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Review of the proposed action of serotonin on the central nervous system and the related effects of iproniazid

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A REVIEW OF THE PROPOSED ACTION OF
SEROTONIN ON THE CENTRAL NERVOUS
SYSTEM AND THE RELATED EFFECTS
OF IPRONIAZID

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I. INTRODUCTION

With the isolation and determination of the structure of serotonin in 1947, humoral substance appeared much in need of a disease. The disease appeared in 1952 when Biorck,[1] Axen, and Thorson reported a 19 year old man with dyspnea and a curious form of cyanosis. This was soon diagnosed to be caused by a malignant carcinoid which was producing excess serotonin. Since this time much interest has been shown in this field and specifically in the effect that neuro-humoral type agents such as serotonin have in relation to brain metabolism and mental illness. Because of the recent advances in tranquilizing agents and psychotropic agents it was thought to be of interest to review the literature on one of the newer psychotropic agents, such as iproniazid, as to its possible metabolic effects most especially in relation to serotonin's central nervous system proposed actions. Also, it was thought desirable to discuss the possible role that serotonin may have in mental illnesses. It is hoped that this paper may encompass what is known up to the present in this field.

II. History

Indolealkylamines had been considered as a group of active substances of rather slight pharmacological interest until it was discovered that one of the indolealkylamines, 5-hydroxytryptamine (5-HT), was identified with both serotonin, the serum vasoconstrictor, and enteramine, the specific secretion product of the enterochromaffin cell system.

The isolation of 5-hydroxytryptamine had been carried out by two groups of independent research workers. In the early 1930 Erspamer[2] and his group were investigating the substance which imparts the histochemical properties to the enterochromaffin cells of the gasterointestinal mucosa. In 1946, the indole nature of enteramine was f und; but, not until 1952 was the enteramine substance identified as 5-hydroxytryptamine by Erspamer. The work of others back as early as 1912 reported that serum contained a vasoconstrictor. This was done by O'Conner, J.M., in Germany. This was farther investigated and in 1947 Rapport[3] and his group isolated from serum a substance which had moderate hypertensive and vasoconstrictor properties. After isolating the serum material which they called serotonin, Rapport went on to identify it as 5-hydroxytryptamine in a creatinine sulfate complex.[3]

Since it was known that serotonin appeared in many tissues, brain was studied by Twarog[4] and Page, in 1947, and this too revealed a low level. The occurrence of serotonin in brain was confirmed by Zettler[5] and Schlosser, and incontestable proof was offered by

Bogdanski et al.[6] using the spectrophotofluorometer.

Isonicotinic acid hydrazide (isoniazid) in recent years had been used as an tibacterial agent more specifically for its tuberculostatic ctivity. It was soon noticed that some patients on this therapy developed slight hyperactivity and euphoria. Its isopropyl derivative, 1-isonicotinyl-2-isopropylhydrazine (ipro-niazid, Marsilid),[7] was noted to also have a tuberculostatic effect, but was seen to produce more of a central nervous system stimula-tory action than isoniazid.
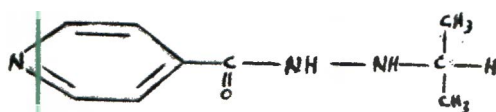
1-isonicotinyl-2-isopropyl hydrazine

Because of this action it was thought feasible to use it as a stimulant such as amphetamine is used and it was coined under the name of a "psychic energizer". It has only been in major clinical use during the last two years.

III. Pharmacology and Metabolism

Rapport[3] and co-workers (1948) reported the isolation of a potent crystalline vasoactive substance from beef serum. Rapport[8] (1949) was able to demonstrate that the substance contained an indole base complexed with creatinine sulfate and that the crystals held one mole of water of crystallinization. On the basis of chemical and physical tests, he postulated the structure 5-hydroxy-3-B-aminoethylindole (5-hydroxytryptamine) which he named serotonin. More recently, Speeter[9] and associates (1951) in the Upjohn laboratories, synthesized the creatinine sulfate of 5-hydroxytryptamine and found it chemically identical to Rapport's natural product.
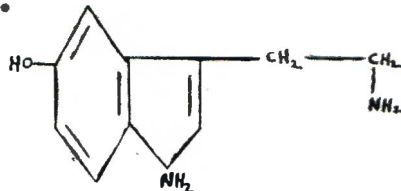
5 - h y d r o x y t r y p t a m i n e

It has been postulated that the metabolic pathway of serotonin is as follows:[15]

