NEUROPHARMACOLOGY OF 5-HYDROXYTRYPTAMINE

By

K. P. BHARGAVA

(From the Department of Pharmacology, K. G. Medical College, Lucknow University)

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The vasoconstrictor principle liberated from the disintegration of platelets in the serum was isolated by Rapport, Green and Page (1947). They suggested that it was closely related to tryptamine and the name 'Serotonin' was given because of its origin and its tensing effect on the smooth muscle. Rapport in 1949 identified the final structure of 'Serotonin' to be 5-hydroxytryptamine complexed with creatinine and sulfuric acid. Hamlin and Fisher 1951) first synthesized the material. Page (1952) showed that the synthetic material was pharmacologically identical with the naturally occuring vasoconstrictor.

Erspamer and his associates, in Italy, working with the enterochromaffin or argentophile cells of gastro-intestinal mucosa of various mammals and salivary glands of mollusks isolated a substance which was very active on isolated intestine and estrous-uterus after atropinization. They published several reports on the distribution, and histochemical properties of a number of indolalkylamines obtained from different biological sources (see Erspamer, 1954a & 1954c). The active substance of the enterochromaffin cells was named "Enteramine". In 1952, Erspamer and Asero found the enteramine to be identical with 5-hydroxytryptamine. As the "Serotonin" of Page and the "Enteramine" of Erspamer have been found to be identical with 5-hydroxytryptamine, it is suggested that the chemical name 5-hydroxytryptamine, abbreviated as 5-HT, be used for either of the names "Serotonin" or "Enteramine".

Excellent reviews published in 1954 by Page and by Erspamer dealing with the various physiological and pharmacological aspects of 5-hydroxytryptamine, have aroused considerable interest in this physiologically occuring substance. The precise significance of the "hormone" has however yet to be established. At least four principal roles, each one virtually independent of the other, have been attributed to 5-HT : one school views 5-HT as a factor influencing hemostasis, another considers it to control vascular tone and therefore the systemic blood pressure, a third regards it a hormone participating in the regulation of renal function, the last attributes to 5-HT an important role in maintaining normal mental processes.

It is the purpose of this review to present the data obtained chiefly in the last three years concerning the role of 5-HT in mental processes. The study

of 5-HT in brain mechanisms has opened a new era. The hallucinogens and the tranquilizers have been partners of 5-HT in the knowledge which has recently been gathered.

DISTRIBUTION

5-HT has been found in chromaffin cells of gastro-intestinal tract (Erspamer 1954), the blood platelets (Rapport, 1949), spleen (Erspamer, 1954) brain of mammals (Twarog and Page, 1953) and ganglia of invertebrates (Florey and Florey, 1953).

The identification and quantitative estimation of the substance in various biological materials has been accomplished by means of its chemical isolation by color reactions, paper chromatography, spectrophoto-fluorimetric procedures, countercurrent distribution studies and pharmacological bioassay methods (Udenfriend *et al.*, 1955 b).

FUNCTIONAL SIGNIFICANCE

Like histamine, there is as yet no clear evidence about the physiological role of 5-HT. The oldest hypothesis assigns a hemostatic role to 5-HT (O'Connor, 1912 and Zucker and Stewart, 1913). The role has been suggested due to release of 5-HT from disintegrating platelets during the process of blood clotting. The vasoconstricting action of 5-HT on neighbouring vessels may help to restrict bleeding until a clot is formed. Fenichel and Seegers (1955) have shown that in bovine plasma 5-HT is probably the most important constituent of platelets concerned with clot retraction. The following points need to be considered before assigning the hemostatic function to 5-HT. It is doubtful if the quantities of 5-HT released at the moment of blood coagulation in any animal species are ever sufficient to constrict the injured vessels. Widely different sensitivity to 5-HT is seen in various vascular areas of the same animal. This is not in accordance with the general and ubiquitous character which hemostatic action of 5-HT ought to have (Brun, 1948 ; 1949). Post-traumatic vasoconstriction occurs even when hemostatic plug formation is prevented by heparin (Huges, 1953). Page and McCubbin (1953) attribute to 5-HT the main function of depressing, through a peripheral mechanism, the neurogenic vascular tone, thus provoking a predominent vasodilatation. This makes 5-HT antihemostatic rather than haemostatic.

Page *et al.*, (1953 a) have recently postulated a physiological regulatory function of 5-HT on neurogenic vasoconstriction. They postulate two opposite actions of 5-HT in the control of arteriolar tone : one a positive spasmogenic action, due to direct stimulation of vascular smooth muscle by pharmacological doses of 5-HT and the other a negative spasmolytic action, which may be attributed to inhibition of the neurogenic vasocontrictor tone, as a consequence of a possible prevention of the release of adrenergic mediators at sympathetic endings in physiological concentrations. In other words, 5-HT is

functionally a "Serohypotonin" not "Serotonin". Objections to this hypothesis are : the failure of the sympatholytic drugs to abolish 5-HT hypotension; the sudden massive release into the plasma of the entire platelet 5-HT of a dog (7.3μg-/kg.)can cause nothing but insignificant and brief pressure change (Spies and Stone, 1952) ; furthermore, there is probably no free 5-HT in circulating plasma (Twarog and Page, 1953; Toh, 1954). Therefore, it appears unlikely that 5-HT plays any part in controlling vascular tone or in regulating the normal tone of smooth muscle elsewhere (Robson and Keele, 1956).

Erspamer and his coworkers (Erspamer and Ottolenghi, 1950 ; 1951 ; 1952 ; 1953 and Erspamer, 1954) have suggested that 5-HT controls renal haemodynamics. They find a renal action of 5-HT in physiological doses of the drug. These doses of 5-HT have no effect on other body functions. Subcutaneous administration of rat serum causes in the rat an evident antidiuretic action (Ginsburg and Heller, 1951 ; and Erspamer and Ottolenghi, 1952). This stable antidiuretic substance, 'Stable ADS', has been indentified as 5-HT (Erspamer and Sala, 1954). Plasma contains no active 5-HT (Twarog and Page, 1953 ; Toh, 1954) hence this action cannot be regarded as physiological unless the renal vessels can remove 5-HT from circulating platelets. Page (1954) believes that the antidiuretic effect can result from the hypotension and is not due to selective renal vasoconstriction.

Since 5-HT is present in the enterochromaffin cells of the intestinal mucosa it has been suggested that 5-HT might exert some influence on glandular activity of the gut (Feldberg and Toh, 1953) but nothing is definitely established about this.

Lembeck (1953) has shown that intestinal carcinoid tumours are rich in 5-HT. The syndrome of argentaffinoma, characterized by pulmonary stenosis, dyspnoea, cynosis and attacks of flushing is associated with "hyperserotinaemia" and presence of another unidentified substance (which may be histamine) in the blood. Of the sixteen cases of presumptive "hyperserotinaemia" none showed evidence of neural disorder or changes in renal function (Editorials, 1954). Pernow and Waldenstrom (1954) attribute some of the changes in the syndrome to histamine liberation. The serum and urine concentration of 5-HT, assayed on isolated rat colon was found to be 12 and 15 μg./ml. respectively i.e. 100 times the normal (Pernow and Waldenstrom, 1954). The estimation of 5-hydroxyindoleacetic acid (5-HIAA), major excretion product of 5-HT, in urine, is a useful diagnostic measure (Udenfriend *et al.*, 1955a). Studies with C^{14} labelled tryptophan administered to a patient of malignant carcinoid tumor, have shown the major metabolic pathway of tryptophan to be through 5-HT to 5-HIAA (Sjoerdsma and Udenfriend, 1955).

Certain reports (Schneider and Yonkman, 1953; McCubbin *et al.*, 1956; Douglas and Ritchie, 1957); indicate that 5-HT excites afferent receptor sites in the thorax and abdomen. Liberation of 5-HT from the disruption of platelets during clotting could produce reflex effects on circulation and respiration in conditions like pulmonary embolism and myocardial infarction by stimulating afferent nerve endings and thus reflex bronchopasm, bradycardia and fall in blood pressure might result (Comroe, 1952; Camroe *et al.*, 1953; Page 1954).

Armstrong *et al.* (1952a, 1952b & 1953) found that 5-HT can produce prolonged pain when applied to the exposed base of a cantharidin blister in human skin. High concentration of 5-HT in wasp venom recorded by Jaques and Schachter (1954) may account in part for the pain of a wasp sting. Collier and Chesher (1956) have identified 5-HT in the nettle (Urtica dioica). Humphery and Jaques (1953) and Waalkes *et al.*, (1957) have shown that an antigen-antibody reaction in rabbit-blood in vitro releases both histamine and 5-HT. Benditt *et al.*, (1955) and Sjoerdsma *et al*, (1957) have identified 5-HT and histamine in mast cells. Langunoff *et al.*, (1957) have shown that mast cells can form 5-HT from 5-HTP. Some protection against anaphylaxis by LSD, an antagonist of 5-HT, has been shown by Pallotta and Ward (1957). The exact implications of these observations are not yet understood but 5-HT may be responsible for some of the features of anaphylactic shock (Weissbach *et al.*, 1957).

