REVIEW ARTICLE

5-HYDROXYTRYPTAMINE

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UNTIL recently acetylcholine, noradrenaline and histamine have been the only three well-defined substances of natural origin which play an important role by regulating the activity of tissues locally. The role of acetylcholine and noradrenaline is principally recognised as that of chemical transmitter or mediator, although these two substances as well as histamine have additional physiological functions independent of nervous activity. The action of many drugs is beginning to find an explanation in their relation to acetylcholine, noradrenaline and histamine. Now there is a fourth substance, 5-hydroxytryptamine, which can be regarded as a local tissue hormone.

The early developments in our knowledge of 5-hydroxytryptamine (5-HT) are due principally to three groups of workers. Erspamer and his colleagues in Italy, described a substance which they called "enteramine" and which imparted to the argentaffin cells of the intestinal tract their histochemical characteristics. Enteramine was regarded as a specific secretion product of this enterochromaffin cell system. The other two groups, Page and his colleagues at Cleveland and Reid and Rand in Australia, arrived at this substance from a different approach. They were interested in the vasoconstrictor substance which appeared in blood under certain conditions and which they called serotonin. Serotonin was isolated by Page and Rapport and their colleagues, and identified as 5-hydroxytryptamine. It was later agreed that enteramine was the same substance. This interesting work, together with many other valuable contributions, has been the subject for review in 1954[1,2]; and, more recently, by Page, 1958[3]. Recently, the British Pharmacological Society organised a symposium on 5-hydroxytryptamine[4] and at this meeting many research workers in the field discussed their latest findings and views. The main outcome from the symposium was that the study of 5-HT has developed from its distribution, localisation and characterisation to the study of its biosynthesis, its fate (how it is stored, transported and released), and its physiological role in peripheral tissues as well as in the central nervous system.

Biochemical Studies of 5-HT

According to the work of Udenfriend and his colleagues[5–7] the essential amino acid, tryptophan, which is present as a dietary constituent, is the origin of the body's 5-hydroxytryptamine. These workers have shown that in toads tryptophan is converted to 5-hydroxytryptophan (5-HTP)[5]

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although this conversion has not yet been demonstrated in mammals. The decarboxylation of 5-HTP to 5-HT has been demonstrated in a number of tissues by Gaddum and Giarman[8]. Clark, Weissbach and Udenfriend[9] proposed pyridoxal phosphate (Vitamin B_6) as the coenzyme in the conversion of 5-HTP to 5-HT, but it remained for Buxton and Sinclair[10] to provide experimental proof. They showed that the activity of kidney 5-hydroxytryptophan decarboxylase was much lower in rats deficient in vitamin B_6 than in normal rats, and further, that the activity was restored by addition of pyridoxal phosphate.

Amino acid decarboxylases are not the only enzymes affected by pyridoxine deficiency. In animals[11] and man[12] suffering from this nutritional disorder, tryptophan metabolism is deranged and this derangement is increased by a high tryptophan intake.

In rats[13] and mice[14], a vitamin B_6 deficiency causes dermatitis of the peripheral parts of the body particularly in the feet, paws, ears and areas around the mouth. It may be significant that these are the sites in these species where large amounts of 5-HT are normally present in the mast cells of the skin[15].

Another site at which the derangement of tryptophan metabolism leads to symptoms which might involve 5-HT is in the brain. There is evidence[14] that pyridoxine deficiency gives rise to a convulsive disorder which appears to be more prevalent in young infants than in older children or adults. It has been found[16] that the pyridoxine deficient rats showed increased brain excitability measured by the decrease in electroshock threshold, while administration of pyridoxine increased the threshold. The possibility that a low concentration of 5-HT in the brain may lead to excitation will be discussed later.

Phenylketonuria is another condition in which there is a defect in tryptophan metabolism. Sandler,[4] following up the finding of Armstrong and Robinson[17] that abnormal indole metabolites were present in the urine of phenylketonurics, has concluded that in these patients phenylalanine competes with tryptophan for the same limited hydroxylating mechanism, eventually causing a diminished 5-HT production.

