooth muscles, PPS produces pain when applied to the exposed base of a blister induced by a cantharidin plaster (7, 8, 126). The activation of plasma by contact with glass is by no means a general phenomenon; dog and sheep plasma, for example, are not activated by contact with glass (88). The intimate, rather complicated mechanism of PPS formation as well as the relation of PPS formation to blood clotting have been thoroughly studied by Margolis (148), to whom reference should be made for details.

Urinary kinin (Substance Z) is closely allied or identical to both kallidin and bradykinin. The rate of kinin excretion in human urine varied between 6 and 18 units/min., or, in terms of pure ox bradykinin, approximately 10 to 30 μg./24 hr. (87, 114). The bradykinin-like substance found in human amniotic liquid is probably attributable to fetal urine (202).

Colostrokinins are produced on the incubation of bovine colostrum with urinary kallikrein (urinecolostrokinin) or calf saliva (salivacolostrokinin). No consistent differences were found in parallel assays between colostrokinins and plasma kinin (96). A colostrokinin (serumcolostrokinin) also appears in a mixture of colostrum with serum. At present it cannot be decided whether it is different from bradykinin or kallidin, as suggested by Werle (210).

Once formed, all the bradykinin-like polypeptides undergo, if left in contact with plasma or lymph, rapid inactivation which is caused by a powerful bradykinin-destroying enzyme present in the globulin fraction of the plasma (99). In this regard guinea pig plasma is a particularly potent bradykinin inactivator (173). Bradykinin inactivation in plasma is usually blocked, in the preparation of the polypeptide, by addition of boiling ethanol.

The first question to be posed at this point is: are all kinins identical or not? The bulk of the available experimental evidence points to the identity or close similarity among the various above-mentioned bradykinin-like substances. In fact (*a*) in parallel pharmacological tests, kallidin, bradykinin,

plasma kinin, and PPS were qualitatively and quantitatively indistinguishable in their actions on smooth muscles, blood pressure, and capillary permeability; (*b*) the similarity of all the above bradykinin-like substances extends to their rate of inactivation by plasma and by lymph of various animals as well as to their chemical and physico-chemical properties (166, 173); (*c*) it seems not unlikely that the release of all bradykinin-like substances found in plasma is attributable to the activation of one and the same enzyme, kallikrein.

Elliott, Lewis & Horton (62) in England and Zuber & Jaques (224) in Switzerland have recently succeeded in isolating pure bradykinin from ox plasma incubated with trypsin (bradykinin T) or with *Bothrops jararaca* venom (bradykinin B). The two bradykinins appeared to be identical. On acid hydrolysis bradykinin T yielded serine, glycine, proline, phenylalanine, and arginine in the molar proportion of 1:1:3:2:2. As already suspected by Rocha e Silva (166), the terminal (N-)residue was arginine. It is evident that the contribution of Elliott and co-workers will be of decisive importance in many respects and that it will allow us in the near future to solve the problem of the identity of the different bradykinin-like substances.

Boissonnas and co-workers (27b, 27c) have completed the work of Elliott and co-workers by the synthesis of six peptides related to bradykinin, one of which, the nonapeptide Arg. Pro. Pro. Gly. Phe. Ser. Pro. Phe. Arg., proved to be identical, under every respect, to natural bradykinin T.

Physiological significance.—Bradykinin-like substances have been considered by some research workers to play an important, if not essential role, in functional vasodilatation, i.e., in the local adjustment of blood flow in relation to activity of tissues, especially glands and muscles. In addition, it has been suggested that they participate in the systemic reactions to some drugs as well as in the vascular and sensory phenomena of inflammatory reaction and are are associated with heightened neural activity. These points will be briefly discussed.

(*a*) Functional vasodilatation: Hilton & Lewis (108, 109, 110) are of the opinion that salivary kalikrein acts as a regulator of blood flow to the salivary glands during salivation by releasing bradykinin from plasma. Thus, this vasodilator polypeptide would have an important physiological role as the agent mainly responsible for the functional hyperemia in the glands.

Similarly, in sweat glands and in the pancreas the bradykinin-like substances formed by the action of the sweat gland kinin-forming mechanism or by pancreatic kallikrein could fully account for the active dilatation of the pancreatic vessels during pancreatic secretion and for the dilatation of the vessels of the human skin which occurs when the body is heated sufficiently to cause sweating (44, 81).