Normally 5-HT does not occur free in spinal fluid but Sachs (1957) has found it in the spinal fluid of patients with head injuries and brain tumors.

The first suggestion that 5-HT acted on autonomic ganglia was made by Gaddum and Hameed (1954) who obtained evidence for the view that this substance acted on tryptamine receptors present in the nervous system of the ileum of the guinea pig. Paasonen and Vogt (1956) using the mollusk heart which is insensitive to epinephrine or norepinephrine, have not been able to detect 5-HT in the isolated ganglia. 5-HT was not found in the extracts of sympathetic ganglia even when steps were taken to exclude the presence of epinephrine in the extracts tested, but this tissue contained large amounts of 5-HT decarboxylase, the 5-HT synthesizing enzyme (Gaddum and Giarman, 1956). This positive result suggests that 5-HT may play a physiological role in this tissue. It may have a sensitizing action on the ganglia as observed by Trendelenburg (1956). Trendelenburg (1956) further provided evidence for the presence of tryptamine receptors, in addition to receptors for acetylcholine and histamine, in the superior cervical sympathetic ganglion of cat. Welsh (1957) showed the existence of 5-HT in the ganglia of certain species by testing the ganglion extract on the heart of *Venus mercenaria*, a clam. Presence of 5-HT in the ganglion

of *Venus mercenaria* has now been established by chromatographic technic (Welsh, 1957) as well as on the highly sensitive atropinized, estrous uterus of the rat according to the method of Erspamer (1952) and Gaddum and Hameed (1954). Very recently however, Gerterner, Paasonen and Giarman (1957) have detected the apparent production of 5-HT in the sympathetic ganglia in situ. The presence of 5-HT was uninfluenced by electrical stimulation of preganglionic nerve and bore no relationship to the contraction of nictitating membrane caused by such stimulation. 5-HT has been found in *Fasciola Hepatica* and a possible role in transmission of nerve impulse in the fluke has been indicated (Mansour, 1956; Mansour *et al.*, 1957). The role of 5-HT in the neurohumoral transmission is open to question.

Stimulation of the central cut end of vagus in dogs is known to release a pressor substance from the brain as demonstrated by Taylor, Page and Corcoran (1951) in cross-circulation experiments. Since this substance was not identified with epinephrine, norepinephrine, or angiotonin, and its pressor effects were antagonized by 1-hydrazinophthalazine, these workers felt that this substance might be 5-HT. However, there was no direct proof for its identity.

The role of 5-HT in mental processes is pertinent to this review and will be discussed in detail.

ROLE OF 5-HT IN CENTRAL NERVOUS SYSTEM

If 5-HT is assigned a role in mental processes the following points must be considered. Firstly, the presence of 5-HT must be shown in the brain and secondly, it should be demonstrated that 5-HT affects the functions of the brain. Next it should be determined whether or not 5-HT has a function in the nervous system and then what is the functional nature of the hormone. Lastly, can the mechanism of the action of drugs affecting the brain functions be satisfactorily explained on the basis of the metabolite?

Identification of 5-HT in the brain.

The presence of 5-HT in the brain was first reported by Twarog and Page (1953). Amin, Crawford and Gaddum (1954) using the rat uterus as assay method reported on the quantitative localization of 5-HT in different parts of the dog's brain. They differentiated it from substance P which also occurs in similar situations, but has no effect on the uterus. The distribution of nor-adrenaline was shown to closely correspond with the distribution of 5-HT in the brain. The 5-HT in the brain is not randomly distributed but is localised in definite areas. It is not found in medulated nerve fibres, but is present in grey matter. The highest concentration is in tissues associated with the central autonomic representation such as the hypothalamus and medulla and in the area postrema.

Bogdanski and Udenfriend (1956) and Bogdanski *et al.*, (1956) have largely confirmed the findings of Amin *et al.*, (1954) regarding the distribution of 5-HT in the brain. These workers have shown that the enzyme 5-hydroxytryptophan decarboxylase, which biosynthesises 5-HT, is present in parallel amounts in regions rich in 5-HT. In addition, the correlation is also held in those areas where 5-HT levels are low or nonexistant as in the cerebellum and in the cerebral cortex (Gaddum and Giarman, 1956). However, anomalous situations have been found in the sympathetic ganglia and in the area postrema. In the former there is a high degree of 5-HT synthesizing capacity (Giarman, 1956) but neither Paasenon and Vogt (1956) nor Gaddum and his coworkers (Gaddum and Giarman, 1956) have been able to detect 5-HT in this tissue. On the other hand, the area postrema contains relatively high levels of 5-HT, but no enzyme for synthesizing it. Presence of chemoreceptor for vomiting in the area postrema, demonstrated by Borison and Wang (1949) may account for the high concentration of 5-HT in this region. The 5-HT is probably not produced locally in this area but reaches it from the blood stream or cerebrospinal fluid.

Biochemical aspects of brain 5-HT

Although, in the intact animal, tryptophan is converted into 5-HT, it is not known in which tissue or tissues the hydroxylation occurs. There is no evidence yet that brain can hydroxylate tryptophan to 5-hydroxytryptophan (5-HTP). However, fixation of 5-HT by brain mitochondria has been demonstrated by Walaszek and Abood (1957) who consider that the actual binding sites of 5-HT exist within the mitochondria.

The 5-HTP can penetrate into the brain from systemic circulation and can raise brain 5-HT level significantly in dogs, rabbits, rats and mice (Bogdanski *et al.*, 1956 and Udenfriend *et al*, 1956). 5-HT formation from 5-HTP has been shown to occur in mast cells (Lagunoff *et al.*, 1957), The ability of 5-HTP to raise the 5-HT level in the brain, makes it a useful pharmacological tool, since 5-HT does not readily penetrate the brain when injected into the systemic circulation. In mice, however, parenterally administered 5-HT produces a rise in brain 5-HT level with high doses (Shore *et al.*, 1957). For conversion of 5-HT to 5-HTP, the enzyme 5-HTP-decarboxylase requires a coenzyme pyridoxal phosphate (Clark, Weissbach and Udenfriend, 1945). In pyridoxine (Vitamin B_6) deficiency in chicks the 5-HT level in brain was reduced to 30% of normal. The amount of 5-HT in blood and other tissues was reduced even more. Thus, the symptoms of pyridoxine deficiency may in part be due to deficiency of 5-HT (Udenfriend, 1957). However, pyridoxal phosphate is involved in several metabolic pathways including those of epinephrine, norepinephrine and histamine and hence the deficiency of 5-HT alone cannot explain the symptoms of pyridoxine deficiency.

Monoamine oxidase, the enzyme which metabolizes 5-HT, is widely distributed in the brain. It has been suggested by Udenfriend (1957) that

monoamine oxidase regulates the action of 5-HT. In the body, 5-HT is metabolised to 5-hydroxyindole acetic acid (5-HIAA) (Hess, Shore and Brodie, 1956). The 5-HIAA is excreted in the urine and can be estimated chemically. Isopropylisonicotinyl hydrazide (Iproniazid) is a potent inhibitor of enzyme monoamine oxidase in tissue slices and in vivo (Zeller and Barsky, 1952; Zeller *et al.*, 1955). In addition to blocking 5-HT metabolism the enzyme inhibitor, iproniazid is known to interact with pyridine nucleotides, and cause deplection of pyridoxine, making it unsuitable for in vivo studies (Udenfriend, 1956). Iproniazid is an efficient inhibitor of overall 5-HT destruction by monoamine oxidase and is quite active in preventing 5-HT metabolism in certain areas of the brain (Weissbach *et al* , 1957 a).

Work with radioactive C^{14} labelled 5-hydroxytryptophan reported to be in progress, suggests that the 5-HT turnover in the brain is more rapid than in the intestine (Udenfriend, 1957).

Functional aspects of brain 5-HT

Undoubtedly, Gaddum (1953) was the first to demonstrate an antagonism of 5-HT by lysergic acid diethylamide (LSD) on the rat uterus. In 1943, A. Hofmann of Sandoz in Basel, Switzerland, noticed the peculiar mental effects of LSD which he had inadvertantly inhaled while he was engaged in its preparation in the laboratory. The 5-HT antagonism and the psychic effects which LSD produces led Gaddum (1954) to suggest, "it is possible that 5-HT in our brains plays an essential part in keeping us sane and that this effect of LSD is due to its inhibitory action on the 5-HT in the brain". The systematic preparation and testing of the antimetabolites of 5-HT by Woolley and Shaw (1954a, 1954b, 1957) also led to the recognition of 5-HT as a "hormone" concerned in the regulation of mental processes. Some antagonists of 5-HT, tested peripherally on tissue (s) cause mental aberrations in man and laboratory animals (Woolley and Shaw, 1954 b).

Thompson and Webster (1955) have shown LSD to be a powerful inhibitor of pseudocholinesterase of human serum and brain. Pseudocholinesterase is located not in the neurones, but in the glial and schwann cells (Cavanagh *et al.*, 1954). Recently, Fried and Antopol (1957) have shown that LSD and 5-HT in low concentrations augments the cholinesterase activity and this potentiation effect has been suggested to be of greater significance than the inhibition with high concentrations, also noted by these workers. The relevance of these findings to the actions of LSD is not yet clear.