TRYPTOPHAN
↓ hydroxylase
5-HYDROXYTRYPTOPHAN
↓ decarboxylase
5-HYDROXYTRYPTAMINE
(Serotonin)
↓ monoamine oxidase
5-HYDROXYINDOLE ACETIC ACID
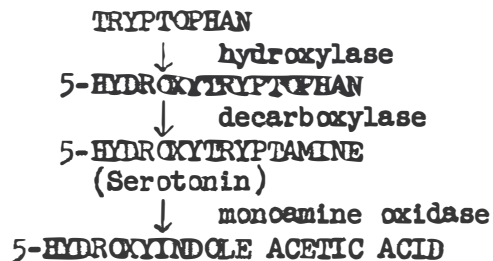
The precursor of serotonin, 5-hydroxytryptophan, was synthesized

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by Ek[10] and Witkop and the biogenesis of serotonin from 5-hydro-
xytryptophan suggested by Udenfriend et al.[11] Clark, Weissbach,
and Udenfriend[12] found a specific decarboxylase (5-hydroxytrypto-
phan decarboxylase) wide spread in plants and animals. This en-
zyme is widely istributed throughout the body. Its function is
to catalyze the decarboxylation of 5-hydroxytryptophan to yield
serotonin and CO_2. As Clark, Weissbach, and Udenfriend[12] had shown
the enzyme is highly specific; even 7-hydroxytryptophan cannot
substitute as a substrate. The optimum ph, 8.1, is unusually
high among amino acid decarboxylases, and this makes it quite
unique. It is also a soluble enzyme and relatively large amounts
of it are found in kidney, liver, and stomach while Gaddum and
Giarman[13] found little activity in spleen, platelets, and bone
marrow. Sympathetic ganglia and some parts of the brain showed
some activity..

Blaschko and Hope[14] found that 5-hydroxytryptophan was oxi-
dized by the amino acid oxidase of cobra venom and preparation of
the digestive gland of Mytilus edulis. Since this amino acid is
readily oxidized by l-amino acid oxidases, they suggested that a
second pathway may exist which would lead from 5-hydroxytryptophan
to 5-hydroxyindole acetic acid without serotonin as intermediate.
This, however, was not demonstrated.

If 5-hydroxytryptophan is decarboxylated to serotonin, then
serotonin is readily inactivated through the action of monoamine

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oxidase. Sjoerdsma et al.[15] concluded that oxidative deamination
by monoamine oxidase is the major metabolic pathway in the metabol-
ism of serotonin. Their evidence is based on the comparable rates
of serotonin and tyramine metabolism in tissues of 3 different ani-
mal species. They demonstrated comparable inhibition of metabolism
of both amines by iproniazid; and also found that serotonin is
metabolized by the mitochondrial portion of liver cells. They
suggest that conclusions based on experiments showing similar
rates of O_2 uptake by tissue homogenates incubated with serotonin
and with tyramine need not be valid.

With the oxidative deamination of serotonin, 5-hydroxyindole
acetic acid is formed. The urinary excretion of 5-hydroxyindole
acetic acid (5-HIAA) is an index of the deamination pathway of
metabolism. Early observations indicated that the excretion of
5_HIAA by patients with the "carcinoid syndrome" was of such mag-
nitude as to require major utilization of dietary tryptophan for
the formation of 5-hydroxyindole compounds. This may show that
the metabolic cycle from tryptophan to 5-HIAA can be of relatively
an excessive amount in this disease.[16]

Cahn et al.[17] have shown that in unanesthetized rabbits ser-
otonin reduces glucose consumption of the brain. Possibly the pro-
longation of action of barbiturates by serotonin is assocaited with
the diminution of consumption of pyruvate lactate and phosphate
caused by serotonin.

Serotonin at concentrations of 5×10^{-3} M inhibits nonspec-

ific human serum cholinesterase about 50% and the specific one of human red cells about 35%. It was also shown by Rapport[3] that serotonin reduces the rate of oxygen consumption of the rat when injected intraperitoneally in doses of at least 1 mgm/kgm. The reduction varied in intensity between 24 and 61% and in duration between 12 and 120 minutes. A similar effect was obtained with tryptamine and histamine at much higher dose levels. The effect was not observed in other experimental animals, even with enormous amounts of serotonin. The phenomenon is attributed, at least to a great extent, to an action of the drug on the central nervous system or on the arterial vessels of the brain. The opposite also has been reported. That is, serotonin increasing the oxygen consumption and causing the appearance of 5 HIAA in homogenated liver, kidney, and brain from pigs and rats. (It is doubtful that accurate comparisons can be made between homogenates and in vivo activity.)

Serotonin in 5 mgm. doses, injected intraperitoneally, increases strikingly the system uptake of P^{32} and the rate of metabolic phosphorylation in experimental animals, but no change in brain uptake occurred. (This may help demonstrate that serotonin does not cross the blood-brain barrier or only so in minimal amounts.)[16,a].

