Serotonin and Related Substances

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THE ROLE OF the vasoconstrictor material in platelets has long been of interest to a number of investigators. As early as 1868, Ludwig (1) noted the vasoconstrictive effect of defibrinated blood. In 1912, the concept was advanced that the vasoconstrictor released from platelets when blood coagulates might participate in the mechanism of hemostasis by its direct constriction of vascular smooth muscle surrounding the clot (2). The substance responsible for this phenomenon remained

unidentified until 1947, when Rapport, Green and Page (3) were successful in isolating a naturally occurring, pharmacologically active substance which had the dual effect of constricting the blood vessels and of stimulating intestinal motility. Soon after its isolation, Rapport *et al.* identified the substance, and Hammlin and Fisher (4), and Speeter, Heinzelman and Weisblat in 1951 (5) were able to synthesize 5-hydroxytryptamine. In 1933, Vialli and Ersparmer (6) reported that a pharmacologically active substance, which they called enteramine, occurs naturally in the enterochromaffin cells of the gastrointestinal tract. The presence of tyramine and histamine in this preparation made it difficult to establish that the stimulation of intestinal and uterine motility was caused solely by enteramine. This difficulty was obviated in 1952 when Ersparmer and Broretty were able to obtain a relatively pure substance which was later identified as 5-hydroxytryptamine and shown to stimulate the intestine and uterus.

Serotonin (5-hydroxytryptamine) and other naturally occurring, pharmacologically active amines are of interest to the chemist, physiologist, pharmacologist and clinician. Not only has a vast amount of research been concerned with the physiologic and pathologic roles of these amines, but inquiry into their routes of formation and degradation has been of equal interest. Ersparmer's and Rapport's contributions stimulated early studies on the function of serotonin, but more recently the discovery that carcinoid tumors contain and secrete this amine and are associated with a fascinating syndrome has given great impetus to further studies. Another impetus has been the evolution of chemical methods which permit a precise, sensitive assay for serotonin and many related substances.

Serotonin occurs in many species and the largest concentration in mammals is present in the gastrointestinal tract distributed along the stomach, small intestine and colon. Lesser, but significant, amounts of serotonin are present in the brain, liver, kidney, spleen, lung and heart. In man, all body fluids contain serotonin in varying concentrations with a range from 0.15 to 0.7 μg./mg. of platelet protein in the blood, and the lowest concentration of serotonin occurring in spinal fluid with levels of approximately .006 μg./ml. In the gastro-

intestinal tract, serotonin is localized to the enterochromaffin cells where it is synthesized and stored. Feldberg and Toh (7) have shown that in the dog as well as in the rabbit, there is no serotonin in the muscular layer of the stomach, while concentrations as high as 10 μg./Gm. are present in the pyloric mucosa of the dog and in the mucosa of the stomach body in the rabbit. In the central nervous system, serotonin occurs primarily in the hypothalamus and midbrain; the cortex and cerebellum contain very little. In blood, serotonin is carried by the platelets, which act as a storage site and vehicle for the amine; however, platelets are unable to synthesize serotonin. This point is supported by the studies of Stacey and Young (8), who showed that, while brain and intestinal serotonin concentrations in the late fetal life of the guinea pig reach approximately 60% of the values present in the adult guinea pig, the serotonin content of the circulating platelets is significantly low. These investigators also suggest that the serotonin fraction carried in the platelets is derived from intestinal depots. The close interrelationship between platelet serotonin and serotonin depots is emphasized by the studies of Zucker *et al.* (9), which show that in carcinoid patients, where the over-all production of serotonin is significantly increased, the half life of serotonin labeled with carbon 14 in platelets is markedly shortened (0.1–1.5 days as compared with a normal half life of 5–6 days), indicating the rapid turnover and the large pool of this amine in this condition. The uptake of serotonin by circulating platelets has been the subject of many interesting studies. For this amine, the uptake is maximal at 37° C. and it is inhibited by the presence of cyanide, iodoacetate and the cardiac glycosides. However, the uptake of serotonin by platelets can be increased by intensifying the concentration of the amine in plasma, and it appears to be related to a cation exchange system by which potassium is displaced from the platelets as serotonin goes into the cell. The same mechanism has been demonstrated for the uptake of serotonin by brain tissue. The movement of serotonin across cell membranes occurs by active transport as it can be accomplished against large concentration gradients.

The subcellular localization of serotonin has been partially elucidated in the past few years. The amine has been shown

to exist in the granule fraction of rabbit platelets, probably in bound form. The platelet membrane does not appear to contain serotonin. The presence of small amounts of free serotonin in the platelets is probably due to mechanical rupture of some of the granules. As the granule fraction of human platelets contains both the alpha granules and the mitochondria, it is not known in which subcellular site serotonin occurs. In the brain, it appears that serotonin, acetylcholine and substance P are located in different microsomal particles which are probably derived from the endoplasmic reticulum, despite early studies which suggested a granule common to both serotonin and acetylcholine. It should be pointed out that the granules containing amines probably are different in the various tissues. For example, it is possible to release all of the serotonin from platelet granules by exposing them to hypotonic solutions, while under similar conditions only 50% of the serotonin in brain granules would be released. (10).

METABOLISM OF SEROTONIN

Tryptophan is the parent amino acid of serotonin, and normally 1% of the absorbed amino acid is converted to serotonin. In pathologic states, however, where the enterochromaffin cell mass is increased, as in the case of the carcinoid syndrome, up to 60% of the absorbed tryptophan may be shunted to form 5-hydroxytryptamine. Tryptophan (Fig. 1) is hydroxylated to form 5-hydroxytryptophan by a hydroxylating enzyme which has been shown to occur in the liver and is possibly present in other tissues. The hydroxylated amino acid, which is pharmacologically active, is decarboxylated to form 5-hydroxytryptamine (serotonin). The enzyme involved in this step is 5-hydroxytryptophan decarboxylase, which is a general aromatic amino acid decarboxylase. In addition, it has been shown that a relatively specific histidine decarboxylase forms serotonin from 5-hydroxytryptophan. Following the administration of 5-hydroxytryptophan, measurable increases of blood and tissue serotonin occur within 15 minutes, reach a maximum in about one hour, and persist for several hours. Following its formation, serotonin is stored in various sites. The

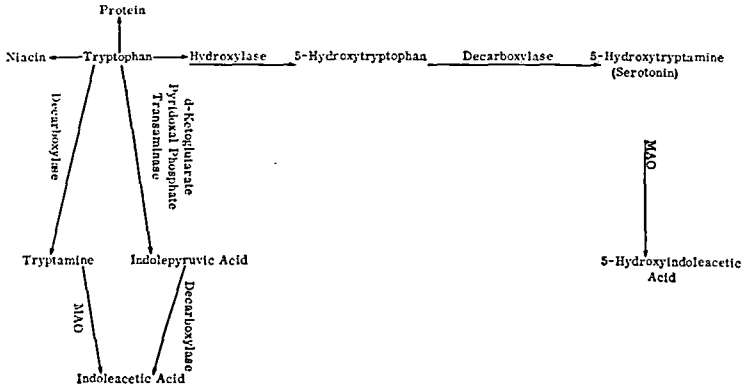


FIG. 1.—Routes of active amine metabolism from tryptophan.

nature of storage of the amine has not been clearly elucidated; some studies, however, indicate that it is stored in a bound form contained within a subcellular fraction or coupled to a protein. Other studies suggest that the amine is retained within the cell in a diffusible form.

The release of serotonin from the storage sites and its presence as a free or pharmacologically active amine have been a subject of great interest to pharmacologists. Reserpine has been a useful tool in the illustration of the mechanism of release of serotonin. Haverback *et al.* (11) have shown that when reserpine is administered to man in small daily doses, platelet serotonin is depleted in about a week and remains so during the course of reserpine administration. Following cessation of reserpine, platelet serotonin slowly returns and reaches the normal level in about 3 weeks. Also, Shore and his co-workers (12) have shown that the administration of single large doses of reserpine to animals, in the order of 1–5 mg. per kilogram, causes the release of serotonin from the three major body depots: namely the blood, intestine and brain. The brain is particularly sensitive to the effect of reserpine, which releases as much as 70% of the amine within 30 minutes following the administration of 1 mg. per kilogram. Serotonin in the brain returns to normal levels after at least one week has elapsed following the administration of reserpine. It would appear that reserpine impairs the mechanism by

which the various tissues store serotonin; the exact mechanism, however, remains obscure. Some experimental work indicates that reserpine acts directly upon the granules, altering their ability to bind amines. Whether this is the sole mechanism or whether reserpine causes the granules to coalesce and rupture, thus releasing the amine, is not known.