Marrazzi and Hart (1955) showed 5-HT to be a powerful inhibitor of central synaptic transmission in the transcallosal fibres. Here, the 5-HT concentration normally is quite low and hence the effect demonstrated by these workers may be a pharmacological action and may not be a physiological one. These workers see mental disturbance as an imbalance between adrenergic or

"Serotinergic" inhibition and cholinergic excitation in the more susceptible cerebral synapses.

An interesting observation on isolated system related to the functions of 5-HT in the brain is that of Benitz *et al.* (1955a,b). The brain cells, oligodendroglia are characterized by a pulsating rhythmical movement. These cells slowly contract and expand. 5-HT has been shown to strongly contract the oilgodendroglia in tissue cultures of human fetal brain. Antagonism by LSD has also been reported on this system. Very recently, similar finding on adult cortical brain cells in tissue culture have been reported by Geiger (1957). 5-HT in concentration of 0.5-2.0 μg./ml. induced repetitive "pumping movement" of neurones hitherto not seen under other conditions. LSD in 0.002-0.001 μ/ml. inhibited the glial movements.

From observations on brain tissue cultures, Woolley and Shaw (1957) suggest a possible explanation of the role of 5-HT in mental processes. The brain is a poorly vascularized tissue in comparison to the kidney and the oligodendroglia are thought to be miniature stirring devices designed to facilitate the circulation of extravascular fluid. In this way the metabolic exchanges of oxygen and nutrients and of waste products is facilitated. An excess of 5-HT could stop these 'stirrers' by causing a tetanic contraction as observed in tissue cultures. Furthermore, if the production and destruction of 5-HT occurs in a rhythmic cycle, then a failure to produce enough 5-HT, i.e. a metabolic deficiency of 5-HT, could slow down the stirring movement and consequently, the metabolism of the brain cells. An antimetabolite of 5-HT would accomplish the same end by bringing about a deficiency. It is well known that hallucinations and other central effects such as convulsions are caused by anoxia and by hypoglycemia. The deficiency of oxygen supply and nutrients may be brought about in the brain by poor circulation in the extravascular fluid which seems to be regulated by the 'stirring movements' of the oligodendroglia. However, no correlation was found to exist between depression of evoked cortical potentials and oxygen tension lowering (due to cerebral vasoconstriction) following the administration of 5-HT (Distefano *et al.*, 1956).

PERIPHERAL ANTAGONISTS OF 5-HT

In drawing conclusions for the central effects of 5-HT from studies on peripheral antagonism the following facts must be realised.

(1) Response to 5-HT is variable depending upon the experimental set up, the organ or tissue in question, the dosage employed, the anesthetic used and most important of all, the species of animal taken. This variability is particularly evident in the vasomotor responses to 5-HT (Schneider, 1953; Schneider and Yonkman, 1953; Erspamer, 1954; Page, 1954; Weidman and Cerletti, 1957).

(2) 5-HT has a polyvalent mechanism of action especially in the vascular reactions (Meier *et al.*, 1957) but in general it is a smooth muscle stimulating agent (Freyberger *et al.*, 1952 and Abrahams and Pickford, 1956a and 1956b).

(3) Studies on peripheral antagonism done in intact animals or even in perfused isolated systems *in vivo* are subject to a number of variables; whereas, studies on isolated tissues *in vitro* have the advantage of being simpler systems.

(4) Antagonists of 5-HT may be *'functional'* or *'specific'*, the latter are supposed to act on the same receptor site as 5-HT and are competitive in nature, i e., reversal of effects can occur.

(5) A peripheral antagonist must reach the central nervous system where also an antagonism should be demonstrable.

Woolley and Shaw (1952; 1953) in an attempt to find anti-5-HT substances synthesized a large series of compounds structurally similar to 5-HT. Their main attempts at first was to find a hypotensive anti-5-HT substance which could be of use in therapy, since it was postulated that the essential hypertension may be due to excessive circulation of the "serotonin" (5-HT) (Woolley and Shaw, 1952; Page, 1954). Woolley and Shaw (1953) showed that 2-methyl-3-ethyl nitroindole a congener of 5-HT prevented the pressor response of 5-HT if fed before the injection of 5-HT in dogs. This finding was not confirmed by Page and McCubbin (1953b) and Page (1954) in normal and neurogenic hypertensive dogs although the sample of the drug was supplied by Woolley and Shaw. Spies and Stone (1952) also failed to change arterial pressure in a hypertensive patient given 33 gm. of antagonist orally for 8 days. Given in this manner, it did not block the action of intravenous 5-HT.

Recently, Shaw and Woolley (1956) and Woolley and Shaw (1957) have synthesized another new and potent anti-5-HT substance capable of overcoming the pressor action of 5-HT in dogs. This new analog, 1-benzyl-2, 5-dimethyl serotonin, abbreviated as BAS, is said to have no mental effects in man since it does not penetrate into the brain. The drug causes 'LSD-effect' in mice if it is introduced directly into the brain. More work on this 5-HT antagonist is expected to be reported soon. However, a hypotensive action of BAS need not be solely due to its antagonism of 5-HT.

Salmoïraghi *et al.*, (1957) reported the effects of LSD and 2-brom-LSD on cardiovascular responses to 5-HT in rats, cats and dogs. Both agents effectively antagonised the pressor-depressor responses to 5-HT in rats. The antagonism was weak and inconsistent in cats and dogs. The antagonism has been shown to be clearly dependent on the species. LSD or brom-LSD

given chronically by mouth had no effect on arterial pressures of chronic renal or neurogenic hypertensive dogs.

Rosell *et al.*, (1957) in Sweden have studied the effects of a number of phenyl and indole derivatives structurally related to 5-HT on respiration and circulation in anesthetized cats. Some of these compounds had no effect on blood pressure and respiration. An attempt to correlate the chemical structures with activity has been made.

A large series of pharmacological agents have been shown to antagonise the effects of 5-HT on perfused isolated vessels of the rear extremities of rabbit (Meier *et al.*, 1957). Drugs which antagonised 5-HT were several adrenergic blocking agents (dihydroergotamine, 'Hydergin', 'Regitin'), antihistaminics (pyribenzamine, promethzine), and a large group which was neither. The latter group included, LSD, caramiphen, chlorpromazine, 'Apresoline', papaverine etc. Unfortunately, no conclusions regarding the central 5-HT antagonism can be drawn from such a study. The only fact which is apparent is that widely divergent groups of drugs can antagonise 5-HT without probably interfering with the natural mechanisms which may be mediated via 5-HT.

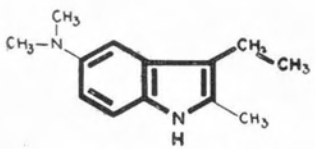
Gaddum and Hameed (1954) have studied the drugs which antagonise 5-HT on three different tissues which are very sensitive to 5-HT, viz. the rat's uterus (Erspamer, 1954b; Gaddum, 1954); the guineapig's ileum (Gaddum, 1953a; Robertson, 1953) and the rabitt's perfused ear (Rapport, Green and Page, 1948). Tests for specificity were carried out by comparing the effect of 5-HT with that of a suitable dose of another drug before and after the addition of the antagonist. 5-HT has been shown to act on tryptamine receptors which are different from the receptors for acetylcholine or histamine (Gaddum, 1953a) in the guinea pig ileum. Similar results have sometimes been obtained in the rat's uterus but it has not been possible to show the presence of the tryptamine receptors in the rabbit's ear. With mepyramine, piperoxane and atropine the effects of histamine, adrenaline, and acetylcholine respectively were abolished without altering the effects of 5-HT. These workers confirmed the results of others (Gaddum, 1953b; Fingl and Gaddum, 1953; Woolley and Shaw, 1953; Page and McCubbin, 1953), that LSD is very active and specific antagonist of 5-HT in experiments on the rat's uterus or rabbit's ear but had little effect on the guinea pig's ileum even when high concentrations were used. The relation between LSD and 5-HT was suggested to be similar to the relation between acetylcholine and atropine. Several other derivatives of ergot have been tested for the antagonism against 5-HT on the rat-uterus (Gaddum and Hameed, 1954; Shaw and Woolley, 1954). The derivatives of lysergic acid, other than LSD, have also been examined for their antagonism to 5-HT (Cerletti and Rothlin 1955; Savini, 1956; Rothlin, 1957).

The most interesting of these compounds is 2-brom-LSD which is as powerful an antagonist of 5-HT as LSD but is devoid of psychotic effects. (Cerletti and Rothlin, 1955; Rothlin, 1955; Rothlin, 1957).