Iproniazid is a compound chemically related to isoniazid, and is noted to have an apparent central nervous system stimulatory effect; because of this effect it was coined "psychic energizer".

The isonicotinic acid derivatives are water-soluble neutral compounds and the hydrazid group is essential, since the corresponding amide and hydroxamic acid are inactive. As to the actual metabolism

of iproniazid little is known. In large concentrations the hydrazid inhibits the oxygen consumption and catalase activity of virulent and avirulent strains of bacteria, but the isopropyl derivative (iproniazid) is much less effective although it is an equally good growth inhibitor. It is known of iproniazid that it can inhibit the action of monoamine oxidase; however, but the exact means by doing so is not known.

Both compounds are rapidly absorbed from the intestine, but higher plasma levels are found for iproniazid because it is excreted more slowly by the kidney. Since it is, probably a larger per cent of it is metabolized. The excretory products found in the urine (about 65% of total dose in 24 hours) are isonocotinic acid and its amide, and some unchanged drug. Other than this little can be said of its metabolic fate.

IV. Serotonin

As was explained previously serotonin is found in many organs of the body including the central nervous system. Rather a moderate amount of experimentation has been done on the actual location and exact concentrations in different areas of the brain. In a recent paper on the subject, Bogdanski, Weissbach, and Udenfriend[18] found the serotonin concentration highest in dog's and cat's brain stem, rhinencephalon, and in the neostriatum. It was not present in the medullated nerve fibers but only in the gray matter. So far preformed serotonin has not been proven to occur in excised sympathetic ganglia of mammals despite the fact that homogenates have great capacity to form it. Recently Gertner, Paasonen, and Giarman[19] showed the apparent production of serotonin by ganglia in situ. Iproniazid was in the fluid perfusing the superior cervical ganglion of a cat. (This was used to keep monoamine oxidase from destroying the serotonin therefore making it easier to identify.) No serotonin was demonstrated in the perfusate until 2-3 hours; therafter it increased progressively.

Serotonin has been detected in the spinal fluid of patients with head injuries, brain tumors, and meningitis in dogs and cats with head injuries. [16,a]

The actual cellular locations of the serotonin in rat's brain is found in the mitochondria. Walaszck and Abood[20] found oxidative phosphorylation independent of the presence of serotonin, nor was it essential for the binding of serotonin by mitochondria. They

believe structural integrity of mitochondria important for sero-
tonin fixation, but the actual binding sites seem to exist within
the mitochondria.

As it can be noted the highest activity for serotonin meta-
bolism as well as the highest content of serotonin is in the auton-
omic nervous system and most specifically within the hypothalamus.
This can be fairly readily seen even by comparing monoamine oxidase
tissue level in relation to it. It has been observed that compar-
able levels of erotonin, monoamine oxidase, and tryptophan decar-
boxylase are found in the same area in relatively the same concen-
trations i.e., that is where one is high the other is high and visa
versa. (This does not prove necessarily that they are always found
in this relationship.) An experiment was done on dog brain. Sero-
tonin was added to homogenized portions of the brain tissue and the
monoamine oxidase of the brain reported as micrograms of added seroto-
nin destroyed per gram per hour. It was as follows: medulla 1,250,
pons 882, cereberllum 970, midbrain 903, hypothalamus 3,154, thalamus
886, and cortex 884. This may help to demonstrate why it is thought
that the serotonin as well as its related enzymes are higher in the
autonomic areas being highest in the hypothalamus.

Erspamer[2] believed earlier that serotonin was synthesized chiefly,
or entirely, in the enterochromaffin cells and released by them, but
the wide distribution of 5-hydroxytryptophan decarboxylase made it

questionable if this were true. It possibly could have been that
these cells made only 5-hydroxytryptophan, which was then decar-
boxylated elsewhere in the body. This would have to be true as
far as brain serotonin levels were concerned since serotonin per
se does not appear to traverse the blood-brain barrier, but there
is now good reason to believe that cerebral serotonin is formed
within the nervous system itself.[16,b]