The principal metabolic pathway for serotonin degradation is oxidative deamination by monoamine oxidase to 5-hydroxyindoleacetaldehyde, followed by dehydrogenation to 5-hydroxyindoleacetic acid by aldehyde dehydrogenase. Monoamine oxidase occurs in the liver in large concentrations. This anatomic arrangement is important for the degradation of serotonin that may be released from those areas drained by the portal vein. Although serotonin is not the only substrate for monoamine oxidase, the enzyme has a high affinity for this amine. In addition, monoamine oxidase is involved in the degradation of tryptamine, epinephrine, norepinephrine, phenylethylamine, N-methyl histamine and possibly histamine. Other pathways for serotonin degradation have been reported, and they include: (1) oxidation by the cytochrome C enzyme system; (2) acetylation by acetyl coenzyme A to form N-acetylserotonin which then conjugates with glucuronic acid; and (3) degradation by human ceruloplasmin which can be inhibited by isoniazid and iproniazid. These secondary metabolic routes become important when the main pathway is blocked, as is the case, for example, during administration of monoamine oxidase inhibitors. In the urine, the major metabolite is 5-hydroxyindoleacetic acid, and its determination has become the most popular method of testing in the diagnosis of the carcinoid syndrome. Serotonin itself can be detected in urine, however, and it is thought that it is derived from renal tissue. Rodnight (13) has presented values of up to .06 μg./ml. of urine in normal individuals. Other urinary metabolites are N-acetyl serotonin in a free or conjugated state, and 5 hydroxyindoleacetic acid, both of which have been reported to be present in the urine of carcinoid patients. It should also be pointed out that 5-hydroxytryptophan can be converted directly to 5-hydroxyindoleacetic acid without previous decarboxylation to serotonin. This route of degradation assumes greater importance in gastric carcinoid

tumors. N-acetyl serotonin has been the subject of several interesting studies which show that it blocks the effect of the melanin stimulating hormones and that it inhibits the effect of ACTH. This substance occurs naturally in the pineal gland and in the peripheral nerves. The functions and the metabolism of tryptamine, another indole amine derived from tryptophan, have been of considerable interest. Mammalian tissue contains enzymes which can decarboxylate L-tryptophan to tryptamine which has been found to occur in mammalian tissues. Tryptamine has also been isolated and measured in normal urine. Its major urinary metabolite is indole-3-acetic acid which can also be formed by decarboxylation of indolepyruvic acid. Interestingly, increased urinary excretion of tryptamine, its metabolite, circulating serotonin, and 5-hydroxyindoleacetic acid have been reported in nontropical sprue, indicating an interrelationship between the gastrointestinal tract and these indoles (14, 15, 16).

PHARMACOLOGY

Central Nervous System

Although serotonin is found in large concentrations in the mucosa of the gastrointestinal tract, hypothalamus and blood platelets, as well as in lesser concentrations in other tissues, the physiologic function of this amine remains unknown.

The presence of large amounts of serotonin in the hypothalamus has caused much speculation concerning its role in brain function, but only few facts are known. Although serotonin in the hypothalamus may be affected by substances which have an effect on the emotions, such as reserpine, monoamine oxidase inhibitors, lysergic acid diethylamide or 5-hydroxytryptophan, it is premature to relate relationships of this type to physiologic function. Not only has serotonin been implicated in brain function, but other naturally occurring amines and their metabolites also affect the central nervous system. The systemic injection of tryptamine can cause chronic convulsions in animals, and this effect is potentiated by monoamine oxidase inhibitors. Convulsions after tryptamine are similar to those produced by administration of 5-hydroxytryptophan and

iproniazid and are centrally induced in contrast to serotonin-induced convulsions, which appear to be secondary to hypoxia. Tryptamine-induced convulsions are not potentiated by the usual analeptics and are not suppressed by the common anticonvulsants. They are suppressed, however, by chloropromazine and other phenothiazine derivatives. The difficulties that are inherent in establishing interrelationships between brain function and amine metabolism in the human are many and obvious. As newer technics evolve which permit the estimation of biochemical changes in the central nervous system by measurements in the periphery, a much better understanding of the function of serotonin and of other related substances in the central nervous system will result.

Platelets

The physiologic role of serotonin present in circulating blood platelets has long been investigated. As early as 1912, O'Connor (2) suggested that serotonin, at that time yet unidentified, had a definitive role in the mechanism of hemostasis by its constrictive effect on the vascular smooth muscle. Furthermore, Zucker and Borrelli (17), after measuring the serotonin content of platelets of patients with thrombocytopathia, pseudohemophilia and thrombocytosis, concluded that serotonin might indeed be involved in hemostasis. However, Haverback *et al.* (11) found no alterations in bleeding time, coagulation mechanisms or capillary fragility when they depleted the platelet serotonin by small daily doses of reserpine. Fenichel and Seegers (18) showed that platelet serotonin enhances clot retraction when added to platelet-poor plasma, suggesting that serotonin is a clot-retraction principle. However, studies by Magalini and Stefanini (19) as well as those by Haverback *et al.* (11) failed to show any abnormality in clot retraction when platelet serotonin concentrations are virtually absent.

Gastrointestinal Tract

The large concentration and wide distribution of serotonin in the gastrointestinal tract have suggested a relationship be-

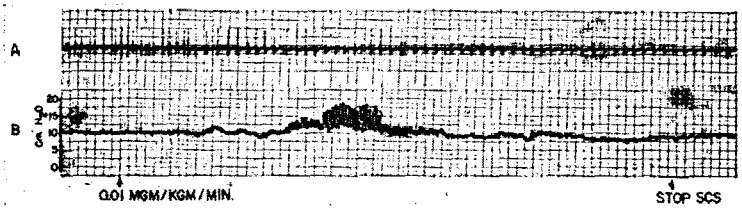
tween motility and serotonin. The anatomic proximity of the enterochromaffin cells to the terminal fibers of the submucous plexus, which was first described by Masson, was another reason to consider a neurohumoral function of this amine on the gastrointestinal tract (20).

With in vitro technics, utilizing isolated intestinal organs of various animals, Ersparmer showed that serotonin has a potent stimulating effect on motility. Some of the organs were especially sensitive to serotonin. The rat duodenum, for example, responded to as little as 0.001–0.005 μg. per ml. of serotonin; mouse jejunum was stimulated by serotonin in dilutions down to one in 3×10^9; guinea pig small intestine responded in dilutions to one in 10^8. Other studies have shown that serotonin increases motility of the intestine in Thiry-Vella dogs or in anesthetized dogs with exposed intestine.

In vitro studies from Gaddum's laboratory (21) showed that serotonin caused contractions of the uterus, duodenum and colon of rats; also the uterus, duodenum, jejunum and ileum of the guinea pig. His findings that mepyramine, piperoxane and atropine inhibited the effects of histamine, adrenaline and acetylcholine respectively, without altering the effects of serotonin support the view that serotonin acts on specific receptors. These have been called tryptamine receptors.

It is abundantly clear that serotonin affects the motility of the gastrointestinal tract. The following investigations were conducted in our laboratory on the effect of serotonin and related compounds on intestinal motility in animals and in

FIG. 2.—Tracing *A* represents respiration, and tracing *B* intestinal motility. Five minutes after the continuous infusion of serotonin was started, increased intestinal motility is apparent; two minutes later tachyphylaxis is noted.



the human. Studies in dogs of the effects of serotonin on jejunal motility and intraluminal pressure were accomplished by passing a tube with an open end through a gastrostomy into the upper jejunum. The recordings were made over several hours while the animals were under pentobarbital anesthesia. Serotonin creatinine sulfate infused intravenously in a dose as low as 0.001 mg. per kilogram per minute stimulated jejunal motility (Fig. 2). This response was not modified by a bilateral cervical vagotomy. In all instances, increasing doses of serotonin (0.01–0.08 mg. per kilogram per minute) evoked increasingly greater responses in jejunal motility. Tachyphylaxis developed during the continuous intravenous infusion of serotonin irrespective of dose, and the increased motility elicited by a given dosage usually ceased within 5 minutes. Within 10 minutes after cessation of the serotonin infusion, the bowel regained its former sensitivity.