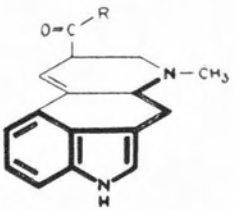
Konzett (1956) showed that LSD and 2-brom-LSD specifically antagonised the effects of 5-HT on tidal air. Atropine and the antihistaminic, 1-methyl-4-amino-N–phenyl-N–(2-Thenyl)-piperidine ('Sandosten') also partly antagonized the action of 5-HT on tidal air, but less specifically than they diminished the bronchoconstrictor action of acetylcholine and histamine, respectively. Similar findings have been reported by King (1957) on isolated perfused guinea pig lungs. Bhattacharya (1955) studied the effects of 5-HT and its antagonists on the bronchial musculature by perfusing the isolated guinea-pig lung. Essentially similar results were obtained, however, the LSD antagonism was irreversible and the antihistaminics 'Phenergan' and 'Neoantergan' were found to be inactive. Epinephrine was antagonistic to 5-HT effects and so were the adrenergic blocking agents 'Dibenamine' and 'Regitine' in high concentrations. Several reports on peripheral antagonism (Sinha and West, 1953; Robertson, 1953; 1954; Kosterlitz and Robinson, 1955 etc.) have been omitted in this review since they do not seem to be pertinent to the central actions of the 5-HT.

Woolley and Shaw (1954a, 1954b, 1955, 1957) have investigated a large series of chemically related synthetic and natural indoles for peripheral antagonism to 5-HT on artery-ring (sheep) preparations and the isolated estrous-uterus (rat) with the view to obtain evidence for the role of 5-HT in mental processes. They consider 5-HT to be a 'hormone' in the central nervous system responsible for the maintenance of normal mental processes. Compounds possessing chemical structures similar to 5-HT can block the specific action of the cell constituent. Thus they produce a specific deficiency of the hormone to which they are chemically related. Such drugs have been called as "anti-metabolites", Some of these structural analogs have been synthesized, others occur naturally. The antagonism of 5-HT by the antimetaboiltes has been studied on isolated tissues *in vitro* and the analogy applied to cerebral tissue *in vivo*. These workers have synthesized several structural analogs of 5-HT with the hope of finding an antagonist. Medmain (Shaw and Woolley, 1954) was one of the first to show antagonism to 5-HT on the rat-uterus, it was unable to antagonize pressor effects of 5-HT in dogs. The only sign that it might antagonize 5-HT in the brain was that it produced convulsions in mice, but these were not prevented by giving excess 5-HT. 2,5-dimethylserotonin (Woolley and Shaw, 1955 and Shaw and Woolley, 1956a) was shown to be a potent antagonist both in isolated tissues (artery rings, uteri) and in dogs. 1,5-dimethylserotonin was found to have actions similar to 5-HT. Woolley and Shaw (1955) conclude that substitution in the 2-position seemed to yield antagonists whereas by substitution in 1-position 5-HT-like derivatives were formed.

Chemical Relationship of 5-HT with Drugs Affecting the Psyche.

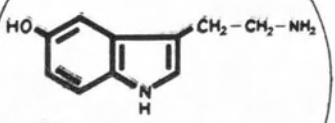


Medmain.

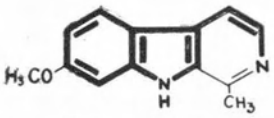


Ergot alkaloid.

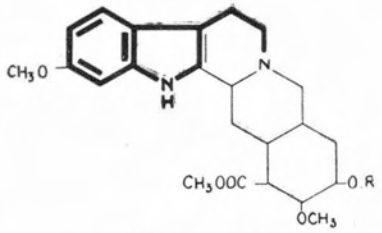
(When R is diethylamino-, =lysergic acid diethylamide LSD.)



5-Hydroxytryptamine. (Serotonin)

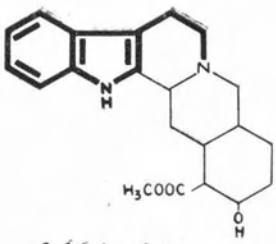


Harmine.



Reserpine

When R = 3,4,5-trimethoxybenzoyl is Reserpine.



Yohimbine.

Three classes of naturally occuring indole alkaloids were shown to antagonise 5-HT on smooth muscles. These were the ergot alkaloids e.g., ergotomine, ergotoxine (Woolley and Shaw, 1953; Fingl and Gaddum, 1953), Lysergic acid diethylamide or LSD (Gaddum, 1954); the harmine alkaloids, such as harmine (Shaw and Woolley, 1953), and yohimbine (Shaw and Woolley, 1953). Structures of these compounds to indicate the similarity with 5-HT are shown in fig. 1. Although these classes of alkaloids differ widely in chemical structure, they have in common an indole nucleus and a substituted aminoethyl side chain (shown in bold type). They all have been shown to act as competitive antagonists of 5-HT on smooth muscles such as those of arteries. Another common feature is that at least one member of each class (LSD, harmine and yohimbine) can cause mental aberrations particularly hallucinations (Woolley and Shaw, 1954). From these findings these drugs were suggested to interfere with the brain 5-HT: the action being comprable to the way they acted on various smooth muscles. However, this cannot be readily accepted since, (1) every antagonist of 5-HT (as measured on isolated tissue) does not elicit the mental changes and (2)5-HT which should reverse the mental effects of the antimetabolites 5-HT does not overcome the neurological effects of medmain or LSD, although it can overcome effects on smooth muscles. In weighing evidence of this type, however, one must consider the penetrability of drugs into the brain and the availability of proper concentration of the drugs at the proper place in the brain where 5-HT receptors are located for their normal function.

A pharmacologically induced cerebral 5-HT deficiency would be the cause of psychotic effect of the antimetabolites—for example the schizophrenia-like condition evoked by LSD. Woolley and Shaw (1954) have suggested that if this view is correct, the psychosis—schizophrenia might well be pictured as resulting from 5-HT deficiency, brought about not by drugs but by failure of metabolic processes which normally synthesize or destroy this 'hormone'. It would seem that 5-HTP, the precursor of 5-HT, which unlike 5-HT readily penetrates the brain (Udenfriend *et al.*, 1956) may prove beneficial in schizophrenia. However, it is not yet known if mental aberrations are due to a deficiency of 5-HT or an excess of 5-HT or neither. More direct evidence in this regard is required.

From the definition of "antimetabolite" of 5-HT, given by Wolley and Shaw (1954), one could see the structural similarity between 5-HT and reserpine and consider it also an antimetabolite (see fig. 1.). Reserpine displaces 5-HT from the tissues that contain it (Shore *et al.*, 1956 b). This is an antimetabolite action (Woolley and Shaw, 1957). This attaractive thesis is difficult to fit in with the finding that one molecule of reserpine can liberate from blood platelets *in vitro* large number of 5-HT molecules (Shore *et al.*, 1956 b).

INTRAVENTRICULAR INJECTIONS

In order to find the role of 5-HT in mental processes and to explain the psychiatric disturbances caused by some of the 5-HT antagonists, it is essential to get the 5-HT into the brain. 5-HT introduced into the brain should overcome the effects of its antagonists. Peripheral injections of 5-HT do not readily raise the level of brain 5-HT, probably because of the rapid destruction by amine oxidase and possibly because of a difficulty in passing 'the blood-brain-barrier'. Consequently one must devise a method of keeping it there, safe from destruction. One more serious problem is that it is only the human subjects who can tell if they are experiencing a mental disturbance. However, one cannot inject 5-HT or its antagonists into the brains of human beings. The risks to health are too great. One has, therefore, to be content with the study of behaviour in terms of a normal animal to specific situations in the laboratory. Another gap in such studies is that we know as little of the functions of cerebro spinal fluid today as was known about blood circulation at the time of Harvey's discovery.

Feldberg and Sherwood (1954) were the first to inject 5-HT into the lateral ventricle of a conscious cat. These workers have observed the effects of several other agents thus injected. Retching, vomiting, defaecation, increased salivation and greatly accelerated respiration leading to panting were common features of many drugs at some stage after intraventricular injection. After epinephrine and nor-epinephrine injection intraventricularly the cats went into a state indistinguishable from light barbiturate anesthesia. After 5-HT (75-500 μg.) the most predominent observation was a loss of muscle power which was evident from the clumsy way the cat walked and fell down. Relaxation of nictitating membrane was seen. Usually the cat was neither sleepy nor drowsy and its eyes wide open, except with large doses the cat lay in the cage with its eyes closed. Tachypnoea and bursts of profuse salivation were also noted. Similar results were obtained by Gaddum and Vogt (1956) from the same type of studies but they interpreted these findings as "a curious lethargy" caused by 5-HT. The cat lost initiative, it became hesitant and retiring and instead of running about it appeared anxious to return to its cage,. Diminished muscular tone was again noted. It seems that most of the behavioral effects could be due to the dimunition of muscular tone which may have been brought about by involvement of the reticular system.

LSD in twenty times the oral psychotic dose of man produced no apparent change in the cat's behavior. A condition like "sham rage" was produced, by 800 times the oral psychotic dose of man, when introduced into the ventricle of a concious cat. 2-brom-LSD, a congener of LSD possessing the same potency of peripheral 5-HT antagonism, failed to produce the "sham rage" and alertness on intraventricular injection; and the 2-brom-LSD did not prevent these effects from being produced by a subsequent injection of LSD (Gaddum and Vogt. 1956).

