A STUDY OF THE TRANSPORT OF 5-HYDROXYTRYPTOPHAN AND 5-HYDROXYTRYPTAMINE (SEROTONIN) INTO BRAIN[1, 2]

SAUL M. SCHANBERG[3]

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut

Accepted for publication November 19, 1962

A study of the uptake of 5-hydroxytryptamine (5-HT, serotonin) and its precursor, 5-hydroxytryptophan (5-HTP), by brain cells is essential for an understanding of both the synthesis of 5-HT and the availability of 5-HT to the brain. An investigation of such transport also is indicated because of recent evidence (Crossland, 1960) that the brain does not hydroxylate tryptophan; rather, it appears to derive its 5-hydroxytryptophan from extraneural sources, probably gastrointestinal mucosa (Dalgliesh, 1958; Cooper and Melcer, 1961) and liver (Freedland *et al.*, 1961).

Furthermore, certain drugs can alter the total level as well as the subcellular distribution of 5-HT in the brain of the rat (Giarman and Schanberg, 1958; Schanberg and Giarman, 1962). The explanation of some of these drug-induced changes might lie in an altered transport of 5-HTP or 5-HT into the brain.

Previous work in this laboratory has shown that there is a facilitated transport of 5-HTP by slices of rat brain (Schanberg and Giarman, 1960). This preliminary study has been extended along three main lines: (1) a comparative study of the uptake of radioactive 5-HT and 5-HTP by brain slices; (2) a study of the effect of a number of pertinent drugs, metabolites and amino acids on the uptake of 5-HTP by slices of rat brain; and (3) an investigation of the uptake of 5-HTP by various regions of bovine and rabbit brains.

METHODS. The techniques used throughout these studies consisted of the measurement of the uptake of DL-5-hydroxytryptophan-1-C^{14} and 5-hydroxytryptamine-1-C^{14} by brain slices incubated in a phosphate-buffered medium, pH 7.4 (Rodnight and McIlwain, 1954). The medium was modified by reducing the level of $CaCl_2$ from 0.11 to 0.022 M. Where indicated, glucose (10^{-2} M) or dinitrophenol (DNP, 10^{-4} M) or both were added to the medium.

Incubation procedure. Brain slices were made sagittally along the central sulcus so as to include both cortical and subcortical areas; of such slices, 200 mg (wet wt) were placed in 2.0 ml of the cold medium. The slices were then preincubated at at 37.5°C for 10 minutes in an atmosphere of 97% O_2 and 3% CO_2 in a Dubnoff metabolic shaker to allow the metabolism of the tissue to come to equilibrium. Radioactive 5-HTP or 5-HT was then added, together with any experimental drugs; the final fluid volume in the incubation flask was maintained at 3.0 ml. In certain experiments the experimental drugs were added before the preincubation period. At the end of the incubation period the slices were removed and rapidly washed in 2 ml of fresh medium.

Extraction and assay of radioactive substances. The tissue samples were homogenized in 10 ml of 70% ethanol and allowed to stand for 1 hour at 4°C; after centrifugation at low speed for 10 minutes, the clear supernatant fluid was decanted. An aliquot of the supernatant fluid was plated, dried and counted in a Nuclear Chicago windowless gas flow counter. Recovery tests showed that approximately 90% of the initial radioactivity of a sample could be estimated in this manner.

The radioactivity measured is regarded as a reflection of the 5-HTP-C^{14} or 5-HT-C^{14} in the tissue, plus any metabolic derivatives bearing the labeled carbon. Chromatographic analysis by means of Dowex 1-formate columns or paper [isopropanol–water–concentrated ammonium hydroxide (100:10:15)] indicated, however, that over 90% of the radioactivity recovered in these studies was still in the original form of the administered compounds, *i.e.*, 5-HTP or 5-HT.

C^{14}-labeled chemicals—DL-5-HTP-1-C^{14} [5(OH) indole-$\overset{*}{C}H_2$ $CHNH_2$] and 5-HT-1-C^{14} [5(OH) indole-$\overset{*}{C}H_2$ $CHNH_2COOH$] were obtained from the California Corporation for Biochemical Re-

Received for publication July 5, 1962.

[1] This work represents partial fulfillment of the requirements of the Yale Graduate School for the Ph.D. degree. It was supported by grant B-940 from the National Institute of Neurological Diseases and Blindness, USPHS.