The ability of several pharmacologic agents to modify the intestinal response to serotonin in the dogs was examined. The intravenous administration of up to 0.75 mg. per kilogram of atropine did not inhibit the serotonin response, nor did administration of 4 mg. per kilogram of hexamethonium ion intravenously. A single intravenous injection of 50 μg. per kilogram of lysergic acid diethylamide caused marked potentiation of the serotonin response and tachyphylaxis was not observed. The potentiation of the serotonin response by lysergic acid diethylamide persisted following bilateral cervical vagotomy. After intravenous administration of 0.5 mg. per kilogram of the potent histamine releasing agent, Compound 48/80, the response of the intestine to serotonin in the dog was variable. In 3 of 5 instances, prior administration of Compound 48/80 inhibited the response to serotonin. In two instances no inhibition was noted. Prior administration of Compound 48/80 more consistently blocked tachypnea and hyperpnea induced by serotonin than the increased gut motility. Administration of the antihistaminic agent, mepyramine, in a dose of 5 mg. per kilogram intravenously, caused a slight stimulation of the intestine but did not alter the serotonin response. The administration of reserpine in a dose of 0.5 mg. per kilogram in two dogs did not stimulate jejunal motility observed over a period of 2 hours, and did

not affect the response of the intestine to serotonin. Studies of the effects of serotonin, administered intravenously, on the gastrointestinal tract in vivo have been limited by the cardiovascular and pulmonary responses to the amine and by its rapid degradation to its inactive metabolite, 5-hydroxyindoleacetic acid. Also, it must be considered that the effect of intracellular serotonin may be different from that of extracellular serotonin. Administration of the serotonin precursor, 5-hydroxytryptophan, results in an increase in intracellular as well as extracellular serotonin. Accordingly, the effect of 5-hydroxytryptophan on intestinal motility in man was investigated.

5-hydroxytryptophan, administered intravenously, in doses of 25–50 mg. over a 30 minute period was found to be a potent stimulus to intestinal motility in man. Within 6–8 minutes after the start of an infusion of 5-hydroxytryptophan, a marked increase in intestinal motility was observed in normal subjects (Fig. 3). Usually there was no marked increase in the intraluminal pressure, but in a few instances increases up to 15 cm. of water were noted and abdominal cramping was experienced. The pressure of the motility waves increased up to approximately 25–30 cm. of H_2O. The increased motility persisted for at least 90 minutes following the start of the 5-hydroxytryptophan infusion. No effect on pulse, blood pressure, respiration or mental status was noted following the dose of 5-hydroxytryptophan used. In three subjects 1 mg. of atropine sulfate was administered during the stimulated phase of intestinal motility, and in each study inhibition of intestinal motility was almost complete. When serotonin creatinine sulfate was administered at this point, however, the stimulus to intestinal motility was not modified (Fig. 3). Brom-lysergic acid diethylamide in an oral dose of from 2 to 6 mg. partially inhibited the intestinal motility stimulated by 5-hydroxytryptophan in the human. This agent was also effective in inhibiting intestinal motility stimulated by serotonin in the dog.

To determine whether serotonin or 5-hydroxytryptophan stimulated intestinal motility when introduced intraluminally, these agents, in doses of 10 and 30 mg. respectively, were administered into the intestine proximal to the recording end

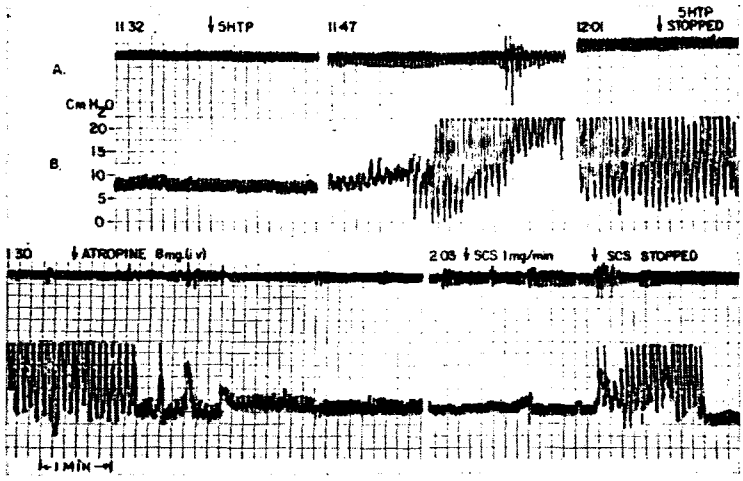


FIG. 3.—Respiration (tracing *A*) and jejunal motility (tracing *B*) in a control subject. Following administration of 5-hydroxytryptophan in a dose of 40 mg., a marked and sustained stimulus to motility occurred. Atropine sulfate inhibited motility stimulated by 5-hydroxytryptophan but not that stimulated by serotonin.

of the intestinal tube. No effect on the intestine was noted.

These studies indicate that intestinal motility is stimulated by a dose of 5-hydroxytryptophan which does not alter respiration, blood pressure, pulse rate or mental status. Thus, the intestinal tract is extremely sensitive to the effects of serotonin. It is not unreasonable to conjecture that the largest store of serotonin in the body performs its function in the organ system where this store is also formed. If the enterochromaffin cells normally influence intestinal motility, it is probable that they do so by releasing a humoral agent which acts on the neural elements within the intestinal wall.

When serotonin is administered by continuous infusion, the gut soon loses its ability to respond despite continued administration of the amine. In contradistinction to this, the administration of 5-hydroxytryptophan provides a sustained stimulus to intestinal motility. It is likely that this is attributable to the effect of intracellular serotonin which apparently is released at a rate that permits a sustained elevated turnover

rate of serotonin locally at the neural effector sites within the intestinal wall.

Bulbring and Lin (22) reported that the introduction of serotonin into the lumen of isolated loops of the guinea pig's ileum in in vitro studies stimulated peristalsis. They found that low concentrations of serotonin administered to the outside of the intestine never increased peristalsis and that high concentrations inside never abolished peristalsis. Their evidence suggested that serotonin may act in vitro by sensitizing the sensory receptors in the mucosa which trigger the peristaltic reflexes.

It is of interest that atropine inhibits intestinal motility stimulated by 5-hydroxytryptophan in a dose up to 40 mg., but it did not modify the response of the bowel to serotonin creatinine sulfate in doses as small as 1–2 mg. As it has been generally accepted that 5-hydroxytryptophan exerts its action only after being converted to serotonin in vivo, this finding could be explained by a difference in dosage or in the site of action of the two compounds. Lysergic acid diethylamide potentiates intestinal motility stimulated by serotonin despite the reported inhibitory properties of this substance on other actions of serotonin. Brom-lysergic acid diethylamide, which does not have the hallucinogenic properties of its analog, inhibits intestinal motility stimulated by either serotonin or 5-hydroxytryptophan. Other studies in our laboratory have shown that reserpine, chlorpromazine, diphenhydramine and tripelannamine do not significantly antagonize the motility stimulated by serotonin in the dog or in man. Recently, a number of compounds have been introduced which are purported to be serotonin antagonists in certain species and on certain organ systems. It has become evident that one may not translate these results to the gastrointestinal tract of man.