The effect of 5-HT was antagonized by LSD, but there was no evidence that the effect of LSD was antagonized by 5-HT. These results raise doubts about the theory that the central stimulant action of LSD is due to interference of natural mechanisms mediated by 5-HT (Gaddum and Vogt, 1956).

Other drugs similarly tested by Gaddum and Vogt (1956) which "aroused" the *cats* from lethargy were morphine, methadone, ergometrine, and subcutaneous amphetamine. Intraventricular injections of amphetamine and methamphetamine, which also antagonise the 5-HT metabolizing enzyme, amine oxidase, caused central depression. Thus amphetamine was shown to act differently when injected into cerebral ventricles and subcutaneously. Methylmedmain (1:2 dimethyl-3-ethyl-5-dimethylamino-indole) and 5-Benzyloxygramine, synthetic analogs of 5-HT had no specific effects on cat's behavior after intraventricular injection.

It must, however, be remebered that the action of intraventricular injection of drugs has not been shown to occur on the neurones. It is quite possible that effects may be due to a constriction of cerebral blood vessels in the neighourhood of the ventricles. The effects of intraventricular injections of epinephrine and 5HT were very similar and may have been due to a local vasoconstriction. Another possibility cannot be ruled out that some drugs administered systemically may change into an active metabolite capable of producing effects centrally.

Essentially similar effects of 5-HT by intraventricular injections in cats (Schwartz *et al.*, 1956) and in dogs (Weinberg and Haley, 1956) were observed. Haley (1957) reported on the effects of intracerebral injection of psychotomimetic and psychotherapeutic drugs into conscious mice.

Effects were obtained at doses ineffective by other routes. 5-HT caused 'central depression' and paroxysms of scratching. LSD produced 'aggressive phenomena'.

Bradley and Hance (1956) conducted an interesting study. They injected 5-HT intraventricularly (Feldberg and Sherwood, 1954) and observed the changes on the electrical activity of brain. With 5-HT (200-250 μg.) electrical activity showed an increase in slow rhythm which still responded to sensory stimuli. An intraperitoneal injection of LSD failed to antagonize the effects of 5-HT but the combination of these two drugs irrespective of the order in which they were given produced an electrical pattern similar to that seen with LSD alone, given intraventricularly, namely, high amplitude rhythmic activity of 4-7 cycles/sec. The 2-brom-LSD produced mild sedation when given intraperitoneally in equivalent doses; the electrical activity showed an increase in slow activity. Even with several times the LSD dose, 2-brom-LSD showed no signs of fast electrical activity as seen with LSD. These results (Gaddum and Vogt, 1956; Schwartz *et al.*, 1956; Bradley and Hance, 1956; Haley, 1957) suggest that the antagonisms studied by intraventricular

injections are nonspecific and have no relation to the antagonism between 5-HT and LSD in their effects on peripheral organs. It seems that the LSD effects observed were the effects of large doses. However, the results do not exclude the possibility that some of the central effects of LSD were due to interference with natural mechanisms mediated via 5-HT.

AGENTS AFFECTING BRAIN 5-HT LEVEL

If 5-HT has to be assigned a role in mental processes, then the agents which affect its level in the brain should interfere with such processes. The distribution of 5-HT in the brain has already been shown to be in a regular pattern (Amin *et al* , 1954; Bogdanski and Udenfriend, 1956). The agents which can influence the brain 5-HT level may be the natural '*precursors*' of 5-HT ; or those which prevent the 5-HT destruction by inhibiting the amine oxidizing enzymes, thus '*preserving*' the 5 HT. Both these classes of agents raise the brain 5-HT level. Structural analogs of 5-HT may act as 'antimetabolites' and thus displace the 5-HT from its functional sites, these agents would seem to lead to a deficiency of 5-HT. Yet another interesting class of agents '*release*' the 5-HT from the sites where it is in a bound form, thus depleting the stores of 5-HT but probably at the same time raising the level of free 5-HT. Lastly there are several drugs which have neuropharmacological effects similar to any of the above classes of agents and which do not influence the 5-HT level. It is indeed often quite difficult to decide if the pharmacological action of a drug was solely the result of a change in the level of the biochemical.

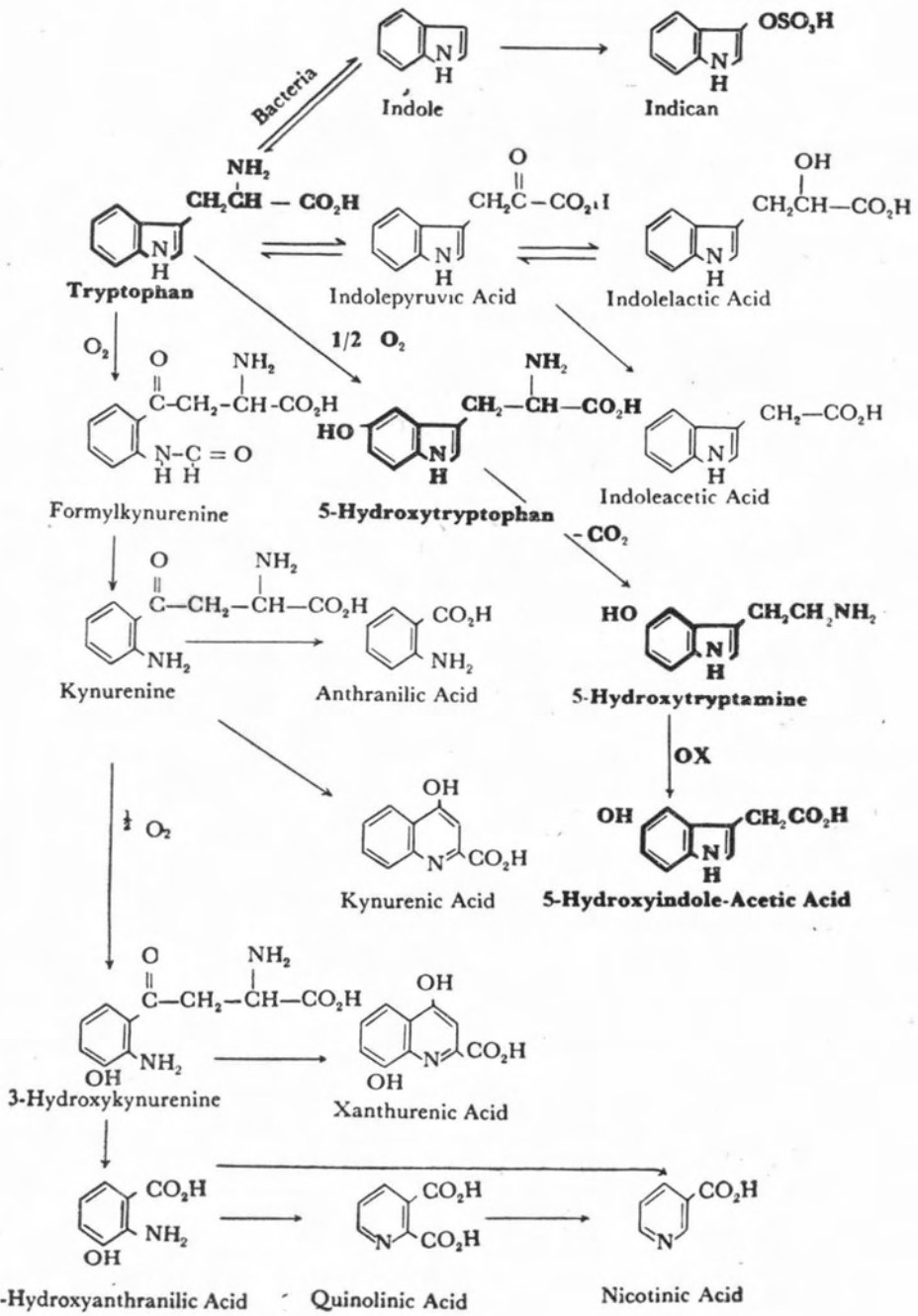
1. 5-HT Precursors.

Tryptophan is the parent substance of most of the naturally occuring indole derivatives. It is normally converted into Kynurenin and then to kynurenic acid. The latter can further be metabolized to the vitamin nicotinic acid which functions as the coenzyme I and II in the intermediary metabolism. Another pathway for the metabalism of tryptophan is by bacterial decarboxylation which leads to tryptamine formation and further degradation to indole and skatole. (See fig. 2).

Ek and Witkop (1953) showed the existence of a new amino acid 5-Hydroxytryptophan (5-HTP) which was resistant to the action of kynureninforming enzymes. Udenfriend, Clark and Titus (1953) demonstrated that the hydroxylation of the five position was the normal pathway of tryptophan metabolism, and 5-HTP was decarboxylated to 5-HT by a specific decarboxylase. Thus, tryptophan and more particularly 5-HTP are the precursors of 5-HT.