As to the actual physiological action of serotonin in the cen-
tral nervous system two lines of thought have been raised. One is
that it is a possible transmitter and the other being an inhibitor,
in the transmission across synapses. Albrecht et al.[21] found a
small but significant decrease in the brain pool of serotonin in
mice prior to the usual time of arousal. Does this mean that the
serotonin is acting as a stimulant or inhibitant here? (The inter-
pretation of this would depend upon whether the sympathetic or para-
sympathetic was thought to be primarily involved.) Marrazzi and
Hart[22] showed that both adrenaline and noradrenaline elicit synaptic
inhibition. More recent work demonstrates that serotonin is the most
active of these inhibitors present in brain. Gluckman, Hart, and
Marrazzi[23] noted that the high inhibitory potency of serotonin is
characteristically limited to the brain. They further showed that
intra-carotid injection of iproniazid reproduced the picture of
serotonin action i. e., reducing both the evoked responses and

the electrocorticogram from the same electrode. What they call
"distortion of synaptic equilibrium" is believed by them to be
the result of hypothetical endogenous psychotogens for which ser-
otonin seems to be the best candidate. Since the electroenceph-
alogram findings and evoked responses are reduced by injection of ipro-
niazid, it is assumed by them that serotonin may be protected
and so accumulate at the synapses, in this way causing the reduc-
tion of activity. It is thus postulated that any exaggeration of the
inhibitory aspects of the chemical control of synaptic function,
either by accumulation of serotonin or by some metabolite resembl-
ing it, or by the increased sensitivity of the responding cells
to what might be normal amounts of inhibitor, could produce abnor-
mal patterns of behavior.

Much work needs to be done before a neurohumoral role for
ser-otonin in lower animals is established. Welsh[24] has pointed out
that serotonin may act as a neurohumoral agent in invertebrates. A
variety of observations, however, none of them in themselves final,
strongly suggests that serotonin has a variety of neurohumoral func-
tions. It may have inhibitory functions, on either the nerves or
non-cardiac muscle; it may have transmitter function in ganglia;
it may have cardio-regulatory functions; it may have all of these, but
it is unlikely that it has none.

Presenting just the opposite proposed activity of serotonin,

Brodie and Shore[25] have the concept that it is the chemical trans-
mitter of the central parasympathetic system, with noradrenaline
the central sympathetic transmitter in mammals. This hypothesis is
being accepted as a better explaination of the action of serotonin than
is the synaptic inhibitory proposal. They suggest that serotonin
may have this function for the following seven reasons:

1. Serotonin is present in the brain. 2. Serotonin is pre-
sent in highest concentration in the brain stem where the major part
of autonomic integration occurs. 3. Monoamine oxidase, which des-
troys it, is especially concentrated in the hypothalamus. 4. 5-hy-
droxytryptophan decarboxylase, which is able to synthesize serotonin,
varies in parallel with the cerebral serotonin content. 5. LSD
antagonizes the central effects of administered serotonin and evokes
marked central sympathetic activity. (d-lysergic acid diethylamide, LSD
is thought to be a competitive antagonist of serotonin and
when given to patients produces symptoms seen in psychotic patients.)[38]
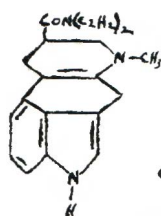
d-lysergic Acid diethylamide

6. When large doses of serotonin are given to mice intraperiton-
eally, a very small amount penetrates the brain and the mice become
sedated; hence the addition of the transmitter to brain has a dem-
onstrable central effect. 7. Inhibiting the inactivating enzyme

by iproniazid and then giving reserpine to release cerebral ser-
otonin does not elicit the usual effects of reserpine in animals.
(Brodie et al.,[36] stated reserpine releases bound serotonin in the
brain. The free serotonin is rapidly destroyed by monoamine oxidase,
thereby resulting in marked depletion of brain serotonin concentration.
Depression and slowing down of activity follows. In 1957, he seems
to contradict this original hypothesis. The contrast is given in
the rest of this discussion.) (Reserpine is a crystalline alka-
loid of Rauwolfia serpentina). On the contrary, the response is typical
of LSD including   violent excitation, mydriasis, exophthalmus, pilo-
erection and other signs of sympathetic activity. Administration
of large amounts of 5-hydroxytryptophan leads to high levels of
free serotonin in brain, again, the animals behave as though they had
received LSD. LSD does not prevent the release of serotonin; it
is presumed to act by blocking liberated serotonin. In 1957, Brodie[25]
suggested reserpine seems not to act by decreasing the total ser-
otonin content of brain. Serotonin is being made continuously, but
because of the reserpine the cells are unable to store it and it
is therefore released. The free serotonin, according to Brodie,[25]
is the cause of the sedation. Thus, reserpine acts by causing
a flow of highly active free serotonin which stimulates the syn-
apses of the central parasympathetic division. As a result, seda-
tion, miosis, hypotension, ptosis, and other signs of parasympatho-
mimetic activity occur. LSD, a serotonergic blocking agent, may

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inhibit nerve ransmission in the central parasympathetic system by anatagonizing serotonin at synaptic junctions.