Various findings suggested that inquiry into the effects of serotonin and other indolic compounds on gastric secretion would be of interest. Reserpine, which contains the indole nucleus, stimulates the volume and acidity of gastric secretion in man. The fact that a time lapse of 45 minutes occurred before gastric acid flow increased raised the possibility that the effect of reserpine was mediated by release of another substance. A logical substance to consider was serotonin, as

it had been established that reserpine released serotonin from the blood platelets, intestines and brain of animals. Another factor which stimulated interest in the effect of serotonin on gastric acid secretion was the increased incidence of peptic ulceration in the malignant carcinoid syndrome in which hyperserotonemia exists. Also serotonin, its precursor 5-hydroxytryptophan and reserpine cause hemorrhagic gastric erosions in the rat. When 5-hydroxytryptophan in a dose of 300 mg. per kilogram is administered either intraperitoneally or subcutaneously, hemorrhagic erosions are noted in the glandular portion of the stomach of the rat 3 hours after injection. Reserpine in a dose of 5 mg. per kilogram produces similar lesions 18 hours after injection. It is interesting to point out that atropine sulfate prevented the gastric erosions produced by 5-hydroxytryptophan in most of the animals. Serotonin in doses of 0.001–0.04 mg. per kilogram per minute intravenously had no marked transient or sustained effect on the volume and pH of gastric secretion from the denervated Heidenhain pouch of the dog. The mean volume was slightly greater and the mean pH lower during administration of 0.01 mg. per kilogram per minute of serotonin than that obtained when saline was given alone (Table 1). The secretory response to serotonin is contrasted with the response to reserpine because of a possible relationship which is discussed subsequently. Reserpine evoked an appreciable secretion of highly acidic juice, although the volume was generally less than that following histamine. In single experiments, 0.01 mg./kg./min. of either sodium acetate or 5-hydroxyindoleacetic acid did not stimulate gastric secretion from the Heidenhain pouch, while the so-called serotonin-antagonist, lysergic acid diethylamide, in a dose of 50 μg. per kilogram produced a secretory response comparable to that of reserpine. 5-hydroxytryptophan in a single dose of 25 mg. per kilogram intravenously inhibited spontaneous gastric secretion in the dog with a gastric fistula and intact vagal nerves. This substance was equally effective in inhibiting gastric acid secretion stimulated by insulin induced hypoglycemia and Urecholine.

TABLE I.—COMPARISON OF THE EFFECTS OF ISOTONIC SALINE, SEROTONIN CREATININE SULFATE, RESERPINE, AND HISTAMINE DIHYDROCHLORIDE ON GASTRIC SECRETION IN THE HEIDENHAIN POUCH DOG*

	Dog I		Dog II		Dog III		Dog IV	
	Vol.	pH	Vol.	pH	Vol.	pH	Vol.	pH
Isotonic saline	0.37	4.2	0.15	3.7	0.05	3.7	0.05	3.7
Serotonin creatinine sulfate (mg./kg./min.)								
0.001			0.04	1.6			0.46	2.2
0.01	0.48	2.4	0.62	1.9	0.16	2.9	0.73	2.1
0.04			0.10	2.4	0.2	7.0	0.05	4.1
Reserpine (0.2 mg./kg.)			3.7	1.0	1.1	1.1	5.1	1.0
Histamine dihydrochloride (0.005 mg./kg./min.)	15.7	1.0	8.8	1.0	2.6	1.0	14.8	1.0

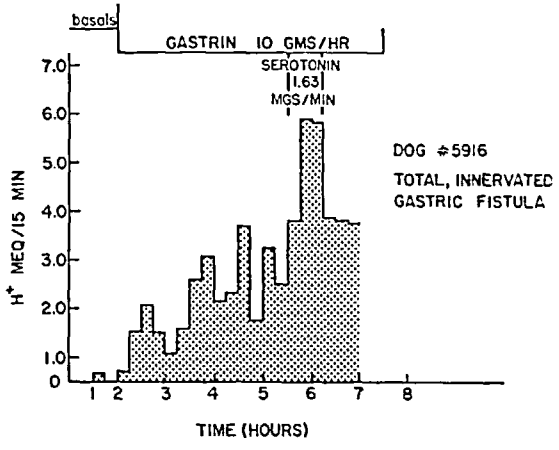
* Each figure for saline and serotonin represents the average value of six 20-minute periods, those for reserpine and histamine three to six 20-minute periods. The volume is expressed in ml./20-minute period.

For years it has been postulated that histamine is a final common mediator for the various stimuli to gastric acid secretion. Strong evidence to support this thesis has recently come from studies by Stubrin *et al.* (23) showing that gastrin, reserpine, insulin-induced hypoglycemia, Urecholine and betazole hydrochloride (Histalog), all release histamine from the gastric mucosa of the rat. Even more important evidence is their demonstration that the administration of enzymes which affect the formation or degradation of histiamine profoundly affects stimulation of gastric acid secretion (24). Histamine is formed by decarboxylation of histidine. Once histamine is released from the storage site, it is catabolized by several mechanisms. The principal routes of degradation are as follows: (1) histamine is oxidized by histaminase to imidazolacetic aldehyde which in turn is degraded by aldehyde dehydrogenase to imidazolacetic acid; (2) methylation of the imidazole ring is accomplished by N-methyltransferase to form 1'4 N-methylhistamine, a compound with very weak histamine-

like activity and which is degraded by monoamine oxidase to 1'4 methyl imidazolacetic aldehyde; this undergoes degradation by aldehyde dehydrogenase to form 1'4 methyl imidazolacetic acid; and (3) a less important pathway of histamine catabolism is acetylation.

The importance of histaminase and of N-methyltransferase in the degradation of histamine varies in different species and in different tissues. Studies in our laboratory have shown that the intravenous administration of histaminase (diamine oxidase) profoundly inhibits gastric acid secretion stimulated by gastrin, insulin-induced hypoglycemia, Urecholine and betazole hydrochloride; also that this inhibition can be blocked by aminoguanidine, a selective inhibitor of diamine oxidase. Of equal interest is the fact that inhibition of N-methyltransferase by the administration of 1'4 N-methylhistamine or serotonin results in marked potentiation of gastric acid secretion stimulated by gastrin (Fig. 4). The serotonin precursor 5-hydroxytryptophan, that in itself inhibits specific and nonspecific histidine decarboxylase, initially inhibits gastric acid secretion stimulated by gastrin, and 45 minutes following inhibition, marked potentiation of the gastrin stimulus occurs, which in time coincides with the conversion of the

FIG. 4.—Serotonin potentiates the stimulatory effect of gastrin when administered in a dose that by itself has no effect on basal secretion.



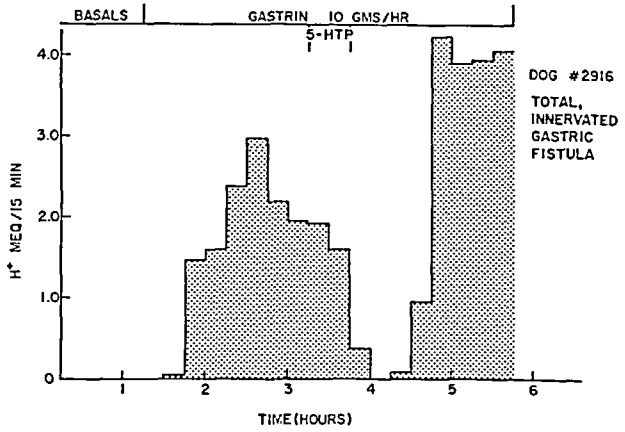


FIG. 5.—It is noted that within 30 minutes following the infusion of 5-hydroxytryptophan the gastrin response is totally inhibited; 30-40 minutes later potentiation of the response to gastrin occurs.

precursor to the active amine. The latter effect corresponds to the action of serotonin itself on gastrin stimulated secretion (Fig. 5). Corresponding studies in the literature have shown that serotonin potentiates gastric acid secretion stimulated by submaximal doses of histamine.