Meager experimental evidence supports, up to the present, the suggestion that bradykin-like polypeptides may be ranked among the chemical

agents responsible for the functional vasodilatation in muscles and in the central nervous system and for the local adjustment of renal blood flow (108, 114).

(*b*) Systemic vascular reactions to drugs: Liberation of bradykinin into the general circulation has been suggested as playing a role in peptone shock (15) and, in reserpine hypotension (168), to be involved in the generalized muscle vasodilatation which develops in man and animals during intravenous infusion of epinephrine and norepinephine (110) and, finally, to interfere with the vasodilatation and sweating occurring in the human forearm during insulin hypoglycemia (3).

(c) Inflammatory reaction: Keele and co-workers (7, 8, 126) first demonstrated that plasma can be activated *in vitro* to produce a substance which induces many of the vascular and sensory features of the inflammatory reaction when applied topically to the exposed area of a cantharidin blister. They further showed that a similar substance was present in the blister fluid after burns and in the joint fluid of rheumatic patients (8). These observations were confirmed and extended by other investigators (44, 51b, 102). Having found that a bradykinin-like material is released in the perfusate of human skin submitted to painful stimuli, Harpman and co-workers (102) suggest that a protease is liberated locally immediately following noxious stimulation and that this protease acts on a globulin to form polypeptides that increase permeability, lower pain threshold, and induce vasodilatation, edema, and other reactions relevant to inflammation. It is possible that every trauma leading to activation of proteolytic enzymes in the blood may also release bradykinin (167). Finally, bradykinin could be also one of the "slow reacting substances" released in anaphylaxis (28c).

Bradykinin and central nervous system.—Two groups of research workers have recently pointed to the possibility that bradykinin-like substances may be in some way related not only to peripheral but even to central neuronal activity.

Chapman and co-workers (44) observed an increased bradykinin-forming enzyme activity in cerebrospinal fluid collected from human subjects with inflammatory or degenerative diseases of the central nervous system or in cases of migraine or chronic schizophrenia. Increased amounts of bradykinin-forming enzyme were also found in the cerebrospinal fluid removed from the cisterna magna of cats following induced convulsions. Apparently the bradykinin-forming enzyme in the cerebrospinal fluid is not plasmin, and it has been hypothesized that it may be a neural intracellular proteinase.

Rocha e Silva and his co-workers (167, 168) found that both intracarotid and intraventricular injections of bradykinin produced, besides a fall in blood pressure and relaxation of the nictitating membrane, tranquillization or, the opposite, restlessness with convulsions. However, it should be noted that, at variance with Chapman and co-workers, Hamberg (100) failed to find in the cerebrospinal fluid from patients with central nervous system disease any protease activity sufficient to release bradykinin.

It is very difficult at present to draw any definite conclusion about the physiological or physiopathological role of bradykinin-like polypeptides. However, if the pessimistic statement of Schachter (173) that "there is as yet no convincing evidence of any specific physiological function of bradykinin" seems in its formulation somewhat crude, noboby can deny that it is substantially true. The main objection which may be raised in regard to the work dealing with the physiological role of bradykinin is that results obtained under particular experimental conditions or in single animal species have too often been unduly generalized. It is all too easy to encounter experimental evidence which does not fit in with general hypotheses and theories.

Pharmacological actions.—These have been described in detail for many years. Hence, only a brief account of some more recent contributions will be given here. The most important of the pharmacological actions is certainly that of Elliott and co-workers (62) who found that pure bradykinin from ox blood was active on a number of preparations and that within the errors of biological variation the increase in activity from the crude material was proportional in all tests. This means that all the activities of crude bradykinin preparations are inherent properties of the pure polypeptide, and this validates at once all the preceding data on the pharmacological actions of crude bradykinin preparations. It was shown that the active concentrations of the pure polypeptide varied between 0.0002 μg./ml. required to contract the rat uterus and 0.4 μg./kg. required to cause a depressor response in cats.

The above results, obtained with bradykinin T(trypsin bradykinin), were confirmed by Jaques & Meier (115b) who used bradykinin B (*Bothrops* bradykinin). Jaques & Meier further found that bradykinin B had no action on the blood pressure of the cock in doses up to 3 μg./kg., and that 0.01 to 0.03 μg. of the substance given intracutaneously in guinea pigs increased capillary permeability, as shown by extravascular leakage of circulating dye.