Gertner, Paasonen, and Giarman[19] have been able to demonstrate the apparent production of serotonin by sympathetic ganglia of cats by injecting iproniazid into perfused superior cervical ganglia. Iproniazid produced an immediate, but partial inhibition of transmission, but no serotonin was demonstrable by the Venus heart method until 2 - 3 hours after beginning the iproniazid perfusion. Thereafter, progressively increasing amounts appeared in the perfusate. The presence of serotonin seemed unrelated to pre-ganglionic stimulation. In the absence of iproniazid, control experiments showed no serotonin for 6 hours. If the method of measurement is delicate enough and serotonin is not too rapidly destroyed, these experiments offer no support to the veiw that serotonin is a transmitter in sympathetic ganglia.

A number of investigators have presumably increased the cerebral tissue serotonin concentration by injecting the drug into the cerebral ventricles. Feldberg and Sherwood[26] found cats become passive, clumsy, and show loss of muscle power and tone, but are not sleepy. Bradley and Hance [27] found in unanesthetized cats similar effects from intraventricular injections of 200ug of serotonin. The electrical activity showed an increase in slow rhythms which still responded to sensory stimuli. Intra-peritoneal injection of LSd failed to antagonize the effects of serotonin. The effects of LSD and serotonin seemed to be synergistic as regards elect-

rical activity.

In conscious cats, Schwarz et al.[28] noted that serotonin in-
jected into the cerebral ventricles produced muscle weakness, rel-
ative motor immobilization, and salivation, without any significant
changes in the electroencephalongram. Intracerebral injection in
mice caused dep ession, itching of the face, tachypnea, micturition
and defecation.

According to Sacchi et al.,[16,c] serotonin injected into the
cisterna magna of dogs produces a profound catalepsy, but injected
intravenously is entirely without such an effect.

Di Stefano Leary, and Feldman[37] studied isolated supra-sylvian
cortices of cats, consluding that no correlation exists between the
depression of evoked contical potentials and oxygen tension lowering
that follows administration of serotonin.

Slocombe, Hoaglund, and Tozian[29] demonstrated that serotonin
resembles adrenaline in its effects on the spontaneous electrical
activity of the brains of albino rats, the effects being chiefly
determined by the type of anesthetic used. In pentothal anes-
thetized animals profound reduction of both frequency and ampli-
tude of electrical activity was found in response in these drugs,
while in ether nesthetized animals there was no significant ef-
fect. They suggested that the site of action of these compounds
is on pentothal-sensitive nonspecific pathways. The site of the
depressive action in animals under pentothal was further defined

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by the fact that both cortical and subcortical structures were equally affected, while respiratory and cardio-regulatory centers were not. The reticular formation seems a likely area for their action.

Benitez, Murray, and Woolley[30] found that in tissue cultures both human and rat oligodendroglia contracted strongly on the addition of serotonin. Woolley believes this is the cerebral counterpart of the effects of serotonin on smooth muscle and, therefore, the results on smooth muslce have a bearing on the reaction of the brain to serotonin and its metabolites. Geiger has also described pumping movements in human and rabbit neurons, initiated by serotonin in concentrations of 0.5-2ug/ml. The time for such a cycle of contraction and expansion varied between 1 and 2 hours. During the contractile phase, the base of the axon often showed a pronounced bulge which seemed due to movement of the cytoplasm under increased pressure. The pumping movements initiated in neurons by serotonin were not normally seen in such cultures.

It has been hoped that it is made clear that while serotonin surely plays some important part in the function of normal brain, it is not known what that function is. The evidence that serotonin is concerned in mental disease is rather small at the present.

V. Possible Participation of Serotonin in Mental Function:

Evidence has been reviewed that serotonin plays an important part in cerebral metabolism, but it must be noted from this evidence that its participation is anything but clear. Yet, the rather generally accepted scientific thought is that when it is known what serotonin does in the brain, it will prove to be important. With this lack of clarity in the understanding of the functions of serotonin in the normal brain, it is easy to see why the addition of the problems of mental disease does not make it clearer.

Investigators should be warned of the danger of inferring mental change from changes in behavior. This seems obvious, but several capable investigators have made unguarded statements such as, for example, that "schizophrenia or schizophrenia-like" states have been produced in animals. Some fail to remember that mental changes in animals do not lend themselves to easy observation. "Abnormal behavior" in animals carries with it certain connotations not all students are willing to grant. Further, it is sometimes difficult to know whether the effects of serotonin-anti-metabolites are due to the effects of the substance itself or to its antimetabolic effect. Because of these forenamed reasons much of the published observations must be reviewed with some reservation and scepticism.

So far the evidence that serotonin metabolism is disturbed in mental disease is not strong. A much more searching analysis of the problem is needed before any sound conclusions can be reached.