CARDIOVASCULAR

Vasoconstriction was the first pharmacologic effect attributed to serotonin; in fact, many publications preceding the identification of this amine refer to it as the vasoconstrictor principle of blood. In addition to the chemical identification of serotonin, Page and his associates (3) have pioneered the basic studies relating to the action of this amine on the cardiovascular system. The vascular response to serotonin is largely determined by the neurogenic tone of the blood vessels during the resting state. Interestingly, the action of serotonin on the cardiovascular system varies in the different animal species. In the cat, the rapid intravenous infusion of serotonin results in prolonged hypotension, bradycardia and apnea. This effect is probably mediated by a reflex mechanism carried via the vagus nerve, and is blocked by vagotomy or adminis-

tration of atropine. The vascular response to the injection of serotonin into the right heart or the vena cava of the cat occurs within 3 seconds, and sometimes as early as .5 seconds. In the dog, the initial response, which also consists of hypotension and bradycardia, is immediately followed by a substantial increase in blood pressure and cardiac output. A third phase is characterized by prolonged hypotension as the result of inhibition of the neurogenic tone. Because of these divergent effects, Page has called the action of serotonin on the arterial pressure "amphibaric." In dogs pretreated with ganglionic blocking agents, the response to serotonin is characterized by vasoconstriction without a vasodepressor phase. The vasoconstrictor effect of serotonin is best demonstrated by its action on strips of denervated arteries. Among the few studies performed in man, those by Baldrighi and Ferrari (25) have shown that, following the administration of serotonin, there is an increase of the pulmonary artery and right ventricular pressures. These effects are associated with an increase of the heart rate and stroke volume. Serotonin also has been shown to be a potent stimulus to the aortic and carotid sinus, and its effect on these receptors is part of the mechanism by which this amine affects blood pressure and heart rate. Destruction of the carotid sinus results in a decreased vasopressor response to serotonin. The amine has also been implicated in the regulation of the glomerular capillary bed of the kidneys. Serotonin decreases both renal blood flow and glomerular filtration rate. These effects occur whether circulating serotonin is increased by exogenous administration or by endogenous secretion by tumors. The cardiovascular effects of serotonin in the human are complex and difficult to interpret. In this regard, Felberg and Smith have shown that serotonin releases histamine from the skin of the cat, and Page *et al.* were able to demonstrate that antihistaminic agents inhibit the vascular response to serotonin, except for the skin vasodilatation. However, recent data point to bradykinin, a naturally occurring nonapeptide, as the substance responsible for the flushing in carcinoid patients. It is possible, therefore, that the vascular changes which follow the administration of serotonin or those present in patients with the car-

cinoid syndrome may be in part caused by other substances, such as histamine or bradykinin, and not solely by serotonin.

Other Actions of Serotonin

As was the case with histamine, multiple and variegated actions have been attributed to serotonin. Although the effect of the action of the amine may be clearly apparent, its physiologic or pathologic significance often is difficult to interpret. Included in this category is the marked increase in capillary permeability that results from the subcutaneous injection of serotonin. Frimmer has shown that the increase in capillary permeability is sufficient to permit large colloidal molecules to escape from the intravascular compartment. Some investigators have implicated serotonin in the formation and organization of fibrous tissue; it remains to be determined whether this action and the increasing capillary permeability have pathogenetic significance with regard to the lesions found in the heart valves and endocardium in patients with the carcinoid syndrome.

The relationship of serotonin to allergic reactions also remains obscure. It would appear from experiments in the mouse and rabbit that changes in tissue serotonin may be associated with allergic manifestations. Although McHaffie has reported that following the treatment with water-soluble antigens elevated levels of serotonin occur in the serum, there is no direct evidence as yet to implicate serotonin in human allergy. It is likely, however, that important interrelationships exist among histamine, serotonin and kinin substances in a human allergy.

CLINICAL SYNDROMES

Carcinoid Syndrome

Prior to the report by Biörck *et al.* (26) in 1954, it is likely that the carcinoid syndrome would not have been recognized clinically. From that time until the present, the clinical symptomatology of metastatic carcinoid tumors has been recognized widely, and the biochemistry of the disease thought to be thoroughly understood. Presently, however, more questions

than answers arise concerning the biochemical mechanisms which are responsible for the symptoms. The carcinoid syndrome, with diverse and seemingly unrelated symptomatology, has been described in association with primary tumors arising from various organs. A consideration of the histology of the tumor, of the biochemistry of the secretory product and of the characteristic site of metastases provides a convenient means for separation of the various carcinoid tumors on an embryologic basis. Often, these features enable one to designate the embryological origin of these tumors from the foregut (stomach, pancreas, proximal duodenum, bronchi), the midgut (lower duodenum, jejunum, ileum, appendix, proximal colon), or the hindgut (descending colon and rectum) (27). Carcinoid tumors originate from argentaffin cells which extend from the cardia of the stomach to the anus. However, in the carcinoid syndrome the most common primary site of the tumor is the terminal ileum. Appendiceal lesions occur more frequently, but metastases rarely occur and the syndrome is infrequently noted. Metastases, but not the syndrome, have been described with rectal carcinoids (27).

Numerous studies have demonstrated the excessive production of serotonin by carcinoid tumors; the elevated levels of the amine have been related to the clinical features of the disease. The major clinical manifestations, including flushing, severe diarrhea, valvular heart disease and telangiectasiae of the face and neck, have been described in great detail. With rare exception, the syndrome occurs only after metastases have occurred in the liver. The large concentration of monoamine oxidase in the liver degrades the serotonin which enters this organ via the portal vein, and it should be pointed out that the metabolite, 5-hydroxyindoleacetic acid, is pharmacologically inert. Liver metastases which synthesize excessive amounts of serotonin liberate this amine directly into the hepatic venous system without exposure to monoamine oxidase in the liver cells. Accordingly, the liver is an effective and important barrier to the expression of the syndrome. As it has been shown that the liver does not degrade all of the serotonin exogenously infused into the portal system and that a percentage of this amine escapes into the systemic circulation, it is conceivable that the carcinoid syndrome may

occur without liver metastases when the primary tumor is quite large or extensive tumor masses are present. It is interesting to note that metastases to the testicle or ovary may be a rich source of serotonin, and in one case removal of the involved testicle caused flushing to cease (28).

Clinical effects of the carcinoid syndrome may occur with only slight elevation of the serotonin concentration in the hepatic vein, which raises the possibility that serotonin is partially or only indirectly involved as a causative factor in the symptomatology, or that another substance may be primarily responsible. The syndrome may also occur with ovarian, testicular or retroperitoneal teratomas which drain directly into the inferior vena cava. As bronchial veins drain directly into the left heart, the syndrome may occur with bronchial carcinoids without liver metastases.

The dermal and vasomotor manifestations observed in patients with the malignant carcinoid syndrome are quite striking. Flushing is a prominent feature and may provide the first indication of the presence of a carcinoid tumor in patients with bizarre or confusing symptoms. The intensity of the flush varies from a delicate pink to an intense brick red erythema, involving the face and neck; the attack may end abruptly or spread to involve the trunk and the extremities. Commonly, white blotches and patchy cyanotic areas, especially on the face, become apparent. The episodes are frequently transient lasting only a few minutes and may occur as often as thirty times daily. Some patients have a chronic rubicund appearance. Piloerection, a sensation of warmth, tachycardia and even mild hypotension may accompany these episodes. Emotional upsets, alcohol, fat, cheese, tea, and lysergic acid diethylamide have been described as precipitating factors for the symptoms. Prominent telangiectasiae are obvious over the face, neck and thorax. Pellagra-like skin lesions, attributed to niacin and protein deficiencies, resulting from abnormal tryptophan metabolism, have been noted. The skin lesions generally are resistant to vitamin replacement.

Substances elaborated by cells of the tumor most likely participate in the pathogenesis of flushing. In fact the severity and frequency of attacks may be alleviated and in some instances completely abolished by surgical removal of tumor

tissue. However, there is sufficient evidence to question whether excessive serotonin production is the sole mediator of the flush. Several investigations have shown that variable effects are noted following the exogenous administration of serotonin to these patients (29). The flush may differ from that which occurs spontaneously and indeed, at times, no flushing may occur at all. In addition to serotonin, other amines including adrenaline, noradrenaline and histamine may be implicated in flushing. The administration of adrenalin and noradrenalin in amounts as small as 5 μg. and 0.25 μg., respectively, precipitates flushing in patients with carcinoidosis, including some patients who do not have the spontaneous symptom with the syndrome. Flushing induced by the exogenous administration of sympathomimetic amines differs qualitatively from the spontaneous type and may be attenuated by adrenergic blocking agents. It has been suggested that the interaction of serotonin and catecholamines is in some way involved in the mechanism of flushing. This concept has some support due to the fact that the combined administration of serotonin and either epinephrine or norepinephrine results in a cyanotic flush which is more characteristic of those occurring spontaneously. The flush observed in patients with tumors of gastric, pancreatic or bronchial origin, which secrete 5-hydroxytryptophan, differs from the typical cyanotic flush caused by tumors which secrete primarily serotonin, being more vivid, patchy, and red. The excessive urinary excretion of histamine by these patients has caused further speculation regarding the significance of this amine in the pathogenesis not only of flushing but also of the other symptoms. The role that histamine may have in these features awaits confirmation.