In man 5-hydroxyindole formation is not a major pathway of tryptophan metabolism. However, in patients suffering with carcinoid tumour the metabolism of dietary tryptophan is upset and in the tumour 5-HT formation is greatly increased[18]. Sometimes this increase is so great as to interfere with other pathways of tryptophan metabolism with the consequent symptoms of pyridoxine deficiency.

Recently an interesting observation was made on tryptophan metabolism[19] in which 5-HTP as well as 5-HT was found in the urine of a patient with argentiffinoma of kidney origin. It was concluded that large amounts of 5-HTP were formed in the argentaffin cells and that some of it was excreted before it could be metabolised to 5-HT. This is the most direct evidence available that the cells of the enterochromaffin system actually produce 5-HT as well as secrete it internally and, in fact, supports the view that the argentaffin cells constitute a diffuse but true endocrine system.

In man and some other mammals 5-HT conversion to 5-hydroxyindoleacetic acid (HIAA) is the final metabolic step. In some species this is a minor metabolic pathway and the main inactivation of 5-HT awaits elucidation. Monoamine oxidase is probably mainly responsible for the oxidative deamination of 5-HT and its importance in man is evident from the urinary excretion of HIAA[20]. Blaschko[21] has suggested that the primary physiological function of this enzyme may be the breakdown of 5-HT. In fact, monoamine oxidase may be for 5-HT what cholinesterase is for acetylcholine. The analogy may even go further. There may be more than one monoamine oxidase just as there are several cholinesterases. For instance, the monoamine oxidase inhibitor, iproniazid, is a poor inhibitor of amine oxidase in peripheral tissues although a potent inhibitor in the brain[22].

The rate of turnover of a substances is often an indication of its participation in the functional activity of a tissue. Some interesting findings have been made[4] on the rate of turnover of 5-HT. The most rapid turnover of 5-HT occurs in the brain where its half life is 1–2 hours. In the intestine the half life of 5-HT is 1 day and in the platelets 1–2 days, a value which is consistent with the survival time for platelets themselves and suggests that the liberation of 5-HT from platelets normally occurs only on their disintegration. There is a slow turnover rate of 5-HT in carcinoid tumours, where its half life is 5–6 days and on this basis it has been estimated that a carcinoid tumour of 2·8 kg. might contain as much as 3 mg. of 5-HT.

The Carcinoid Syndrome as an Indication of the Functional Significance of 5-HT

If we take the view of Masson[23] and Erspamer[2] that the enterochromaffin cells constitute a diffuse endocrine system designed for the production and storage of 5-HT then an indication of the physiological function of its secretion may be gained from observations on carcinoids, because in this condition there is hyperfunction of this diffuse endocrine organ.

Lembeck[4] has suggested that the two cardinal signs of carcinoid patients, vascular changes and diarrhoea, give an indication of the physiological role of 5-HT. The vascular changes consist of a flush sometimes accompanied by oedema and occasional petechial haemorrhages. 5-HT infused intra-arterially in man produces the same effects[24]. In addition, reserpine, which releases 5-HT (see page 537) causes flushing accompanied by nasal congestion in man[25]. The local vascular effects observed in carcinoid patients are usually not associated with systemic blood pressure effects.

Diarrhoea and increased intestinal motility are seen in most carcinoid patients. 5-HT is known to stimulate isolated smooth muscle and in cats close arterial injections into the coeliac artery of small amounts of 5-HT increase intestinal motility[4]. Injected subcutaneously into rats, 5-HT increases faecal excretion[26]. Lembeck discusses the possibility that the enterochromaffin cells secrete 5-HT to act on the neurons of Meissner's

plexus so that this 5-HT constitutes a physiological stimulus to intestinal motility.