It is conceivable that the dementia associated with pellagra may be due to a deficiency of 5-HT, since the source of both 5-HT and nicotinic acid is tryptophan (see fig. 2). If the requirement of nicotinic acid is increased as in pellagra it is possible that a deficiency of 5-HT would concomittantly result.

Tryptophan Metabolism



As yet no evidence is available that the brain can hydroxylate tryptophan to yield 5-HT. It is known that parenterally administered 5-HT does not lead to appreciable increase in brain 5-HT level in dogs and rabbits. Slight increase in brain 5-HT level with large doses of parenteral 5-HT in mice has been observed. Further evidence that peripheral 5-HT does not readily penetrate into the central nervous system comes from studies on patients with malignant carcinoid tumors. The 5-HT produced by these tumors is about 100 times the normal, the blood 5-HT levels are about 20 times normal and free 5-HT (not bound to platelets) is found in the blood. Normally, the circulating 5-HT is exclusively in the platelets. No 5-HT is detected in the spinal fluid and no mental disturbance is associated with this disorder. Thus it seems that the 5-HT found in brain is not brought from peripheral sources (Udenfriend *et al.*, 1957).

Udenfriend *et al.*, (1956) have demonstrated that 5-HTP penetrated the blood brain barrier and thus resulted in a 10-20 fold increase in the brain 5-HT level. At these high levels of brain 5-HT the animals showed marked tremors and excitement which were similar to the effects observed after the administration of LSD. The ability of 5-HTP to elevate brain 5-HT level, makes it a useful pharmacological tool for the study of neuropharmacological agents which are supposed to act via the mediation of 5-HT in the brain. Although 5-HTP, 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) appeared in the brain after 5-HTP administration, the observed pharmacological effects paralleled the 5-HT level, this indicates that 5-HTP acts through its conversion to 5-HT (Bogdanski *et al.*, 1956).

2. 5-HT Preservers.

5-HT is normally catabolized to 5-hydroxyindole acetic acid (5-HIAA) which is readily excreted in the urine (Udenfriend *et al.*, 1955). The tissue catalyst responsible for this metabolism of 5-HT is mono-amine oxidase, and this enzyme is present in the brain (Sjoerdsma *et al.*, 1955). The 5-HT is rapidly oxidized by preparations containing amine oxidase (Blascho, 1952). The inhibitors of monoamine oxidase have been studied by Zeller *et al.*, (1955) and these workers report that 1-isopropyl-2-isonicotinyl-hydrazide (Iproniazid) is a potent inhibitor of the enzyme. Thus Iproniazid is an agent which would preserve the destruction of 5-HT by monoamine oxidase. The value of Iproniazid as a tool in investigation of 5-HT is obvious. The inhibition of monoamine oxidase by Iproniazid has been demonstrated *in vivo* in rats and rabbits since administration of Iproniazid increased the brain 5-HT level by three folds and the 5-HT in other tissues remained unaffected. Except in the brain the Iproniazid did not penetrate to the site of monoamine oxidase while the cells were intact (Weissbach *et al.*, 1957). However, the enzyme inhibitor is not too specific in its action and this makes the interpretations of *in vivo* studies difficult.

Chessin *et al.*, (1956) made a study on mice, dogs and cats before and after treatment with Iproniazid in relation to doses of either 5-HT or reserpine. Reserpine is known to liberate 5-HT from tissues including the brain, and causes sedation (Pletscher *et al.*, 1955; Shore *et al.*, 1956a; Pletscher *et al.*, 1956). Iproniazid-treated mice given reserpine, rather than being sedated, became restless, hyperactive and aggressive. 5-HT given intravenously to Iproniazid pre-treated mice failed to cause excitation but intracerebral 5-HT did produce the excitation. Dogs and cats (anesthetized or unanesthetized) following treatment with Iproniazid respond to reserpine with marked changes from the normal.

In vitro studies of brain homogenates from Iproniazid-pretreated mice demonstrated an inhibitory effect of 5-HT on endogenous oxygen uptake (Chessin *et al.*, 1956).

Although Iproniazid has been found useful in many studies on 5-HT, it leaves much to be desired. In addition to blocking the amine metabolism, it is known to interact with pyridine nucleotides (Kaplan *et al*, 1954), and like Isoniazid, to cause depletion of pyridoxine (Biehl and Vilter, 1954), making its *in vivo* action difficult to interpret especially in chronic studies (Udenfriend *et al.*, 1957).

3. **5-HT Releasers.**

These include "mast-cell depleters" and the Rauwolfia alkaloids.

Histamine releasers.—Histamine and 5-HT have both been identified in mast cells (Benditt *et al.*, 1955; Sjoerdsma *et al.*, 1957). Bhattacharya and Lewis (1956a) have shown that the histamine liberator 48/80 releases 5-HT in addition to the histamine from the mast cells in rat. This release of 5-HT is also affected by other histamine releasers like propamidine and morphine. Histamine caused no release of 5-HT. Under the circumstances the histamine liberators may better be known as "mast-cell depleters" (Bhattacharya and Lewis, 1956a). Feldberg and Smith (1953), on the other hand, found that tryptamine and 5-HT liberated histamine to a limited extent. They considered the possibility that the release of histamine might in some way be preceded by the release of 5-HT. However, histamine is liberated by 48/80 in those species in which there is no release of 5-HT. There is no evidence for the release of 5-HT by 48/80 from the perfused tissues of cats, dogs and rabbits. No mast cells have been detected in these species.

Rauwolfia serpentina alkaloids.—Reserpine, the purified alkaloid of Rauwolfia serpentina, possesses marked sedative and mild hypotensive properties. That reserpine releases 5-HT from the tissues, has been demonstrated by Shore, Brodie, Pletscher and others working at the National Institutes of Health, Bethesda, Maryland and others (Naess and Schanche,

1956). Pletscher, Shore and Brodie (1955) showed that reserpine released 5-HT from its major body depot, the intestinal tract. Later on reserpine was shown to release 5-HT from platelets, without causing their disruption or interfering with haemostasis (Shore *et al.*, 1956a), and from the brain (Pletscher, Shore and Brodie, 1956; confirmed by Paasonen and Vogt, 1956). Reserpine seems to be a general 5-HT liberator, but the brain was found to be most sensitive (Pletscher *et al.*, 1956), 80% decline occured within 30 minutes and maximum decline (90%) occured within 4 hours (Brodie *et al*, 1955). The property of reserpine to release 5-HT from brain mitochondria was shown to be unrelated to its action as an inhibitor of oxidative phosphorylation (P/0) at 10^{-5}M., (Walaszek and Abood, 1957). Bhattacharya and Lewis (1956b) report that in rats when they had been pretreated with intraperitoneal injection of reserpine (5.0 mg./kg.) for two or three days, intra-arterial injection of the histamine liberator 48/80 into the perfused hindquarters of the animal released the usual amount of histamine no 5-HT. Thus an inter-relationship between 5-HT and reserpine was shown to exist.

An interaction between reserpine and 5-HT was shown, more directly by giving reserpine (5.0 mg./kg.) to dogs. A marked increase lasting about 12 hours, in urinary excretion of 5-hydroxyindole acetic acid, 5-HIAA, (Shore, Silver and Brodie, 1955 a), a major metabolite of 5-HT (Titus and Udenfriend, 1954) was shown. A second dose of reserpine 24 hours after the first failed to cause another rise in 5-HIAA excretion in urine (Shore *et al.*, 1956). Apparently the 5-HT stores were depleted and had not yet replenished.

5-HT by itself in a dose of 20 mg. per kilogram produced a mild depression, but doses as high as 100 mg. failed to produce hypnosis in mice in the hands of Shore, Silver and Brodie (1955); and 5-HT was found to markedly potentiate the hypnotic action of hexobarbital in mice. These workers thus showed that 5-HT causes a subhypnotic dose of barbiturate to become hypnotic. Similar action of increasing the sensitivity of central nervous system to barbiturates was observed with chlorpromazine and reserpine.

Considering the 5-HT releasing property of reserpine together with the observation that both 5-HT and reserpine potentiate the action of hexobarbitone in mice, Shore, Pletscher, Brodie and their collaborators came to the conclusion that the central actions of reserpine might well be mediated through the release of 5-HT in the brain (Pletscher *et al.*, 1956 a). Further support of the theory was obtained by the finding (Pletscher, Shore and Brodie, 1956; Shore, Carlesson and Brodie, 1956; Brodie, Shore and Pletscher, 1956) that the 5-HT releasing property of the Rauwolfia alkaloids was limited to those alkaloids (reserpine, rescinnamine, deserpidine and raunescine) which

possessed tranquilizing action Isoreserpine, an inactive stereoisomer of reserpine and isoraunescine, an inactive isomer of raunescine, had no effect on the brain 5-HT level. Shore *et al.*, (1957) further rule out the possibility that a metabolic product of reserpine, not measured by analytic methods, may persist which may be responsible for the reserpine action. They think this to be unlikely, firstly because one of the metabolic pathways of reserpine *in vivo* is hydrolysis to methyl reserpate and trimethoxybenzoic acid, neither of which products exerted 5-HT releasing or sedative action. Secondly, brain 5HT declined to the same extent whether 1 or 5 mg. of reserpine was administered to rabbits. The same intensity and duration of effects and the same time for restoration of 5-HT levels was observed with the two doses. If a metabolite of reserpine was responsible the effects would be proportional to the dose. Evidence that the intact reserpine molecule can release 5-HT has been obtained from studies *in vitro*, that a molecule of reserpine affected a release of hundreds of molecules of 5-HT indicating that the release of 5-HT is not a simple displacement by reserpine (Shore *et al.*, 1956 b).