Although serotonin, adrenaline, noradrenaline and histamine have been implicated in one way or another with flushing, cogent arguments can be raised against each of these substances being the sole mechanism of the symptom. Concerning serotonin and histamine, blood levels of these amines have been determined before, during and after flushing, and no significant changes have been noted (30). Many investigators have shown that there is no increase in circulating levels of catecholamines or of urinary levels of vanillylman-

delic acid in this syndrome. If none of these amines causes the flush, what does? Recent investigations have indicated that increases in circulating kinin substances are associated with the symptomatology of the syndrome (31). The kinins which are found in the circulation have the following properties: (1) vasomotor activity (causing hypotension); (2) bronchoconstriction; (3) increased capillary permeability; (4) production of pain; and (5) stimulation of migration of leukocytes. Of the different kinins, the nonapeptide bradykinin has been studied most extensively. It is formed from kallidinogen, an alpha-2-globulin, either directly or via the intermediate kallidin which is a decapeptide (lysyl-bradykinin). Glandular kallikreins which are proteolytic enzymes found in the pancreas, salivary gland and kidney convert kallidinogen to kallidin. The lysyl group is hydrolyzed by an aminopeptidase in plasma to form bradykinin. The kallikrein found in plasma converts kallidinogen directly to bradykinin (32). Studies by Oates *et al.* have shown that bradykinin in hepatic venous blood becomes remarkably elevated in patients with the carcinoid syndrome following the administration of small amounts of adrenalin (31). Carcinoid tumors contain kallikrein, and it has been suggested that catecholamines either activate or release kallikrein which in turn would catalyze the formation of the active kinin. Whether bradykinin or some other peptide, such as substance P, is responsible for the flushing awaits further study.

One of the most intriguing features of the carcinoid syndrome is the pathologic change that involves primarily the tricuspid and pulmonic valves. The most frequently encountered clinical manifestations which accompany the pathology are tricuspid regurgitation and pulmonic stenosis. In addition to the heart valves on the right side, involvement of the valves on the left side, of the endocardium of the heart chambers, of the intima of the great veins, the coronary sinus, and less frequently the large arteries, may be present (33). Cardiac involvement is a late feature in the carcinoid syndrome. Usually pathologic changes occur in the heart only in association with hepatic metastases when the disease has been present for many years. The pathology consists of focal or diffuse collections of a peculiar type of fibrous tissue, free of elastic

fibers, which is deposited on the endocardium of valves and chambers. The fibrotic lesions are generally confined to the endocardium of the right atrium and ventricle if no intracardiac communication exists. The lesions are characteristic and can be distinguished by experienced pathologists from the alterations found in other cardiac conditions. In the 9 patients with cardiac pathology reported by Roberts and Sjoerdsma (33) the pulmonic and tricuspid valves were equally affected; in 3 patients left-sided cardiac lesions were also noted. Pathologic changes involving only the left side of the heart have been reported in a patient with a bronchial carcinoid. Precordial systolic murmurs, distended neck veins, a pulsating liver, edema and ascites have all been observed. However, functional murmurs, ascites and edema may occur with tumor metastases and protein depletion without cardiac involvement and should be distinguished from these findings caused by valvular heart disease. In some patients, the measured cardiac output is significantly elevated in either the presence or absence of valvular defects, which may result in heart failure. Although the cardiac pathology can be recognized easily, the pathogenetic mechanisms remain obscure. It is unlikely that serotonin alone produces the cardiac lesions for the following reasons: (1) there is no difference in serotonin production measured by the urinary excretion of 5-hydroxyindoleacetic acid in patients with and without cardiac lesions; and (2) the long term administration either of serotonin or of its precursor, 5-hydroxytryptophan, did not produce cardiac lesions in animals. It is interesting to point out that right-sided endocardial lesions, differing from those which occur in carcinoid patients, may be produced in guinea pigs made tryptophan- or niacin-deficient following subsequent intraperitoneal administration of serotonin for 6–8 weeks (34).

The smooth muscle of the bronchi and intestine may be stimulated in this syndrome to produce wheezing respirations and diarrhea. Generally the diarrhea is mild and episodic, but occasionally it may be incapacitating, resulting in as many as 20 or more stools daily. The stools are watery, brown, and usually devoid of gross blood, but may be positive for occult blood when tested chemically. Although steatorrhea is un-

common, intestinal malabsorption has been reported in the syndrome. Usually this occurs in association with hyperserotonemia and increased urinary excretion of 5-hydroxyindoleacetic acid; in one patient, however, normal levels of urinary indoles have been reported. Although this facet of the syndrome is very interesting, little speculation and lesser data concerning the mechanism have been presented.

One of the serious, complicating diseases occurring in the carcinoid syndrome is peptic ulceration of the stomach and duodenum, with hemorrhage in some cases. The incidence of peptic ulcer has been reported to be as high as 38% in one series of 21 cases (35). In our own experience the incidence of peptic ulcer has been approximately 25%. The mechanism for the increased incidence of peptic ulcer is not clear, especially when one considers the fact that basal and histalog-stimulated gastric acidity has not been elevated in these patients. Also, studies in our laboratory have clearly shown that serotonin and its precursor, 5-hydroxytryptophan, inhibit basal gastric secretion in the rat, dog and man. Administration of both serotonin and 5-hydroxytryptophan, however, cause hemorrhagic erosions in the glandular portion of the stomach despite the decrease in gastric acid (36). Whether these erosions occur in man and serve as the nidus for peptic ulceration in the foregut remains unanswered. As noted in the foregoing section, serotonin increases gastric secretion stimulated by either gastrin or histamine probably by inhibiting the histamine degrading enzyme N-methyl transferase. In this regard, it would be of great interest to determine gastric secretion in carcinoid patients following the ingestion of a meal at which time endogenous gastrin is released. Although in some patients with the malignant carcinoid syndrome, histamine is elevated in both blood and urine, no correlation of the elevation of the levels of this amine has been made with the presence of peptic ulceration. Also in the syndrome of multiple endocrine adenomatosis with a fulminating form of peptic ulceration, histamine and serotonin content in both blood and islet cell tissue was not significantly elevated.

The refinement in the methods to measure small quantities of circulating humoral agents secreted by tumor cells, or their urinary metabolites, has provided the clinician with a valuable

tool to diagnose the carcinoid syndrome. Elevations of blood serotonin and of urinary 5-hydroxyindoleacetic acid are the most consistent and reliable biochemical abnormalities found in these patients. In our experience platelet serotonin levels invariably have been elevated in all patients with the syndrome; 4 patients in this group had normal levels of urinary 5-hydroxyindoleacetic acid. In our laboratory, the normal range of platelet serotonin is 0.2–0.7 μg./mg. of platelet protein and that for urinary 5-hydroxyindoleacetic acid is 2–16 mg./24 hours. No correlation exists between the levels of circulating serotonin or urinary 5-hydroxyindoleacetic acid and the severity or type of clinical symptoms. With very few exceptions, large elevations of circulating serotonin (2–5 μg./mg. of platelet protein) indicate the presence of the carcinoid syndrome. A mild elevation of the blood serotonin level is not diagnostic for the carcinoid syndrome as this value may be slightly elevated in a few other conditions; these include untreated nontropical sprue, some instances of intestinal malabsorption of other etiology, following the ingestion of monoamine oxidase inhibitors. However, a careful history and appropriate diagnostic studies usually enable one to differentiate these particular disorders from carcinoidosis.

Although normal levels of urinary 5-hydroxyindoleacetic acid occasionally occur in the carcinoid syndrome, most patients excrete large amounts of this indole. An increasing level of urinary 5-hydroxyindoleacetic acid likely means an increase in the amount of metastatic tissue mass. Moderate elevations of 5-hydroxyindoleacetic acid may occur in patients without the syndrome following the ingestion of foods which contain large amounts of serotonin (bananas, tomatoes, red plums, avocado, eggplant) (37). However, a 24-hour urinary level of 5-hydroxyindoleacetic acid greater than 25 mg. is highly suggestive of the presence of the tumor. The significance of increased blood and urinary levels of histamine in the carcinoid syndrome remains unknown. Abnormally high histamine levels have been more frequently encountered in patients with tumors secreting 5-hydroxytryptophan of gastric, pancreatic or bronchial origin, than with primary ileal tumors. The indoles which are excreted from patients with the variant carcinoid tumors are different from those excreted from pa-

tients with the more usually encountered tumor in that substantial amounts of serotonin and 5-hydroxytryptophan accompany 5-hydroxyindoleacetic acid.