The possibility that 5-HT is involved in the peristaltic reflex has also been considered by Ginzel[27], Kosterlitz and Robinson[28] and by Bülbring and Lin[29]. Ginzel as well as Kosterlitz and Robinson observed that 5-HT blocks the peristaltic reflex of the isolated guinea pig intestine. Bülbring and Lin however, introduced the 5-HT into the lumen of the isolated intestine and found the opposite effect. They observed a lowering of the threshold of the pressure required to elicit peristalsis and showed that the contractions so elicited were more frequent and expelled a larger volume of fluid. Further small amounts of 5-HT appeared in the fluid passing through the lumen of the isolated loop of intestine at rest, but increased two-fold when the intestine became active during peristalsis. When 5-HTP, the precursor of 5-HT, was added to the perfusing fluid a greater conversion of 5-HT occurred during activity. Bülbring and Lin concluded that there is an increased production of 5-HT in the intestinal wall during peristaltic activity, but they did not exclude the possibility that its release of 5-HT occurs as a result of the motor activity, that is by mechanical extrusion rather than by an increased formation. According to their view the increase in the intralumenal pressure releases 5-HT which sensitizes the sensory receptors in the mucosa which trigger the peristaltic reflex.

Action of 5-HT on Peripheral Nerve and Muscle Structures

The conclusion that 5-HT acts on ganglia or nerve endings in the intestine is in accord with a number of recent observations about an action of 5-HT on nerves and ganglia.

Trendelenburg found that it excited the cells of the superior cervical ganglion and that very small amounts of 5-HT potentiate the effects of preganglionic stimulation.[30] No 5-HT has yet been detected in the superior cervical ganglion, but 5-hydroxytryptophan decarboxylase, which is the enzyme that forms 5-HT, is present. The absence of 5-HT may only mean, as suggested by Dalgliesh, that in the ganglion 5-HT exists, in an active form, such as 5:6-dihydroxytryptamine, a substance which is extremely labile. In that case the very fact that it is so labile could explain the difficulty of detecting 5-HT as "active" 5-HT in the superior cervical ganglion. To settle this problem specific metabolic inhibitors are required which prevent its rapid destruction. If 5-HT were to play a role in ganglionic transmission we would have to assume that it is rapidly destroyed near its site of action. By analogy with acetylcholine the "active" form of 5-HT might elude detection without the aid of a potent and specific inhibitor of amine oxidase. However, the only experiments of this type carried out by Gertner, Paasonen and Giarman[31] with the aid of metabolic inhibitors do not support the idea that 5-HT is a ganglionic transmitter. They perfused the cat's superior cervical ganglion with a solution containing monoamine oxidase inhibitor iproniazid and found no 5-HT in the effluent after preganglionic stimulation. However, 5-HT appeared in the effluent after the ganglion had been perfused for 2–3

hours, but the significance of this finding must await further experimental results.

Gaddum and Picarelli[32] in studying the action of 5-HT and its antagonists on smooth muscle and peripheral nervous structures have found that the antagonists lysergic acid diethylamide (LSD), ergot alkaloids and dibenzyline were active 5-HT antagonists on the guinea pig ileum, whereas the drugs like atropine, morphine, and cocaine which did not antagonise the 5-HT response on rat uterus or had only an unspecific action of this kind, were effective antagonists on the guinea pig ileum. These differences may be associated with the fact that the rat uterus is composed only of smooth muscle whereas the guinea pig ileum contains, in addition, a complex nervous system.