Poloni[31] claims to have found an excess of substance like serotonin in the blood of schizophrenia patients. But Rodnight and McIlwain[32] found the urinary excretion level of serotonin and related substances normal in a groups of mentally ill subjects; patients receiving 100ug LSD intravenously excreted less serotonin than normal during the succeeding 24 hours.

A newly described, genetically determined, disease, called Hartnup's disease, is characterized by pellagra-like skin rash along with intermittent cerebellar ataxia and mental retardation. There is a constant gross amino aciduria with a typical amino acid excretion pattern and an equally typical excretion of indole derivatives. Hartnup's disease seems to represent a diversion of tryptophan away from its conversion to nicotinamide. Study of the serotonin metabolism in this disease does not appear to have been carried out, and should be most interesting. It will be recalled that nicotinamide deficiency has been suggested as possibly occurring in carcinoid. The fact that mental deterioration is a part of the syndrome is also of importance in relation to indole metabolism of the brain.[16,d]

To recapitulate, after it was shown that serotonin occurred

in the brain, Woolley and Shaw[34] suggested that there might be an association chiefly on the basis that many of the antimetabolites of serotonin produced mental disturbances. Second, it was shown by Marrazzi and Hart's[22] work that serotonin, more effectively than any other agent so far tested, depressed synaptic transmission within the brain, suggesting that it is this interference with communication that causes mental disturbance. Third, Woolley and Shaw[33] showed that 5-hydroxytryptophan crosses the blood-brain barrier and is converted to serotonin by the decarboxylase in the brain. Administered iproniazid protects the serotonin from destruction. The serotonin content of the brain in animals so treated rises 3 or 4 fold and is associated with behavioral changes. Some, however, may think this due to increased synaptic transmission instead of depression. Lastly, there is a less impressive body of evidence that other indole-alkylamine-like substances can produce psychosis.

Concurrent with the growth of knowledge of the biochemistry of the brain, serotonin has taken its place. This substance, along with the hallucinogens, tranquillizers, and psychic energizer, seems to have formed a nidus around which new disciplines are forming. Serotonin was gotten out of the blood because of its nuisance value in the search for the vasoactive, angiotonin. It has proven to be a nuisance of quite a different sort.

VI. Iproniazid

As was explained earlier iproniazid is rather a new
deri-vative of isonicotinic acid having a psychic energizer action.
The ability of iproniazid to do this has advanced clinical med-
icine as well as giving research a good tool to help determine a
great deal more about serotonin. It is known to be an inhibitor of
monoamine oxidase and as such should advance the knowledge of
serotonin in the same manner that cholinesterase inhibitors have
advanced the knowledge of acetylcholine.

The ability of administered iproniazid to inhibit monoamine
oxidase activity in homogenates (Zeller et al.[34]) and to
potent-iate the pharmacological actions of 5-hydroxytryptophan
(Uden-friend et al.[35] has been taken as evidence that this drug is
a potent inhibitor of the actions of this enzyme in vivo. However,
if iproniazid is as effective in vivo as it is in homogenates then
one would expect it to produce striking pharmacologic effects when
administered alone due to increased levels of unmetabolized amines.
Actually, iproniazid produces little pharmacologic effect when
ad-ministered in quantity sufficient to inhibit almost completely
the enzymatic destruction of serotonin and other amines in homo-
genates. (This apparently is not true and is rather confusing
since it will be seen later that it does produce a pharmacologic
effect. Also actions on homogenates and intact animals cannot be

be taken as identical data.) The contradictory nature of these findings led to the following study concerning the efficiency of iproniazid as an inhibitor monoamine oxidase in vivo.

In previous studies on the biochemistry of 5-hydroxytryptophan it had been shown that administered iproniazid increases endogen-ous brain serotonin. It is apparent that in both rats and rabbits, iproniazid increased brain serotonin to levels 2 to 3 times normal. Of interest, too, is the rapidity with which the effects appeared, the highest level being attained in 3 to 5 hours. However, this increase was not maintained and the levels started to drop within a few hours, although the effects of administered iproniazid, as determined by inhibition of amine oxidation by homogenates, per-sisted for more than 24 hours (Zeller et al.[34]) When repeated doses of iproniazid were administered over several days, the brain sero-tonin levels were not above the level produced by a single dose. In the same animals in which brain serotonin levels were markedly increased, no increase in any of the other tissue depots was observed i. e., blood, stomach, and intestine. (One may interpert this as meaning that the iproniazid activity may possibly have been selective being present only in the central nervous system.)