An appealing diet, high in protein, is essential for these patients. Although restriction of tryptophan in the diet may result in partial relief of symptoms, the effect is transient and incomplete and may enhance the tendency for the development of pellagra-like symptoms. Another reason against the restriction of dietary tryptophan is that a variety of pharmacologic agents is available to antagonize the peripheral effects of serotonin as well as some to inhibit the synthesis of this amine. In theory, inhibition of the synthesis of serotonin may permit more of the available tryptophan to become incorporated with needed protein. In our experience, a particularly effective agent in the repression of symptoms has been chlorpromazine in a dosage which has varied from 50 mg. to 200 mg. daily. Administration of the larger doses of chlorpromazine may be accompanied by the usual untoward effects of the drug and these should be looked for. Chlorpromazine has been particularly effective in relieving the flushes. Another drug which has been helpful in these patients is cyproheptadine in a dose from 8 to 40 mg. daily. This substance has been shown to have both antiserotonin and antihistamine properties and has been of particular help in the relief of diarrhea and cramps, as well as of other symptoms of the syndrome. Methysergide, another antiserotonin preparation, administered in a daily dosage of 6–24 mg. has been reported to be effective, but in our experience the results have been somewhat disappointing. The combined use of chlorpromazine, methysergide and cyproheptadine in modified dosage may be indicated in some patients. Drugs which inhibit synthesis of serotonin have been only partially effective in our experience. Administration of alpha-methyl dopa has often caused excessive drowsiness and weakness with only slight regression of the troublesome symptoms. However, this drug has been reported to be more effective in patients with tumors originating in the foregut producing 5-hydroxytryptophan (38). It is hoped that many current investigations relating to the discovery of drugs which are nontoxic, potent inhibitors to the various decarboxylases will result in more

effective, specific therapy. Also of great interest will be the application of inhibitors to kinin peptides in this disease. In our experience, chemotherapeutic agents that include 5-fluorouracil, cyclophosphamide and nitrogen mustard have not been particularly effective. Whether intrahepatic catheterization with administration of the chemotherapeutic agent directly to the metastatic area will be helpful remains to be determined. Also radiation has not been particularly helpful as carcinoid tumors are resistant to radiotherapy.

Carcinoid Syndrome with Lung Tumors

All of the major manifestations of the carcinoid syndrome have occurred in patients with metastatic bronchial adenoma. The occurrence of the syndrome with this tumor brings up the question of the cellular genesis of the bronchial adenoma. As the foregut is the embryologic precursor of the bronchi, it is logical to think that the cells of bronchial adenoma are true argentaffin cells derived from the same tissue as those occurring in the gastrointestinal tract. However, there are distinct differences, in that silver-staining granules are characteristically found in the argentaffin cell of gastrointestinal tract carcinoids, whereas it is the rare exception to demonstrate these granules in cells of the bronchial adenomas. There is little question, however, that the bronchial adenoma and its metastases synthesize and secrete serotonin, for the amine has been found to be elevated in tumor tissue as well as in the circulation. Also the urinary metabolite, 5-hydroxyindoleacetic acid, is generally elevated in these patients. Because of the unique anatomic position of the primary tumor, is it possible to have the carcinoid syndrome without metastases? From our experience and those of others, it would appear that the syndrome is present only after metastases occur. However, Warner *et al.* (39) have reported two cases of bronchial carcinoid without discernible distant metastases and without the syndrome; in both, the hyperserotonemia and increased urinary excretion of 5-hydroxyindoleacetic acid returned to normal following resection of the primary tumor. This feature assumes added significance in a consideration of therapy for this type of lesion. The clinical findings differ somewhat from

those associated with intestinal carcinoids. Flushing may be more severe and left-sided heart involvement is more common. Left-sided valvular heart defects, in the absence of right-sided valvular heart defects, have been described only in a patient with a bronchial carcinoid tumor (40). Another interesting aspect of bronchial carcinoid tumors is the rather frequent association with endocrine disturbances. The associated endocrine abnormalities include Cushing's syndrome, acromegaly and adenomas of the pituitary and the parathyroid glands. Also the association of bronchial carcinoids with multiple glandular adenomatosis has been reported (41).

In one patient with bronchial adenoma, the primary tumor was described as showing some features of an anaplastic carcinoma (42). In 1957 the carcinoid syndrome was described in association with oat cell carcinoma of the lung (43). The carcinoid syndrome has been reported in about 6 patients with lung cancer of a type other than bronchial carcinoid. We have had an opportunity to study two such patients (44). One of them was a 69-year-old male who developed diarrhea consisting of 15–20 watery, yellow stools daily, flushing which included the face, neck, shoulders, abdomen and legs and, terminally, left ventricular heart failure and bronchopneumonia. The patient had smoked a package of cigarettes daily for many years. The pertinent physical findings included a moderate erythema of his face and palms, and enlargement of the liver which extended 7 cm. below the right costal margin. Autopsy revealed a grayish-white firm tumor mass of the left lower bronchus with metastases to the tracheobronchial lymph nodes, the liver, the mesenteric nodes, left adrenal gland and the brain. The tumor was a typical oat cell carcinoma. The second patient was a 49-year-old female who developed diarrhea and facial flushing which occurred several times daily. The watery bowel movements contained mucus but no blood. She smoked 2 packages of cigarettes daily for a period of 20 years. Physical examination was normal except for erythema of her face, upper extremities and back. The liver was enlarged 6 cm. below the right costal margin. A chest film was interpreted as showing a neoplasm involving the superior segment of the right lower lobe with hilar lymphadenopathy. Biopsy of the right scalene

lymph node revealed a metastatic tumor growth of undifferentiated carcinoma compatible with bronchogenic origin. The pertinent blood, urinary, tissue-amine data are listed in Tables 2 and 3. It is clear from these data that the patients had elevated levels of serotonin in the blood and tumor tissue, as well as increased levels of urinary 5-hydroxyindoleacetic acid. The syndrome also has been seen in patients with tumors originating from less common sites, namely, the pancreas, gall bladder and parotid gland. It is obvious that one cannot distinguish the primary site of the tumor from the peripheral symptoms or from the changes in the level of blood serotonin or its metabolite in the urine.

Flushing, Diarrhea, and Peptic Ulcer without Carcinoidosis

Investigators who have been interested in the carcinoid syndrome encounter many patients whose symptomatology includes flushing, but who on detailed examination do not have the carcinoid syndrome. Warner (45) has reported on 9 patients who had all or some of the following symptoms: abdominal pain, nausea, vomiting, diarrhea, weakness, psychiatric symptoms. Two of the 9 had flushing in addition to these symptoms. Most of these patients had elevated serum serotonin levels and low urinary levels of 5-hydroxyindoleacetic acid. In some of these patients, he reported that the administration of reserpine precipitated an attack of the symptoms. Over the past five years we have encountered 11 patients who have had flushing, a rubicund appearance and telangiectasiae of the face and neck, diarrhea, abdominal cramps, and in 7 the demonstration of peptic ulceration (9 of the 11 patients were females). The flushing occurred many times daily lasting from 30 seconds to 20 minutes and was associated in many instances with the ingestion of cheese, minute amounts of alcohol, and other foods. Physical examination revealed no remarkable findings except for the flushing and telangiectasiae. Routine laboratory and x-ray studies were within normal limits, except for the demonstration of peptic ulceration in the majority. Although carcinoidosis

or systemic mastocytosis was suspected as a possible diagnosis in each of them, none of the many studies revealed the presence of either of these two diagnoses. Carcinoidosis was virtually excluded by the normal levels of platelet serotonin and urinary 5-hydroxyindoleacetic acid. In all but 2 patients, the whole blood histamine levels were elevated with values extending from 0.1 to 0.29 μg./ml. (normal is .02–0.08). The blood histamine was determined by organic extraction of the amine and coupled with o-phthalaldehyde to form a fluorophor, which was then measured spectrophotofluorometrically by the method of Shore *et al.* (46). Urinary histamine was determined in 8 of these patients using the method of Oates *et al.* (47), and in 3 patients was found to be elevated tremendously with levels in the range of 300–500 μg./24 hours, the normal value being less than 60. Interestingly, 2 of the 3 patients had normal blood histamine levels (48, 49). Urinary tryptamine was either normal or in the high normal range, and the urinary tryptamine metabolite indole-3-acetic acid was normal in all patients.