As already stated, Boissonnas and co-workers (27b, 27c) prepared by synthesis six polypeptides bearing strict resemblance to natural bradykinin, more precisely, a nonapeptide, four octapeptides, and one esapeptide. Two of these synthetic peptides, and especially the nonapeptide appeared to possess an intense biological activity. In fact, the guinea pig ileum and the rabbit duodenum were contracted by concentrations of the nonapeptide as low as 0.001 μg./ml., and 0.001 μg. was sufficient to lower the permeability of skin capillaries in the guinea pig. To produce a fall of blood pressure in rabbits, rats, guinea pigs, cats, and dogs, as well as a bronchoconstriction in guinea pigs, the dose ranged from 0.2 to 0.4 μg./kg. Removal of the prolyl residue in position 3 produced a 100-fold decrease in the biological activity; removal of the prolyl residue in position 7, a practically complete disappearance of activity (155b, 180b). Once again it should be stressed that the synthetic nonapeptide is in all probability identical with the natural bradykinin isolated by Elliott.

Some synthetic peptides with only slight structural resemblance to natural bradykinin were studied by Tritsch & Woolley (198). They possessed a negligible bradykinin-like activity.

Another important contribution to the pharmacology of bradykinin is credited to Rocha e Silva and co-workers (167, 168), who observed that the vasodilator effects of bradykinin were strongly potentiated by sympatholytic drugs as well as by some centrally acting hypotensive drugs, such as apresoline, chlorpromazine, and reserpine. Although most of the potentiation observed might be explained on the basis that the return of the blood pressure to normal level was impaired by peripheral sympatholysis or by depletion of the stores of catecholamines in their peripheral stocks, it is possible that in addition some central component participates in the potentiation by apresoline and reserpine. Since many of the effects produced by reserpine have also been observed by intraventricular injection of bradykinin, it is suggested that bradykinin too, should be considered seriously as a possible mediator of the actions of reserpine. Finally, the recent experimental results of Collier and co-workers (49b) should not be passed over in silence. These investigators demonstrated that bradykinin is a potent bronchoconstrictor agent in the guinea pig (at least as potent as histamine weight for weight) and that bradykinin bronchoconstriction is antagonized by acetylsalicylic acid in doses (minimum 2 mg./kg., intravenously) which give concentrations in blood that are much lower than those reached in man during salicylate therapy.

The above observations raise the questions whether bradykinin or a related polypeptide may participate in human asthma and whether suppression of response to bradykinin or to a related polypeptide contributes to the antirheumatic action of acetylsalicylic acid and other nonsteroid agents.

Bioassay of bradykinin-like substances is usually carried out on the blood pressure of cats and rabbits, on guinea pig and rabbit ileum, and on the duodenum and uterus of the rat. To these preparations the intestine of cats and dogs may be profitably added. Both preparations are fairly sensitive and show an excellent dose-response relationship (Erspamer, unpublished observations).

Substance P

Substance P is a polypeptide of unknown chemical structure and amino acid composition first described by Euler & Gaddum (73) in extracts of many tissues. The purest known preparation of substance P contains about 3000 units per mg. (161).

Distribution.—Substance P has been found in large amounts in gastrointestinal tract, retina, brain, posterior roots of spinal nerves, and preganglionic sympathetic nerves. Some activity was also detected in many other nerves (6, 58, 86). It is now generally accepted that the substance P in brain and nerves is the same substance as the substance P in intestine (58, 86).

In the alimentary canal maximum concentrations of substance P were found in the duodenum and jejunum of all species. The absolute values, however, greatly varied according to animal species, being greatest in the monkey and smallest in the pig intestine. Stomach contained very little substance P; the rectum, on the contrary, fairly large amounts (58).

Substance P seems to be present in all parts of the central nervous system, but its concentration in the brain varied over a range of more than 100-fold. The highest concentrations were found in human substantia nigra (700 units/gm.), in the ala cinerea, area postrema, central midbrain, hypothalamus, and caudate nucleus; the lowest in the cortex and cerebellum. There are wide differences in the concentration of substance P in different brains of the same species (58, 220). Attention has been repeatedly called to the fact that large amounts of substance P are contained in the dorsal roots, the posterior columns of the spinal cord, and the nuclei gracilis and cuneatus, structures all containing the same actual nerve fibers and little or no acetylcholine or choline acetylase (58, 136).