It was shown by Gaddum and Picarelli that the response of the guinea pig ileum to 5-HT was reduced to nearly 50 per cent by low concentrations of dibenzyline, but increasing its concentration even 100 times caused no further reduction. The same result was observed when morphine was used as inhibitor for 5-HT. But when morphine and dibenzyline were used together, the response to 5-HT was abolished. These results suggest that each of these two inhibitors acted on a specific type of receptor both of which were stimulated by 5-HT. They have been called D and M receptors. The D receptors are antagonised by large doses of tryptamine, LSD, dibenzyline and gramine, while the M receptors are blocked by morphine, atropine, or cocaine. It seems likely that the D receptors are located in the smooth muscle fibres and are present in both the rat uterus and in the guinea pig ileum while the M receptors form part of a nervous structure, possibly the ganglion cells, and are present in the guinea pig ileum but not in the rat uterus. On this assumption the action of atropine may be due to antagonism of acetylcholine liberated at the endings of nerves stimulated by 5-HT, cocaine may act on the nerve fibre, while morphine probably exerts its antagonistic action by preventing the release of acetylcholine from the excited nerve endings. As morphine was shown by Trendelenburg to block the stimulating action of 5-HT on the superior cervical ganglion it seems likely that in the intestine the same mechanism may hold and that part of the effect is due to stimulation of ganglion cells. Thus if 5-HT plays a part in the physiological regulation of intestinal motility the constipating action of morphine may perhaps be explained in this way. Vane[33] has provided evidence that sympathomimetic amines which do not possess a phenolic hydroxyl group, like mescaline and amphetamine, act upon tryptamine or D receptors in certain smooth muscle preparations. He found that these amines contract the isolated rat stomach like tryptamine and 5-HT whereas sympathomimetic amines having phenolic hydroxyl groups, like, noradrenaline and adrenaline, relax this smooth muscle. Further, the contractions produced by mescaline and amphetamine are abolished by the antagonists of 5-HT, bromo-LSD and dibenamine whereas the relaxations caused by adrenaline and noradrenaline are unaffected by 5-HT antagonists. Another smooth muscle preparation, the guinea pig ileum responds by contracting to mescaline and amphetamine and also to large

doses of adrenaline, but only the contractions caused by mescaline and amphetamine are inhibited by bromo-LSD. Vane has pointed out that the sympathomimetic amines which act upon tryptamine (D) receptors are those which cause stimulation in the central nervous system. The possibility therefore exists of the presence in the central nervous system of tryptamine receptors on which these analeptics act.

Gaddum has drawn attention to the resemblance of the peripheral actions of 5-HT to those of acetylcholine. Both drugs have two receptors. Atropine antagonises the action of acetylcholine on smooth muscle revealing ganglionic effects and LSD antagonises the action of 5-HT on smooth muscle revealing effects which might well be ganglionic. Hexamethonium specifically blocks the ganglionic effects of acetylcholine while morphine is less specific and blocks also the effects of 5-HT.

In addition to effects on autonomic ganglia, 5-HT stimulates another type of nerve structure, the endings of sensory nerves. Armstrong, Dry, Keele and Markham[34] have shown in man that it causes pain when applied to the base of an exposed blister. Although human skin contains only minute amounts of 5-HT which could be liberated and stimulate nocireceptors it is possible that a high concentration in blood may occur in the vicinity of these receptors after the damage of platelets in an injured tissue.

Another type of sensory nerve ending stimulated by 5-HT is found in the chemo- and baroreceptors. Douglas and Toh[35] showed that the hyperpnoeic response to 5-HT was abolished by sinus nerve section. Since then many investigators have found evidence that it stimulates both the chemoreceptors and the baroreceptors. Ginzel and Kottegoda[36] showed in cats that 5-HT injected into the carotid sinus region caused a hyperpnoeic response accompanied by a fall in arterial blood pressure, the latter out-lasting the respiratory stimulation. Both responses were the direct outcome of stimulation of the receptors in the carotid sinus since the depressor response was unchanged when the animal was artificially respired and the respiratory response persisted when a constant pressure apparatus was used to neutralise the fall in blood pressure. Direct evidence of chemoreceptor stimulation was provided by McCubbin, Green, Salmoiraghi and Page[37] who demonstrated that there was an enormous increase in the number of impulses in the carotid sinus nerve when 5-HT was introduced into the carotid artery.

The response of the chemo- and baroreceptors to 5-HT disappeared when small doses were repeatedly injected in quick succession or after administration of a single large dose. Such tachyphylaxis is also encountered with the vascular responses of 5-HT[36] and the question arises, is its action on the sensory endings due to local vascular effects? This question has not yet been settled but it is interesting to note in this connection that Malcolm[4] found under certain experimental conditions that 5-HT depresses cortical responses evoked by stimulation of a sensory nerve and this effect ran parallel to oedema formation which occurred in the brain after the 5-HT. He pointed out that depression of the cortical responses may be a result of the vascular changes which follow intra-arterial injection of 5-HT.