TABLE 2.—THE MAIN BLOOD AND URINARY FINDINGS IN TWO PATIENTS WITH CARCINOMA OF THE LUNG AND THE CARCINOID SYNDROME

PATIENT	DATE	BLOOD		URINE 5-OHIAA
		Histamine (gammas/ml. whole blood)	Serotonin (gammas/mg. platelet protein)	(mg./24 hr.)
A. G.	4-1-64	.085		8.2
	4-2-64	.07	1.02	22.2
	4-10-64	.105	1.17	54.2
	5-1-64	.105		
M. L.	3-30-64	.085	1.73	
	4-4-64	.045	.56	16.5
	4-9-64	.06	.920	16.2
NORMAL VALUES:		(.02—.08)	(.2—.7)	(less than 8 mg.)

TABLE 3.—THE CONCENTRATIONS OF HISTAMINE AND SEROTONIN IN NECROPSY TISSUE FROM A PATIENT WITH METASTASIZING OAT CELL CARCINOMA OF THE LUNG AND THE CARCINOID SYNDROME*

	TUMOR NODULE IN LIVER	MEDIASTINAL NODE WITH TUMOR	NORMAL LUNG	LUNG TUMOR	ADRENAL METASTASIS
Histamine	1.6	2.2	10	2.2	0.6
Serotonin	6.1	34.2	1.4	9.0	6.4

* Values are expressed in gammas per Gm. of tissue.

Do the bizarre and seemingly unrelated findings in these patients constitute a clinical entity or do they merely represent unrelated events without a common biochemical abnormality? The evidence would appear to be weighted in favor of the first possibility for the following reasons: (1) the symptomatology and findings were similar in each of the patients; (2) all patients had a demonstrable significant abnormality in the level of either the blood or urine histamine; (3) all had normal levels of circulating serotonin and of the urinary excretion of its metabolite; and (4) 64% had peptic ulceration. Although histamine metabolism was abnormal, it is unlikely that this abnormality constitutes the primary biochemical defect for the following reasons: (1) elevation of the blood levels of histamine remained constant despite the presence or absence of symptoms; and (2) the usual antihistaminic preparations generally were ineffective. It is likely that the abnormality in histamine is but a peripheral manifestation of a more basic biochemical defect. An investigation of the enzymes which synthesize, as well as degrade, naturally occurring, pharmacologically active amines and measurement of the circulating vasoactive kinins would likely be rewarding in elucidating the underlying mechanism.

SEROTONIN METABOLISM FOLLOWING MONOAMINE OXIDASE INHIBITION

Monoamine oxidase is a stable mitochondrial enzyme that is widely distributed in animal and human tissues. Many naturally occurring amines, such as tyramine, tryptamine,

serotonin, dopamine, epinephrine, norepinephrine, metanephrine and normetanephrine, are partially or completely degraded by monoamine oxidase. Monoamine oxidase metabolizes amines by oxidative deamination according to the following general equation:

$$R.\ CH_2NH_2 + O_2 + H_2O \rightarrow R.\ CHO + NH_3 + H_2O_2$$

Metabolic changes that occur when monoamine oxidase is inhibited include increases in platelet serotonin, brain serotonin and norepinephrine, urinary tryptamine, metanephrine and normetanephrine and a reduction in urinary 5-hydroxyindoleacetic acid, indole-3-acetic acid and vanillylmandelic acid.

Monoamine oxidase inhibitors are of two types: hydrazine derivatives, which inhibit both monoamine oxidase and diamine oxidase, the latter enzyme being important in the metabolism of histamine; and nonhydrazine compounds which inhibit monoamine oxidase but have no effect on diamine oxidase (50).

We have studied the amine metabolism in 4 patients who had attempted suicide by the ingestion of large amounts of tranylcypromine (Parnate), a potent nonhydrazine and monoamine oxidase inhibitor. Initially, their behavior was characterized by random, uncoordinated, purposeless muscular activity, incoherent speech and hyperthermia. Subsequently, coma ensued and was accompanied by an appearance of decerebrate rigidity, myoclonic seizures, carpopedal spasm, hyperactivity of all reflexes, dilated, fixed pupils, an intense facial flush and marked diaphoresis. At this time marked monoamine oxidase inhibition was indicated by the following alterations in amine metabolism: marked elevation of blood serotonin; elevation of urinary tryptamine, metanephrine and normetanephrine; decrease in 5-hydroxyindoleacetic acid and vanillylmandelic acid; and slight elevation in blood histamine. These changes reflect a block in the degradation of serotonin and tryptamine, a change in the route of metabolism of epinephrine and norepinephrine and a slight effect on the degradation of histamine (51).

It is clear from these studies that the enzymes involved in

the synthesis and degradation of amines are important not only in the normal routes of amine metabolism but in the mechanism of action of many drugs.

SEROTONIN METABOLISM IN INTESTINAL MALABSORPTION

As the gastrointestinal tract has the largest store of serotonin, it was natural to focus attention on serotonin metabolism in patients with gastrointestinal tract diseases. Accordingly, blood serotonin and urinary 5-hydroxyindoleacetic acid levels were determined in patients with nontropical sprue, Whipple's intestinal lipodystrophy, regional enteritis, and in one with resection of the colon and almost all of the small intestine. In the last patient the blood serotonin levels were in the range of 0.03–0.05 μg./mg. platelet protein and the urinary 5-hydroxyindoleacetic acid levels in the range of 0.3–1.0 mg./24 hours. These findings would indicate that the gastrointestinal tract serves as the major source for circulating serotonin and consequently its major excretory metabolite. The futility of attempting to evaluate the status of brain serotonin by similar measurements is apparent. In some patients with untreated nontropical sprue, blood serotonin levels are elevated being in the range of 0.8–1.2 μg./mg. of platelet protein and the urinary 5-hydroxyindoleacetic acid levels are correspondingly high, with the range being from 9 to 20 mg./24 hours (15). Following gluten restriction, the levels of both serotonin and the urinary metabolite revert to normal, usually in association with clinical remission of the disease. Although there has been much speculation concerning the mechanism for the increase in 5-hydroxyindoles in patients with nontropical sprue, the only convincing evidence to explain these findings was recently reported by Swanson and Thomassen (52). Their detailed studies clearly established that in tropical sprue the argentaffin cells in the small intestine are markedly increased in both size and number. It is likely that similar increases are found in the intestine of patients with nontropical sprue. No suitable explanation exists for this finding. Patients with ulcerative colitis and regional enteritis did not demonstrate abnormalities in the blood or urine levels of 5-hydroxyindoles. In other diseases

such as chronic pancreatitis, peptic ulceration and cystic fibrosis of the pancreas, 5-hydroxyindoles were also normal. Chromatographic analysis of urine from patients with nontropical sprue revealed the presence of an elevation of another indole substance in addition to 5-hydroxyindoleacetic acid. This substance, indole-3-acetic acid (see Fig. 1), may be formed from tryptophan by either of two metabolic pathways. One of these routes involves transamination of tryptophan to form indolepyruvic acid which is then degraded to indole-3-acetic acid. Tryptophan also may undergo decarboxylation to tryptamine which is degraded by monoamine oxidase to indole-3-acetic acid. The normal urinary levels of indole-3-acetic acid range from 5 to 20 mg./24 hours. Although increased levels have been noted in the urine of patients with nontropical sprue, this finding is nonspecific, for similar levels may be noted in steatorrhea associated with pancreatic disease and tropical sprue. However, normal levels have been reported in ulcerative colitis, regional enteritis and in the irritable bowel syndrome. Because sterilization of the intestinal tract substantially reduces urinary excretion of indole-3-acetic acid, it is probable that a change in the intestinal bacterial flora is responsible for much of the alteration in tryptophan metabolism.

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