Substance P is not confined to mammals; it has been found in the intestine of fishes and even ascidians and in the central nervous system of all vertebrates (52, 54). The distribution of substance P in different areas of the central nervous system of the dogfish was the same as in mammals (54).

Enormous amounts of substance P were found in the brain of amphibians (up to 500 to 1000 units/mg.) (52, 93), but it is doubtful whether in this case all the activity on atropinized guinea pig ileum is actually caused by substance P. Other slow-reacting substances of unknown nature probably contribute to the stimulation of the gut. Following fractionated centrifugation of cat brain homogenates, 80 per cent of substance P was found in the sediment, especially, in the mitochondrial fraction (138).

Pharmacological actions.—For the actions of substance P on blood vessels and on extravascular smooth muscles we refer to the review articles of Pernow (160b, 161). Here it should only be remembered that there is as yet no clear understanding of the mechanism of action of the polypeptide on the intestinal muscle. The opinion of Pernow that substance P acts on two different sites—directly, by stimulating the smooth muscle fibers and, indirectly, by stimulating the nervous elements of the intramural reflex arc—is not completely shared by Kosterlitz (133) who could not find unequivocal evidence that the muscular contraction caused by the polypeptide was attributable to stimulation of nervous structures.

Since primary interest has recently been centered on the actions of substance P on peripheral nervous structures and central nervous system, these points will be discussed in some detail here.

(*a*) Action of substance P on peripheral nervous structures: Beleslin and co-workers (14) found that intraarterial injections of substance P in doses from 10 to 30 units potentiated the response of the nictitating membrane to submaximal stimulation of the sympathetic preganglionic nerve,

whereas higher doses usually depressed the response. The stimulatory actions of acetylcholine on the superior cervical ganglion and of epinephrine, norepinephrine, and tyramine on the nictitating membrane were also potentiated by the polypeptide.

(*b*) Central actions of substance P injected into the general circulation: Substance P injected subcutaneously in mice, in doses of 500 to 3000 units/kg., caused an inhibition of spontaneous movements and sedation. This effect was also obvious in animals pretreated with psychomotor stimulants, such as morphine, methamphetamine, and amino-β,β-dipropionitril (191, 223). Similarly, a definite, long-lasting taming effect has been observed in wild hares given intravenously very low doses of the polypeptide (100 to 300 units/kg.) (192). At rather high dose levels (8000 to 16,000 units/kg., subcutaneously) substance P was further found to potentiate in mice barbiturate hypnosis and bulbocapnine catatonia, to antagonize harmine tremors as well as the convulsant activity of strychnine, picrotoxin, and tetanus toxin (not that of caffeine, nicotine, ammonium salts, or electroshock), finally, to produce hyperalgesia, and to counteract morphine analgesia (222, 223). In guinea pigs, substance P (12,000 units/kg., subcutaneously) at first also antagonized the morphine-induced depression of the respiratory center, but later it deepened morphine paralysis. In untreated guinea pigs, substance P alone displayed a clear depressant effect on respiration (223).

Not all the above results of Zetler could be confirmed. In fact, Stern & Hukovic (194) found that whereas crude preparations of substance P possessed exactly the actions described by Zetler, purified substance P (270 units/mg.), given intraperitoneally in doses of 1000 to 10,000 units/kg. did not protect the mouse against strychnine convulsions nor did they prolong evipan narcosis. Similarly, whereas small doses (1000 units/kg.) of purified substance P caused, like unpurified preparations, hyperalgesia, high doses (10,000 units/kg.) did not produce this effect. It is evident that if the above observations of Stern & Hukovic are correct, the whole problem of the central effects of substance P should be re-examined with pure polypeptide.

(*c*) Central effects of substance P injected into the cerebral ventricles: Following intracisternal or intraventricular injection of 5 to 50 units substance P in cats or rabbits, a variety of symptoms made their appearance, the most constant autonomic effect being a long-lasting stimulation of respiration, and the most marked behavioral change a general inhibition of spontaneous activity (76).

(*d*) Action of intracarotid substance P on cortical and hippocampal EEG: Intracarotid injections of 30 to 100 units substance P in rabbits caused a decrease in amplitude and an increase in frequency in the cortex, and a synchronization of the hippocampal activity. It was concluded that the ascending activating system of the reticular formation was stimulated by the polypeptide (135).