Cardiovascular Effects

As 5-HT can produce both hypertension and hypotension in the same animal, Page and McCubbin[38] have introduced the term "amphibaric" to describe this blood pressure response. Page has given a comprehensive review[3] of the work carried out largely in his own laboratory on the cardiovascular effects of 5-HT. Some of the factors determining its effect on the arterial blood pressure are stimulation of baro- and chemoreceptors, an action on the vessels causing either vasoconstriction or dilatation, or both, as well as inhibition of neurogenic vasoconstriction. There is considerable species difference in the blood pressure response to 5-HT depending on which of the above factors plays the greater role.

The blood pressure response to 5-HT appears to depend largely on the vasomotor tone. When this is low, as for example after ganglionic blockade or destruction of spinal cord, the response is pressor, whereas when the vasomotor tone is high, for example, after section of buffer nerves, the response is depressor. Page has conceived the idea of 5-HT as a humoral antagonist to neurogenic vasomotor tone and speaks of a "chemical buffering system".

Erspamer[39] concluded that the antidiuresis caused by 5-HT was due to a preferential vasoconstriction of the afferent glomerular arterioles and that it was a hormone designed for the physiological regulation of renal function. But Abrahams and Pickford[40] studying its effect in the dog concluded that the antidiuresis was due to its action on the vascular system in general and not on the kidney vessels in particular. They found that antidiuresis occurred only when 5-HT raised the systemic blood pressure and that injected arterially into the kidneys a much larger dose was required to cause antidiuresis than on intravenous injection. 5-HT was even more effective in causing antidiuresis in rats when given by the subcutaneous route than when injected intravenously. Since in rats it produces a local oedema on subcutaneous injection, retention of fluid produced in this way may account for at least part of the strong antidiuretic effect seen under these conditions.

The Role of 5-HT in Anaphylaxis

The local oedema formation in rats at the site of a subcutaneous injection of 5-HT was first observed by Rowley and Benditt[41] and has been confirmed by Parratt and West[42]. It is known that in rat skin, oedema is a characteristic feature of the effects of histamine liberators, substances which produce their effects by disrupting mast cells and releasing the histamine contained in them. However, Benditt, Wong, Arose and Roeper[43] showed that the mast cells of rat contain not only histamine but 5-HT as well, and Bhattacharya and Lewis[44] have shown that 5-HT is released by histamine liberators. The oedema produced in rats by histamine liberators is in fact mainly the effect of the released 5-HT. This view is supported by the finding that after reserpine which depletes the mast cells of their 5-HT leaving their histamine intact[45], histamine releasers or, better, mast cell depleters, cause little or no oedema.

It seems likely that 5-HT plays a role in anaphylactic shock in rats because Lewis and Mota[46] found that 5-HT as well as histamine was released into the blood stream of rats sensitised with horse serum, after injection of antigen. However, no positive evidence has so far been obtained in perfusion experiments. When the antigen was injected into the hind-quarters of sensitised rats perfused with Locke's solution, neither 5-HT nor histamine appeared in the venous effluent.

5-HT is present in the mast cells of the skin of only mice and rats[47]. It seems possible, as suggested by Feldberg, that in the rat in particular, it takes over some of the functions which histamine has in other species. Certain human allergic states resemble the reaction in the rat and although normal skin does not contain appreciable amounts of 5-HT there is a possibility that the 5-HT content is increased in such conditions. There is no experimental evidence in favour of this view; in fact, West has observed that in a case of urticaria pigmentosa which is due to an increase of mast cells in the skin, the 5-HT content of the skin was not increased although the histamine content was raised considerably.

It is also not possible to explain the anaphylactic contractions of the histamine insensitive rat uterus by 5-HT because Brocklehurst[4] showed that 5-HT antagonists do not affect these contractions. Further, he showed that 5-HT does not contract but relaxes the medium sized bronchioles which are mainly responsible for the bronchospasm in human asthma. It therefore seems unlikely that it plays a role in these conditions.