The following experiments were carried out with intact mice. Serotonin or its precursor, 5-hydroxytryptophan, were administered to mice, both normal and treated with iproniazid. At intervals the mice were sacrificed, homogenized with their excreta, and assayed

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for serotonin. It was noted that the administration of ipron-
iazid had little, if any effect on the metabolism of serotonin.
This was true whether the compound administered was serotonin or
its precursor. (Although a single dose of iproniazid was well
tolerated, repeated daily administeration produced severe toxi-
city making it ifficult to use the animals for experimental pur-poses.
200mgm/kg were given rabbits, and 240mgm/kg were admin-istered rats.)

Although the serotonin formed from 5-hydroxytryptophan was
not significantly increased in the whole animal by administration
of iproniazid, the iproniazid-treated animals did show central ef-fects
suggesting that metabolism of exogenous serotonin might be inhibited
centrally. Accordingly 5-hydroxytryptamine was adminis-tered to mice
following iproniazid and the brains and carcasses
were assayed separately. It was seen that although iproniazid had
no effect on the levels of serotonin in the carcass there was a large
increase in brain serotonin. (It should be understood that the amount
of serotonin in the brain is very small compared to the
total body serotonin. Therefore, a substantial increase in brain
serotonin would not significantly increase total body serotonin.) Thus,
once again, the ability of iproniazid to influence serotonin metabolism
in viro was demonstrable only in the central nervous system.

In rats, too, iproniazid had little effect on the metabolism
of administered serotonin. Nine mgm/kg of serotonin were injected

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into control and iproniazid treated rats and the plasma levels were assayed at intervals. Levels of approximately 1 to 2 microgm./ml of plasma were obtained in 15 minutes and in both cases there was a 70% drop at the end of one hour. (This may also point to the selective central nervous system action of iproniazid.)

Although he destruction of injected serotonin in the intact animal was unaffected by iproniazid, homogenates prepared from tissues of these same iproniazid treated animals were practically de-void of any ability to destroy serotonin. Such findings were most puzzling and suggested that either a much more potent catalyst for serotonin destruction existed in intact cells before homgenization, or that iproniazid did not reach the site of monoamine oxidase act-ivity in intact cells. There was nothing to support the first poss-ibility since he rate of destruction of serotonin in homogenates appeared to be consistent with its rate of destruction in the intact animal. It has also been shown that 5-hydroxyindoleacetic acid, the expected end product of the action of monoamine oxidase on serotonin, is the major product of serotonin metabolism in vivo (Udenfriend et al.[11]) The second possibility, that iproniazid did not reach the site of enzyme action in the intact cells, was therefore considered more likely. To investigate this possibility monoamine oxidase activity was measured by Udenfriend[35] in slices and homogenates from iproniazid treated animals and these values were compared with those obtained on tissues from untreated control animals. It was

seen that both liver and brain slices from iproniazid treated animals
were capable of metabolizing serotonin at virtually the same rates
as slices from the untreated controls. On the other hand, homogenates
prepared from portions of the same tissues from the iproniazid treat-
ed animals were completely devoid of activity.

It should be pointed out that the initial activity of ipron-iazid
slices was comparable to that of controls but started to decrease
after 15 to 30 minutes. This decline in rate has been
taken to signify disintegration of the tissue slices. For this reason
they found it difficult to establish a difference in the behav-ior of
slices and homogenates towards iproniazid when the inhibit-
or was added in vitro. For this inhibitor to be effective in the
in-cubator flask, it must be preincubated with the tissues for up to
30 minutes before the substrate is added. Unless such preincuba-tion
is carried out little inhibition is observed (Zeller et al.[34]) However,
the time required for in vitro preincubation is as long

as that found to abolish most of the differences between slices
and homogenates observed on tissues from animals to which iproniazid
had been administered in vivo. Even with these difficulties slices
were found to be inhibited to a much lesser degree than homogen-
ates by the addition of iproniazid in vitro.

Because of the observed effect of iproniazid on brain sero-tonin
levels it was thought that at least in this tissue there
would be a demonstrable effect of iproniazid on the intact cells.
However, it is apparent that brain slices do not differ from liver

slices in this respect. The failure to observe such a difference may indicate that only certain areas of the brain are responsible for the observed in vivo effects of iproniazid on serotonin in the central nervous system.

Monoamine xidase can act on a variety of amines and tyramine has been widely used as a substrate in studies on this enzyme. To determine whether the effects described above were unique for serotonin (Udenfriend et al.[35]) or occurred with other amines comparable experiments were repeated with tyramine. It was found that iproni-azid had little influence on the destruction of parenterally ad-ministered tyramine in intact mice. Furthermore, as with serotonin, slices from iproniazid treated rats were able to metabolize tyramine although homogenates prepared from these same tissues were devoid of such activity.