(*e*) Action of local, epicortical application of substance P on the elec-

trical activity of the rat brain: The local application of 4 to 10 units substance P on the rat cortex produced a displacement of the zero potential of the cortex to the positive side and increased the dendrite potentials. These effects were eliminated by cathode polarization and by γ-aminobutyric acid (40).

(*f*) Correlation between reserpine sedation and substance P: Having observed that reserpine provokes an increase in the substance P concentration of the brain of rats (from 14 to 19 to 70 units/gm.) and rabbits, Stern & Kocic-Mitrovic (193) suggest that the tranquillizing action of reserpine may partly depend upon the rise in the level of substance P which is considered a "physiological tranquillizer." Unfortunately, the results of the Yugoslav investigators are at variance with those of Paasonen & Vogt (157) who failed to find any influence of reserpine and various other drugs on the substance P concentration in brain.

At this stage it may be said that, in spite of all the above experimental efforts, the assumption that exogenous substance P exerts distinct direct effects upon the central nervous system still lacks any satisfactory experimental basis. The main objection which should be raised against the validity of all or nearly all the above experiments is that the investigators have completely overlooked the powerful vasodilator action possessed by substance P. Doses of up to 8000 to 16,000 units/kg. were administered parenterally without any apparent control of systemic blood pressure and of temperature. Yet, in human beings the infusion of quantities of substance P as low as 30 to 50 units/min. (i.e., 0.5 to 1 unit/kg./min.!) was sufficient to bring about a bright red flush in the head immediately following the start of the infusion (56b, 146), and in dogs intravenous doses of substance P as low as 0.2 to 0.5 unit/kg. were capable of producing a fall of blood pressure of 40 to 60 mm. Hg (Erspamer, unpublished observations).

Although results obtained in humans and dogs cannot be generalized to other animal species without adequate experimental support, it seems imperative to exclude, before speaking of true central effects of substance P, any interference by systemic or local vasomotor changes produced by the polypeptide.

Physiological significance.—(*a*) Intestinal substance P: It has been suggested that substance P in the alimentary canal is associated with the intramural nervous plexuses and that it controls or participates in the control of the activity of the intestinal smooth muscle. More precisely, substance P would produce, like 5-HT, a reflex contraction of the longitudinal muscle coat by stimulation of sensory nerve endings (13). Without constituting definite evidence, some facts support this view. Substance P is a powerful stimulant of intestinal motility in numerous animal species both when given by intravenous route and intraluminally (13, 89). In man a marked increase in the segmental and peristaltic movements of the small intestine was obtained following intravenous infusion of substance P in doses as low as 50 units/min. (146). Moreover, Ehrenpreis & Pernow (57)

found low concentrations of substance P in the inactive, aganglionic part of the gut in Hirschsprung's disease and abnormally large amounts in the hyperactive gut above this point.

(*b*) Substance P in peripheral nerves and dorsal roots: Since substance P is present in large amounts in the posterior roots of spinal nerves and in the dorsal columns of the spinal cord, structures which contain little or no acetylcholine or choline acetylase, it has been suggested by Lembeck (136) that the polypeptide might be the chemical transmitter liberated by the first sensory neurone. The opinion has also been advanced by Holton (112) that substance P might be associated with the transmission of nervous impulses from nerve endings. This view was mainly based on the assumption that substance P was formed in the nerve cells of the spinal ganglia and moved down the axon to nerve endings. However, in more recent experiments, Holton (111) found that after dividing the sciatic nerve of rabbits, the distal end of degenerating nerve contained residual substance P varying in different animals from 5 to 73 per cent of that in the control nerve. Obviously, this important fraction of substance P could not be concerned with normal nervous activity.

(*c*) Substance P in the central nervous system: The pharmacological properties of substance P and its prevalent distribution in brain areas which contain relatively low concentrations of choline acetylase led Kopera & Lazarini (132) to the view that substance P is a noncholinergic transmitter in the central nervous system. A similar opinion is shared by Zetler (223) who considers the polypeptide as an inhibitory transmitter, and by Stern & Milin (192), who consider it a "physiological tranquillizer," displaying its depressive action mainly on the hypothalamus. Actually, as Zetler admits, all suggestions about a possible transmitter function of substance P are merely hypothetical.

To conclude, we can only say at present that substance P should have some function in the tissues in which it is formed. What this function is, and how important, is largely obscure. This is particularly true for the substance P present in the brain.