Unlike 5-HT, 5-HTP readily enters the brain where it is decarboxylated to yield the 5-HT (Udenfriend *et al.*, 1956). One hour after 5-HTP administration to normal rabbits, the brain 5-HT level rose significantly but in the case of reserpine-pretreated animals the rise in brain 5-HT level was impaired even when reserpine was no longer detectable in the brain. The possibility that reserpine had blocked the action of 5-HTP decarboxylase, was ruled out as the activity of this enzyme in the brain was unchanged (Shore *et al.*, 1957) and 5-HT continues to be formed although the 5-HT binding sites are impaired by reserpine (Kuntzman *et al.*, 1956). When rabbits were pretreated with Marsalid (iproniazid), the monoamine oxidase inhibitor, and reserpine subsequently injected intraperitoneally, the animals became hyperactive and showed other signs similar to LSD administration. This was in contrast to reserpine alone in which case they were only sedated. Marsalid alone had no obvious effects (Brodie, Pletscher and Shore, 1956). The excitement observed with iproniazid, followed by reserpine administration, is explained by Brodie and Shore, (1957) to be presumably due to excess of 5-HT (free) which counteracts its own action. The action is supposed to be similar to acetylcholine in this respect which also counteracts its own action when in excess.

Moreover, the pharmacological actions of reserpine persisted long after reserpine had disappeared from the brain and these actions were correlated with changes in the brain 5-HT (Hess *et al.*, 1956 a ; 1956 b). A cumulative action of reserpine, *in vitro*, on platelet 5-HT level has been shown (Haverbrock *et al.*, 1956). Furthermore, it was found in the mouse that LSD antagonized the synergism between hexobarbitone and 5-HT in the same way as between hexobarbitone and reserpine (Shore, Silver and Brodie, 1955 b). In experiments done on cats given 5-HT into cerebral ventricles or reserpine intraperitoneally, it was shown that LSD interrupted the lethargy produced by

either 5-HT or reserpine (Gaddum and Vogt, 1956). These facts add circumstantial evidence to the view that some, at least, of the central effects of reserpine are indeed mediated by a raised level of circulating 5-HT. However, it would be interesting to find out if the sedative and hexobarbitone potentiating and sedative effects of chlorpromazine, which does not liberate 5-HT, are not blocked by LSD.

Shore *et al.*, (1957) and Brodie *et al*, (1957 b) believe that reserpine acts as follows : it rapidly enters the brain and in some unknown way, irreversibly affects the 5-HT binding sites, and then disappears. As a results, 5-HT is available in free form and it is this free 5-HT which accounts for the reserpine action. This is however, soon metabolized by monoamine oxidase. 5-HT continues to be formed although it cannot be bound and hence the low 5-HT level that persists presumably represents a balance between its rate of synthesis and rate of destruction. The effect of reserpine persists until the binding sites regain their binding capacity or until new sites are formed.

Low levels of 5-HT after reserpine correspond well with the various pharmacologic effects including sedation, miosis, hypothermia and hypotension (Deming *et al.*, 1956). The sodium retaining property of reserpine, in dogs, may even be related to the 5-HT release (Blackmore, 1957). No direct evidence is available for the possibility that reserpine-hypotension is due to release of 5-HT. Bhargava and Borison (1955 a & b) employing the stereotaxic technic for eliciting central vasomotor responses showed the differences between the actions of reserpine and alseroxylon (a purified fraction of Rauwolfia serpentina). The latter was found to be more potent hypotensive and had actions at all levels of the neuraxis including the spinal cord ; whereas, reserpine was mildly hypotensive and the site of action was only supraspinal, the medulla being more important than the hypothalamus. On the basis of these differences various alkaloids of Rauwolfia were studied. 5-HT was also included in the subsequent study. Bhargava and Borison (1957) demonstrated that the central hypotensive action of 5-HT was similar to the hypotensive action of reserpine and rescinnamine. These authors emphasize that the more important hypotensive alkaloids of Rauwolfia contained in alseroxylon do not seem to mediate their actions through 5-HT. However, it is possible that the central hypotensive action of reserpine may be mediated through the 5-HT release. Cronheim and Gourzis (1956) reported an augmentation of fall of blood pressure and heart rate in dogs pretreated with reserpine and infused with 5-HT. No effects were observed in control animals with the 5-HT infusion (10 μg./kg./min.). They interpret these cardiovascular actions to be due to the 'free' 5-HT in the brain. In cross-circulation experiments, perfusion of the isolated head with 5-HT caused fall in blood pressure in the recepient cat (Costa and Aprison, 1957). This finding is in further support of a central hypotensive action of 5-HT.

Reserpine (10μg.) given intraventricularly did not produce any obvious effects but the intraperitoneal injection of reserpine (0.25-0.5 μg. per kg.) resulted in marked sedation in cats (Gaddum and Vogt, 1956). Unlike 5-HT, however, with reserpine the pupils were constricted, nictitating membranes relaxed and the ataxia was more pronounced. These effects were all antagonized by LSD. Either the reserpine given systemically is more likely to reach sites inaccessable to the drug injected intraventricularly or these effects of reserpine are not mediated via 5-HT release (Vogt, 1957).

Many side effects of reserpine such as flushes, nasal congestion and diarrhoea do seem to be due to the peripheral release of 5-HT and are also noted in patients with carcinoid tumors. Although, they can be partially antagonized by antihistaminics, now one should seek to employ the antagonists of 5-HT instead. This should be practicable with small doses of the antagonists which antagonize the peripheral actions of 5-HT whereas central actions are either not antagonized or only slightly influenced by high doses (Gaddum and Vogt, 1956). However, the trials of 2-brom-LSD, a peripheral antagonist of 5-HT as potent as LSD but devoid of central actions of LSD, in the control of symptoms of carcinoid tumours have been disappointing. Waldenstrom (1957) administered doses upto 1.5 mg. (1500 μg.), without causing side effects of psychic changes, but also without producing any significant decrease in the symptoms of the disease. In one patient, Snow *et al.*, (1955) administered upto 7.5 mg. of 2-brom-LSD daily over a period of 8 weeks. No changes in psyche were noticed and the therapeutic effects on the symptoms were insignificant. These results are disappointing and hard to explain.

Let us now discuss whether the main actions of reserpine could, in the light of present knowledge, be due to the release of large amounts of 5-HT from its storage depots. It is needless to emphasize that such a discussion will be handicapped by the incomplete knowledge of role of 5-HT and the conflicting data accumulated on the subject. We are not yet aware of the possible effects on the tissues (which normally store 5-HT) of the loss of this cell constituent.

Feldberg (1957) is not at all certain whether the central effects of reserpine are an action of released ('free') 5-HT or whether the sedation as well as the hypotension result from the fact that the brain is depleted of its 5-HT and there is therefore too little left for its normal function. There is general agreement that reserpine does release 5-HT from the brain. It is, however, not yet certain if the pharmacological actions of reserpine are due to its affecting the 5-HT level in the brain. Is it the presence of free 5-HT or the loss of bound 5-HT, brought about by reserpine, which is responsible for the reserpine effect? This is not yet clear. More direct and convincing proof of the existence of the two pools

of 5-HT (bound or free) is required to prove the hypothesis. The fact that the action of reserpine is long lasting, suggests that the central effects, if they have anything at all to do with 5-HT, are more likely to be associated with the loss of 5-HT from these structures; and on the other hand the peripheral effects like flushing and diarrhoea may well be the effects of released 5-HT.

Paasonen and Vogt (1956a) showed that amphetamine reduces the 5-HT content to one-half in the dog's hypothalamus. The signs exhibited by these dogs are, of course, those of excitement and therefore opposite of those after reserpine.

The complexity of the phenomena involved in the action of reserpine is illustrated by the fact that not only is the 5-HT content of the hypothalamus reduced by reserpine, its norepinephrine content is also considerably lowered (Holzbauer and Vogt, 1956), a definite drop occured within 15 minutes and maximum decline (90%) occured within 4 hours (Brodie *et al.*, 1957b) and then slowly returned to normal values. The almost identical pattern of effect of reserpine on 5-HT (Brodie *et al.*, 1955) and norepinephrine with regard to duration and response to various doses of reserpine suggest that the release of these biogenic substances are closely linked. Peripheral norepinephrine was also shown to be depleted following reserpine administration (Brodie *et al.*, 1957a). The emerging picture of reserpine as a releaser of 5-HT, norepinephrine and epinephrine thus seems more complicated than hitherto imagined.