Iproniazid has been found to be particularly useful in the treatment of depression (manic, involutional, or reactive); however, this therapy should be reserved for those depressed patients who have not responded to the milder central nervous system stimulants. It also may be employed to stimulate appetite and weight gain in debilitated and postoperative patients, and in patients with chronic debilitating disorders. The drug may be useful as adjunctive ther-apy in rheumatoid arthritis when associated with depressed psychomotor activity. It stimulates physical and mental activity, appetite and weight gain without objective joint changes. However, the use

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of iproniazid should be restricted to the severe disabling form of the disease in which the risk inherent in this therapy seems warranted. The side reactions which have been noted are over stimulation, jaundice, constipation, paresthesias, dizziness, sweating, dryness of the mouth, and delay in starting micturition. Postural hypotension of quite severe nature has been noticed on occasions, especially in doses in the ex-cess of 100mgm daily. This should be treated by withdrawal of the drug and the routine shock therapy. Mild hypochromic anemia some-times may occur early in the institution of the therapy but is self limited upon withdrawal of the drug. Some of these side effects mentioned seem to respond also quite well to administeration of vitamin B_6 (pyridoxine HCL) which tends to agree quite well with what might be expected from some of the experimental evidence.

The main contraindications known at present are lack of cor-rect knowledge of the drug action on the part of the therapist, patients with a history of previous liver disease or with impaired liver function. Occasional liver function tests should be run while administering the drug. Also known epileptic patients definitely should not use this drug.

Since iproniazid has a cumulative action, the dosage should be reduced after improvement is evident. This may not be noticed for several days. Generally a starting dose suggested is 50 mgm a day and then withdrawal to maintenance of 10 to 25 mgm a day as the patient responds. Withdrawal effects have been noted as the drug is stopped and are usually present within 48 hours. The symptoms

may include headache, nervousness, vertigo, insomnia, and depression. These symptoms are much less severe if the withdrawal is gradual.[7]

VII. Summary

1. The short history of serotonin and iproniazid was reviewed revealing that serotonin was first identified in human plasma while actually studying angiotonin. Much later its possible action and location in the brain was noted. Iproniazid is an isopropyl derivative of isoniazid and was synthesized for its antibacterial activity primarily. It was found to be rather weak in this respect but its "side reaction" of possible central nervous system "stimu-latory effects" becomes its primary importance at the present.

2. The limited knowledge of serotonin metabolism as related to the brain is presented. That is, that it is produced indirectly from tryptophan $\longrightarrow$ 5-hydroxytryptophan $\longrightarrow$ serotonin, and its degradation product being 5-hydroxyindole acetic acid. Mention is made of the enzymes that predominate in these transformations. It is also noted that serotonin reduces glucose consumption of the brain, slightly increases uptake of P^{32}, and reportedly increases and decreases oxygen consumption of the brain by independent workers. As to the exact location of serotonin in the brain tissue, it was demonstrated to be highest in the hypothalamus and related auton-omic centers. The exact physiological action here is not known since it has been thought by many to be both a synaptic transmitter and inhibitor. Good explaination given by one group of researchers suggests that it may be a parasympathetic transmitter and noradrenaline being the sympathetic synaptic transmitter.

3. The pharmacology and metabolism of iproniazid was review-ed which was found to be somewhat limited at the present. Its ef-fect as to monoamine oxidase inhibition, inhibition of catalase activity, and oxygen consumption are discussed. Its action in cen-tral nervous system physiology has been studied and apparently it functions indirectly by its inhibition of monoamine oxidase and the secondary increase of serotonin. The physiological findings that follow are both subjective and objective in humans and are rather hard to evaluate. Supposedly it produces increase emotional stimu-lation and mood lifting which from experimental evidence could be expected. The clinical uses and dosages for humans are given.

4. Very limited work and theory has been presented as to the possibility of serotonin being involved in mental illness. The evidence at present is small and no definite conclusions were drawn.

VIII. Conclusion

The review of the literature on serotonin in the central nervous system up to the present is very interesting, but with some confusing facets. This, of course, is easy to understand since any biochemical studies of the brain tend to be such and so much more so when working on the chemistry and supposed relation it may have to mental functions. The small quantity of the seroto-nin and related enzymes in the brain makes the work even more tedi-ous. Then trying to correlate the objective chemical findings with the physiological, emotional, and subjective findings adds great difficulty in good scientific evaluation.

Very definitely, the research on serotonin and iproniazid has only begun. What is learned by one magnifies what can be theorized and learned about the other. About all that definitely can be said now is that serotonin must be an intimate part of the complex chemical machinery of the brain; the exact function and action not known but thought to be related to synaptic transmission. Its location quite well established in the autonomic, hypothalamic center of the brain, may help explain why iproniazid has the effect it does of a mood lifter or "psychic energizer".

The research in this field in the future may definitely shed a new light into the understanding and treatment of mental illness.

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