In the guinea pig 5-HT causes a shock syndrome characterised by vascular collapse and bronchospasm. There is a rapid tachyphylaxis and it is interesting that during the period when the guinea pigs are insensitive to 5-HT, antigen does not produce an anaphylactic shock[48]. However, there is no evidence that 5-HT plays a role in anaphylaxis in the guinea pig, and 5-HT antagonists have no effect on the course of anaphylactic shock.

Humphrey and Jacques[49] found that 5-HT is released from platelets when antigen is added to blood from sensitised rabbits and this has been confirmed *in vivo*[50]. In spite of the fact that rabbit mast cells do not contain 5-HT[44] it is still possible that it plays some role in anaphylaxis in this species.

Transport and Release of 5-HT

Page has stressed the importance of the chronic effects exerted by the continuous presence of minute amounts of 5-HT in the blood although there is no evidence of free 5-HT in plasma, all that in plasma probably being bound to platelets[51]. It seems possible that there is a continual release of 5-HT into the circulation and that this excess is taken up at once by the platelets which seem to control the amount available in plasma and its transport in the blood stream. The degree of absorption in plasma varies considerably in individual subjects and although patients with blood diseases often show a low 5-HT content in the platelets, there is no clear relationship between any pathological condition and degree of absorption

of 5-HT by the platelets. Born, Ingram and Stacey[52] found a proportionality between the concentrations of adenosine triphosphate (ATP) and 5-HT in platelets and discussed the possibility that ATP plays a role in the binding of 5-HT. Further, blood clotting leads to the disappearance of ATP as well as 5-HT from platelets[53]. But there are at least two conditions where there is no relation between the release of 5-HT and of ATP from platelets. Firstly, Hardisty and Stacey[54] found that in a case of myeloid leukaemia, a deficiency in platelet 5-HT was not accompanied by a corresponding deficiency in ATP. Secondly, reserpine greatly reduces the platelet 5-HT but does not alter the platelet ATP.

There is another more likely mechanism by which ATP may be involved with 5-HT in platelets. ATP may provide the energy for an active transport mechanism for concentrating 5-HT in platelets. Brodie and Shore[55] explained the action of reserpine in releasing 5-HT from platelets by this mechanism. Reserpine is known not only to release the 5-HT from platelets but also to prevent its accumulation in platelets. It is possible that it acts by upsetting this active transport system allowing the 5-HT present in the platelets to diffuse out and prevent further concentration until the transport mechanism is replenished.

Reserpine and several other rauwolfia alkaloids release the 5-HT not only from platelets and rat mast cells, but they also deplete the stores in the intestinal tract and brain[56–58]. At all these sites 5-HT is released, probably destroyed by monoamine oxidase as soon as it is freed, and the tissue remains depleted of it for many hours after reserpine has disappeared from the body. As in the case of the platelets, reserpine prevents the binding of 5-HT at its normal sites in the brain and intestine.

Brodie has developed the thesis that reserpine alkaloids exert their sedative effect by releasing 5-HT in the brain. Part of this evidence is derived from experiments with the reserpine alkaloid, rescinnamine, which provides a good example of the relationship between the intensity of the pharmacological effects and 5-HT release. This alkaloid induces slight sedation in the mouse where it causes only a small release of 5-HT, but in the rabbit where it causes marked sedation it causes a large release of 5-HT.

The rauwolfia alkaloids do not specifically release 5-HT but other substances as well. From the platelets, reserpine releases histamine, although it does not do so from the mast cells of rats[45]. On the other hand, reserpine releases catechol amines in most species[59–62], for instance, it releases the noradrenaline and adrenaline from the brain, the suprarenal glands and from sympathetic adrenergic neurones. It is possible that the effect on the suprarenals is central in origin since the depletion of the amines does not occur when reserpine is given after the glands have been denervated.