Other Active Polypeptides

Angiotensin, substance P, and bradykinin-like substances are the main representatives of the pharmacologically active polypeptides. However, several other active substances belonging to this group have been described. Since these additional polypeptides are as yet poorly defined and characterized or appear of doubtful biological importance, they will not be discussed here in detail.

Active polypeptides formed in serum by pepsin and by acidification.— The hydrolysis of blood serum with pepsin produces the liberation of substances with conspicuous pharmacological effects resembling those of angiotensin and the hormones of the neurohypophysis. These substances are

pepsitensin, possessing vasoconstrictor effects similar to those of angiotensin; pepsitocin, producing a tonic contraction of the atropinized rat and guinea pig uterus; and pepsanurin, causing a powerful antidiuretic action. The problem whether these substances are merely artifacts or whether they have some role in homeostasis is still open, as is the problem of their individuality (53b). The same may be said for the oxitocic and pressor polypeptide originated in the serum from the globulin fraction by acidification (53c).

Substance A.—The incubation of fraction IV-4 of plasma protein with α-amilase produces the appearance of a hypertensive smooth-muscle-stimulating polypeptide. From a pharmacological point of view, substance A is very similar, if not identical, to angiotensin (205b).

Hysterotonin.—A pressor substance found in highest concentration in decidua and amniotic fluid in patients with toxemia was given the name hysterotonin by Hunter & Howard (114b). This substance seems to be a polypeptide formed by an enzymatic reaction. It is claimed that it differs from every other previously described polypeptide.

Vasodilator and spasmolytic polypeptide of lymphoglandular origin.—This factor has been found by Vallette and co-workers (201b) in the hydrolysate obtained by controlled action of papain on ox lymphatic ganglia.

Pharmacologically active polypeptides are now at the center of enormous interest and fervid biochemical and pharmacological research. We are dealing with a group of compounds of relatively simple character (their molecular weight falls generally between 1000 and 2000) which possess chemical structures that have already been established or are near being elucidated, and which produce tremendously intense pharmacological effects bearing comparison with those of the most powerful neurohumoral amines. Some of the pharmacologically active polypeptides have already been reproduced by synthesis, thus permitting extensive and profound research with absolutely pure compounds.

Recent biochemical and pharmacological development will certainly help, in a decisive way, to solve the central problem in the investigation concerning the active polypeptides, that of their particular significance in physiology and pathology.

LIPID-SOLUBLE ORGANIC ACIDS

Prostaglandin (59, 203) is a mixture of lipid-soluble organic acids found in the vesicular glands (not in the prostate!) of sheep and in the semen of sheep, goat, and man. The substance present in the seminal fluid from man is secreted exclusively from the seminal vesicles.

Prostaglandin is present in the tissue in an inactive form and is secreted into the seminal fluid in a free, active state. Under appropriate conditions of incubation, the amount of free active prostaglandin in sheep's ground

vesicular glands can be increased by 40 times. The human seminal vesicles are capable, in some cases, of replenishing their content of prostaglandin within 14 to 24 hours.

Prostaglandin stimulates both *in vitro* and *in vivo* the intestine and uterus of various species and produces a long-lasting fall in arterial blood pressure. However, both tonus and spontaneous activity of the isolated nonpregnant human uterus are depressed by the substance.

Recently, two crystalline compounds, both nitrogen-free, unsaturated acids, have been isolated from the vesicular glands of sheep (16). Characteristic differences in the activity ratio of the two crystalline fractions, named Prostaglandin E and Prostaglandin F, were noted on different organs. Prostaglandin E, for example, lowered the rabbit blood pressure in doses from 0.5 μg., whereas no action was observed with prostaglandin E in doses up to 10 μg. The approximate threshold dose of both prostaglandin E and prostaglandin F on the rabbit jejunum (a preparation which is frequently used in the bioassay of prostaglandin) is 0.003 to 0.01 μg. per ml. nutrient liquid (17).

Chemically pure prostaglandin E infused in doses of 0.2 to 0.7 μg./kg./min. in healthy human subjects over periods of 4 to 10 min. produced tachycardia, reddening of the face, headache, and an oppressive feeling in the chest. Systemic arterial blood pressure and cardiac output fell only moderately (18).

As already stated, it is probable that prostaglandin is not an unitary substance but a mixture of various related compounds. This opinion is corroborated not only by the isolation of prostaglandin E and prostaglandin F, but also by the fact that the biological actions of partially purified preparations of prostaglandin from the sheep vesicular glands could be largely, but not wholly, explained by assuming that they contained a mixture of prostaglandin E and prostaglandin F (17, 59).