Tolerance to the effects of 5-HT develops but no such tolerance seems to arise from prolonged treatment with reserpine (Wilkins, 1954). This finding is not compatable with the hypothesis that the reserpine-action is mediated through the release of 5-HT.

In a very recent report, in man, on the urinary excretion of 5-HIAA, a major metabolite of 5-HT, Forrest (1957) did not observe any correlation between the administration of reserpine and the excretion of 5-HIAA. Shore *et al.*, (1955) found in their dogs, that the maximum rise in the excretion was at 2-4 hours lasting about 12 hours. This might well happen in human subjects given comparably large doses, but even then it would be difficult to correlate it with the accepted facts that the effects of reserpine (as regards the mental state, at any rate,) do not appear for several days.

Chessin *et al.*, (1957) confirm the findings of Brodie *et al.*, (1955) that 5-HT release was responsible for the hypotensive effects (in cats and dogs) and the central effects (in mice, guinea pigs and rabbits). Depression after reserpine was explained by a diminished concentration of 5-HT in the brain (Chessin *et al.*, 1957) and not by increased concentration of liberated 'free' 5-HT (Shore *et al.*, 1957). Iproniazid-pretreated mice given reserpine rather than

being sedated became restless, hyperactive and aggressive (Chessin *et al.*, 1956). Failure to cause excitation by reserpine in the absence of Iproniazid may have resulted from the extremely rapid destruction of 5-HT by monoamine oxidase. This lends support to the concept that 5-HT is a central excitant (Chessin *et al.*, 1957). The situation still is uncertain what actually causes the signs of reserpine-action, depletion or flooding or neither.

If the effects of reserpine were due to the released 5-HT, the effects of intraventricular 5-HT should have been similar to intraperitoneal reserpine. 5-HT did not produce ptosis, relaxation of nictitating membrane or miosis. The sedation due to 5-HT was largely explanable on the basis of muscular weakness (Feldberg and Sherwood, 1954). Moreover, reserpine has been found to be active *in vitro* in releasing 5-HT (Shore *et al.*, 1956 b) but was found to be inactive when injected into the cerebral ventricles (Gaddum and Vogt, 1956).

5-HT has an anticonvulsant action as shown by tests on electroshock, reserpine on the other hand, is known to lower the threshold to electroshock (Chen *et al.*, 1954; Everett *et al.*, 1955). The antiepileptic drugs have been shown to raise the brain 5-HT level (Bonnycastle *et al.*, 1957).

Cerletti and Rothlin (1955) report that both LSD, which produces psychic disturbances, and 2-brom-LSD, which does not produce abnormal mental reactions, are strong inhibitors of 5-HT actions *in vitro* and *in vivo*. Both LSD and 2-brom-LSD have been detected in brain tissue, but only LSD is found to be active as an analeptic on reserpine sedated mice. These data do not appear to be consistant with Brodie's hypothesis that the sedation produced by reserpine is mediated through 5-HT. LSD and 2-brom-LDS are both effective agents for inhibiting the prolongation of barbiturate hypnosis by 5-HT. LSD and amphetamine are potent agents for inhibiting the prolongation of barbiturate hypnosis by reserpine. It is again significant to note that 2-brom-LSD which inhibits the prolongation of barbiturate hypnosis due to 5-HT, does not inhibit prolongation of the barbiturate hypnosis due to reserpine (Burton, 1957).

Fastier *et al.*, (1957) observed a significant increase in the sleeping time of chloral hydrate when it was combined with other agents like epinephrine, norepinephrine, 5-HT, and histamine. They believe that this increase in sleeping time of chloral hydrate is due to a hypothermic effect, since 5-HT + chloral hydrate or epinephrine + chloral hydrate lowered the temperature more than with chloral hydrate alone. It would be interesting to study the effects of chloral hydrate when it is combined with LSD or 5-HTP which are known to cause hyperthermia (Horita and Gogerty, 1957).

4. Miscellaneous agents

Only few compounds have been discovered which alter the brain 5-HT level. 5-HT releasers like reserpine which lower the brain 5-HT level have

been discussed. The agents which raise the 5-HT level in the brain are either the 5-HT precursors, mainly 5-HTP or the 5-HT preservers, mainly Iproniazid which inhibits the 5-HT metabolizing enzyme, monoamine oxidase. A new class of agents which are pharmacologically related have been shown to increase the brain 5-HT in the rat (Bonnycastle *et al.*, 1957 a). Several antiepileptic agents: diphenyl hydantoin, trimethadione, paramethadione, phenacetamide, primidone, milontin, phenobarbitone and sodium bromide, have been shown to raise the brain 5-HT level (Bonnycastle *et al.*, 1957 b). It has been shown that the effect upon 5-HT is restricted to the cerebral tissue. The mechanism of change is not yet known. However, preliminary studies suggest that it is not the result of monoamine oxidase inhibition (Bonnycastle *et al.*, 1957 a). The existence of a relationship between an increase in brain 5-HT and the anticonvulsant action of these drugs is open to question. Convulsing procedures like electroshock, administration of leptazol, picrotoxin, and CO_2, did not change the brain 5-HT level (unpublished data quoted by Bonnycastle, 1957 a). Furthermore, administration of 5-HTP or Iproniazid, to raise the brain 5-HT level afforded no protection against leptazol convulsions. Hence, the significance of the raised brain 5-HT levels with the anticonvulsant drugs has not added much to the clarification of the role of 5-HT in mental processes.

Recently another group of pharmacologically related but chemically unrelated drugs have been used successfully in the treatment of neurosis and psychoses. The commonly used tranquilizing agents: reserpine, chlorpromazine, benactyzine and meprobamate, have been tested for their interaction with 5-HT (Berger *et al.*, 1957). Of these reserpine has been shown to decrease 5-HT level in the brain (Pletscher, Shore and Brodie, 1956). They have also shown that the excretion of 5-HIAA, a metabolite of 5-HT, was consequently markedly increased by reserpine administration (Shore, Silver and Brodie, 1955 a). Chlorpromazine, benactyzine and meprobamate did not cause an increased excretion of 5-HIAA in rats (Berger *et al.*, 1957). Varying effects of the tranquilizing agents were observed on the brain potentials (Berger *et al.*, 1957). No correlation was found between the central effects of the drugs affecting the psyche and their peripheral antagonism on isolated rat uterus (Costa, 1956) or the rat colon (Berger *et al.*, 1957). Chlorpromazine, reserpine and benactyzine powerfully antagonized contractions evoked by acetylcholine or 5-HT in the isolated rat colon, benactyzine was most potent against acetylcholine induced contractions, while chlorpromazine and reserpine showed little specificity. Meprobamate antagonized the stimulants only in large doses (Berger *et al.*, 1957). Costa (1956) demonstrated on the rat uterus that reserpine, chlorpromazine and azacyclonol (Frenquel) antagonized 5-HT contractions without affecting the sensitivity of acetylcholine or oxytocin. Mescaline, a hallucinogen like LSD, caused facilitation of 5-HT activity on the

rat uterus. Both mescaline and 5-HT activity on the rat uterus was antagonized by LSD in high concentrations and facilitated by LSD in low concentrations. Thus the findings obtained from the rat uterus cannot be transferred to the mental effects. One is inclined to agree with Woolley and Shaw (1957) that the same compound can have both proaction and antiaction depending on the test object and concentration used.

Although chlorpromazine does not seem to act via 5-HT interaction in vivo, it has been shown to possess definite 5-HT antagonizing property on the rat uterus (Gyermek, 1956) and the rat colon (Benditt and Rowley, 1956). The brain 5-HT level was not affected by chlorpromazine, (Brodie, Shore and Pletscher, 1956), yet the effects of chlorpromazine, and reserpine, which is known to release 5-HT, are quite similar on the psyche. The differences between the modes of action of reserpine and chlorpromazine can perhaps be explained by assuming that they act on physiologically antagonistic systems in the brain stem (Brodie, 1957 b). 5-HT is assumed to be the chemical mediator of the central parasympathetic system. Drug induced paralysis of one system would unmask the other system and allow it to predominate. Reserpine is postulated to stimulate the parasympathetic system by effecting a persistent release of 5-HT from its storage depots. LSD by blocking the action of normally released 5-HT at the central synapses would unmask the action of the opposing sympathetic system and thus produce central excitation. Chlorpromazine is assumed to inhibit the sympathetic system blocking the chemical mediator of the system viz. norepinephrine. Thus in effect it would unmask the action of the opposing parasympathetic system and the results would be the same as with reserpine namely sedation. Mescaline (trimethoxyphenylethylamine), which is structurally related to epinephrine rather than 5-HT, might be considered to act centrally and mimic the action of norepinephrine. Thus mescaline which produces effects like those produced by LSD, may be assumed to act not by blocking "Serotinergic" or parasympathetic brain centres but by stimulating the reciprocal "adrenergic" or sympathetic brain centers. This, rather oversimplified, concept of central synaptic transmission is however, wholly hypothetical. In this regard it may be recalled that norepinephrine when introduced into the ventricle produces a state similar to light barbiturate anesthesia (Gaddum and Vogt, 1956) and not excitement as would be expected from Brodie's hypothesis.

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