Role of 5-HT in the Brain

There are a number of papers which deal with the problem of explaining the central pharmacological actions of reserpine by release of 5-HT. The problem, however, becomes even more complex by the knowledge that the catechol amines are also released in the brain.

One method of studying the sedative action of reserpine in animals developed from the finding that it lengthened the barbiturate sleeping time. It is interesting that large amounts of 5-HT also increase the barbiturate sleeping time, since with these injections some of the 5-HT gets into the brain. These results are in favour of the view that the sedation following reserpine is due to the released 5-HT, although there are many drugs which lengthen barbiturate sleeping time[63].

Marazzi and Hart[64] reported that minute doses of 5-HT inhibited central synaptic transmission. They evoked activity in the cat cortex by transcallosal stimulation of the optical cortex and found that after intracarotid injection of 5-HT the response was greatly reduced. However, this appears to be a non-specific effect as they found the same effect with small doses of LSD, mescaline, bufotenine, adrenaline, noradrenaline and adrenchrome. Malcolm[4] also used intracarotid injections of 5-HT in cats and found that these injections inhibited the responses evoked by somatic sensory nerve stimulation only if large doses (200–300 μg. as a 1/1000 solution) were given.

Although 5-HT does not readily pass the blood brain barrier its precursor 5-HTP does so, and in this way the 5-HT content of the brain increases up to tenfold after administration of large doses of the precursor[6]. A further increase occurs when iproniazid, a potent inhibitor of amine oxidase is first administered. These facts have been used in a recent investigation by Lewis and Malcolm[65] in an attempt to throw some light on the role played by 5-HT in the central nervous system. They found that when the 5-HT level is raised by giving a large dose of 5-HTP (30 mg./kg.) the electrical responses evoked by cutaneous and muscle afferents in all areas of the brain explored are depressed. The responses were restored to normal by injection of reserpine. After previous iproniazid treatment 5-HTP caused a much greater depression which usually resulted in complete depression of the whole central nervous system and death. The depression in the latter case could not be reversed with reserpine. 5-HTP, iproniazid and reserpine produced a marked fall in blood pressure. They ruled out the possibility that hypotension was responsible for the central depression, since the effect also occurred when the blood pressure was kept constant by an automatic compensator.

These results showed that an increase in the level of "bound" 5-HT in the brain results in depression of central activity, but when this "bound" 5-HT is lowered by reserpine the activity returns to normal. This suggests that it is necessary for 5-HT to be present in a "bound" form to exert its central effects.

The sedation caused by reserpine in the anaesthetised animal therefore may not be due to its action in releasing 5-HT but to some other action, for instance, the release of catechol amines. Bogdanski, Weissbach and Udenfriend[66] reported that when the brain 5-HT is raised by administration of 5-HTP to unanesthetised cats, there was marked central excitation. This has not been confirmed in the experiments of Lewis and Malcolm, the only sign of excitation was some respiratory stimulation. The usual effects were slight depression after 5-HTP and after iproniazid the cats

showed a tendency to lie down until disturbed. The effects were similar to those reported by Feldberg and Sherwood[67] after injection of 5-HT into the cerebral ventricles of cats. Lewis and Malcolm found that there was never any sign of sham rage in cats after 5-HTP (30 mg./kg.) as reported by Udenfriend and his colleagues[66] or after iproniazid 100 mg./kg. followed by 5-HTP 30 mg./kg.

As the changes in electrical activity occurred in many areas of the brain and not particularly in those areas with a high 5-HT content, it was possible that the distribution of brain 5-HT was related to a cell type rather than to general anatomical areas. An attempt to determine its distribution at a cellular level by injecting ^{14}C labelled 5-HTP (0·1 mc./kg.) and examining the distribution on autoradiographs did not give very clear autoradiographs as the dose employed was insufficient. However, it indicated that 5-HT is distributed fairly evenly throughout the brain and is not related to nervous elements. The possibility exists that 5-HT is contained in the connective tissue cells—the glial cells—and its function is an action on the blood vessels around which glial cells are concentrated.

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