Prostaglandin does not seem to have a general biological significance since it is absent in the seminal fluid and male accessory genital glands of a number of mammals (stallion, bull, boar, dog, cat, rabbit, guinea pig). It has been suggested that, in human beings and sheep, prostaglandin may promote ejaculation by inducing peristalsis of the male genital tract, may stimulate the motility of the spermatozoa, and may facilitate the migration of spermatozoa in the female genital tract (17). Of the three suggestions the last seems to be less hypothetical.

An acid substance present in the semen and in the vesicular glands and the prostate of the monkey, vesiglandin resembles prostaglandin in many respects but differs from it in having a relatively stronger hypotensive action and a weaker muscle-stimulating effect (74).

Darmstoff is an active principle extracted by Vogt (203) from bath fluids in which a piece of intestine had been suspended or from intestinal tissue itself. In the intestinal wall most of the substance appears to be bound and to become easily extractable only after the disintegration of the tissue.

The properties of darmstoff are those of a lipid-soluble acid, and it has recently been characterized as a mixture of closely related and, as yet, unseparated acidic phospholipids. It is possible that the major component of the mixture is an acetalphosphatidic acid.

Darmstoff increases the tone of the longitudinal muscle of the intestine of all species investigated, including those which do not react to histamine. The isolated rabbit duodenum is particularly sensitive (threshold concentration for purified preparations is less than 0.001 μg./ml.) and widely used in the bioassay of the substance. The isolated uterus and the urinary bladder are also contracted. The mechanism of action of darmstoff on the gut is not completely understood and it may be different in different species. Vogt believes that the substance does not act directly on the smooth muscle fibers: a noncholinergic action being predominant in the rabbit intestine and a cholinergic, but not necessarily neuronal, action predominating in the guinea pig ileum. Darmstoff does not produce any remarkable effect on the circulatory system.

On the basis of its occurrence and action, Vogt suggests that darmstoff plays a physiological role in the motor activity of the gut and, more precisely, that it is the postganglionic transmitter for atropine-resistant vagal impulses. Thus, lipid-soluble acids also may participate, together with biogenic amines and polypeptides in the control of intestinal motility.

Watery extracts of rabbit iris contain an unsaturated lactonizing hydroxy-fatty acid, irin, which is capable of contracting iridial and intestinal smooth muscles (4). The histamine-insensitive rat or hamster colon preparation is used for the routine estimation of irin; it is capable of detecting the activity corresponding to 0.25 to 1 mg. of iris per ml. organ bath (equalling 0.1 to 1 μg. purified irin). On the isolated ox iris preparations, doses of purified irin as small as 0.3 to 1 μg./ml. produce a strong and long-lasting contraction of the sphincter muscle. Irin-like activity is present not only in iris tissue, but also in aqueous humor, in the conjunctiva, in trigeminal nerve, ventral and dorsal ear skin, and muscularis externa of the intestine.

So far irin has not been obtained in a pure form. However, watery crude extracts have been remarkably purified through ether, acetone plus chloroform extraction, and electrophoresis. Irin possibly plays a role in the function of the iris and since the substance is occasionally released into the aqueous humor after stimulation of the trigeminal nerve, Ambache (4) suggests that it is the transmitter for the atropine-resistant miosis which occurs on antidromic stimulation of the trigeminal nerve.

G-Acid is a straight-chain, unsaturated monocarboxylic acid, probably identical with 3-octadecenoic acid, which has been isolated by Gabr (84) from the G2 fraction of human plasma. G-Acid, which appears to be preformed in plasma or loosely bound, produces a slow and delayed contraction of the isolated guinea pig and rabbit jejunum, possesses a marked hemolytic action on human red cells, and displays a vasoconstrictor activity on rabbit

coronary arteries. The biological significance of the acid is completely obscure.

One of the hemolytic agents occurring in blood and tissue, especially brain, has been characterized by Laser (134) as an unsaturated monocarboxylic acid, possibly the *cis*-11-octadecenoic acid (*cis*-vaccenic acid). The compound possesses strong hemolytic effects and causes a delayed contraction of the guinea pig ileum. The function of *cis*-vaccenic acid in brain is completely obscure. It has been suggested that the acid occurring in red cells may have a bearing in the physiological destruction of aged erythrocytes.

Arachidonic acid, a highly unsaturated fatty acid normally present in body lipids, possesses a powerful smooth-muscle-stimulating effect and causes pain when applied to human skin, in concentrations as low as 3 to 30 μg./ml. According to Jaques (115) this acid, too, may play some physiopathological role.

The so-called slow-reacting substance A is an acid lipid-soluble, smooth-muscle-stimulating principle which appears, together with histamine, in the venous effluent of perfused sensitized guinea pig, rabbit, or monkey lungs on injection of the antigen (29), and in the incubation fluid of tissues from sensitized guinea pigs on incubation with the antigen (42). It was also possible to recover the same, or a closely related principle, from the perfused cat paw after the injection of compound 48/80 (43). No slow-reacting substance A or histamine was detected in the effluent from perfused lungs of rats on the addition of antigen (29).

Since on the one side there is a close correlation between the amounts of histamine and slow-reacting substance A obtained from different tissues, and, on the other, a good proportionality between the content of the two substances and the mast cell population, it has been suggested that the tissue mast cells may be the common source of both histamine and slow-reacting substance A (28b). According to Brocklehurst (29), its formation occurs chiefly in lung and vascular tissue. The isolated atropinized guinea pig ileum is the preparation most suitable for the bioassay of slow-reacting substance A.

It is highly probable that this substance contributes to the symptoms of anaphylaxis, and it is possible that it may also be of significance in human asthma. In fact, Brocklehurst found that it is formed by the action of allergen in human asthmatic lung and that it produces bronchospasm in man (29).

Other substances possibly related to lipid-soluble acids are fraction X found, like darmstoff, in intestinal extracts (203); eutocine, occurring in uterine muscle tissue and amniotic fluid of man at the time of delivery (101); the plain muscle stimulant principle present in the menstruum (162); and pressor or smooth muscle contracting substances found in the skin (5, 145) and brain (221).

As pointed out by Vogt, who has recently written an excellent review article on the subject (203), the lipid-soluble organic acids have attracted

general attention only in the last few years. In fact, it is only since modern analytical and preparative methods have become available that considerable progress has been made in the study of these substances. It is quite probable that the list of the compounds discussed in the present paper is destined to lengthen in the near future, and it is hoped that future research will help to elucidate the many obscure points concerning biosynthesis, liberation, fate, and, above all, biological significance of the pharmacologically active lipid-soluble acids occurring in the mammalian organism.

It is evident, however, that the necessary requirements for any progress in this field will be the exact characterization and individuation of the different compounds. It is probable that very often we have to deal with mixtures instead of with individual substances (prostaglandin, darmstoff, etc.), and one cannot avoid the impression that not infrequently the same activity has been attributed to different compounds by different investigators.

Generally, lipid-soluble acids are considered local hormones, but their distribution is often difficult to reconcile with the idea of a specific local function (a darmstoff-like substance occurs in skeletal muscle and in brain; irin-like activity occurs in the skin and in the intestinal wall, etc.). Vogt rightly believes that they may have a more general function. Lipid-soluble acids are substances with the general property of stimulating smooth muscles. Since stimulation of excitable cells is brought about by depolarization, i.e., by equilibration of concentration differences of cations inside and outside the cell, Vogt suggests that it is conceivable that acids capable of forming complexes with cations might act as carriers of negative charges in the cell membrane, thus interfering with both the excitability and the excitation of the cell.

CONCLUSION

The three groups of active substances discussed in the present paper, while showing decisive differences in their chemical structure, share important pharmacological properties. In fact, all possess powerful actions on vascular and extra-vascular smooth muscles.

The kindred pharmacological character of amines, polypeptides, and lipid-soluble acids emphasizes the opportunity of considering all three groups of compounds when trying to interpret the action of crude tissue extracts or explaining the pathogenesis of physiological and pathological processes involving changes in caliber or permeability of blood vessels, changes in tone and motility of extra-vascular smooth muscles, or both. It may be that representatives of the three groups of substances are liberated contemporaneously or successively in the same physiopathological process or following the administration of the same pharmacological agent.

The antigen-antibody reaction, for example, may set free not only histamine and 5-hydroxytryptamine, but also bradykinin and the slow-reacting substance A; reserpine, in its turn, has been claimed to liberate not only indolealkylamines, catecholamines, and histamine, but also bradykinin, and to increase the brain concentration of substance P.

These elementary concepts and facts are very often overlooked.

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