[2] Preliminary reports of this work have appeared in: Biochim. biophys. Acta **41**: 556, 1960; Fed. Proc. **20**: #1, 1961.

[3] The work was done during the tenure of a USPHS pre-doctoral fellowship.

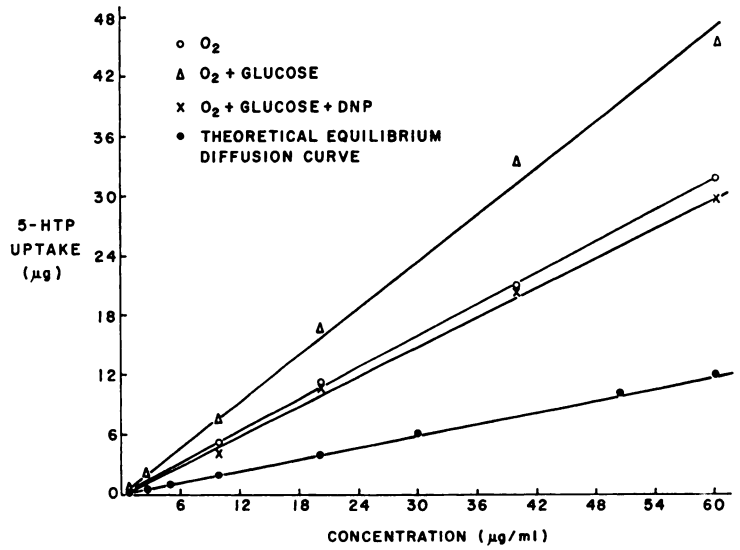

FIG. 1. Relationship of concentration of DL-5-HTP (1-C^{14}) to its uptake by slices of rat brain. Incubation time was 60 minutes. Theoretical equilibrium diffusion curve was calculated on the assumption that the tissue is 100% water, and, therefore, represents 0.2 ml.

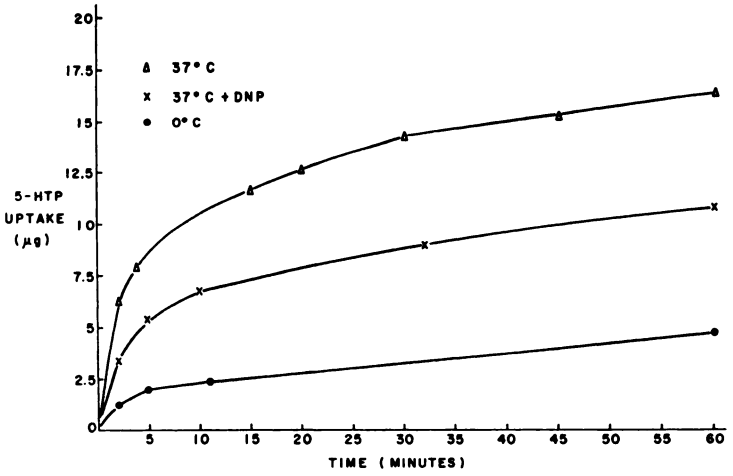

FIG. 2. Rate of uptake of 5-HTP by slices of rat brain. A fixed amount of 20 μg DL-5-HTP (1-C^{14}) was used and glucose (10^{-2} M) was present in all three experiments.

search and the Nuclear Chicago Corporation. Radiochemical purity was 99% as determined by paper chromatography. The specific activities of these substances as used in these experiments were approximately 7.5×10^5 cpm $mmol^{-1}$ of 5-HTP and 2.3×10^6 cpm $mmol^{-1}$ of 5-HT.

RESULTS. *Comparative uptake of radioactive 5-HTP and 5-HT.* Glucose and O_2 each increased the uptake of 5-HTP-1-C^{14} by slices of rat brain, and dinitrophenol (DNP) inhibited the combined effect of O_2 and glucose (fig. 1). About 65% of the total uptake of 5-HTP occurred within 10 minutes (fig. 2). After that, a slow uptake continued at the same rate as that observed at 0°C. This slow uptake probably represents diffusion. Lowering the temperature to 0°C was more effective than DNP in blocking the uptake of 5-HTP (fig. 2).

5-HT-1-C^{14} was not transported into brain slices by a similar energy-requiring system. Al-

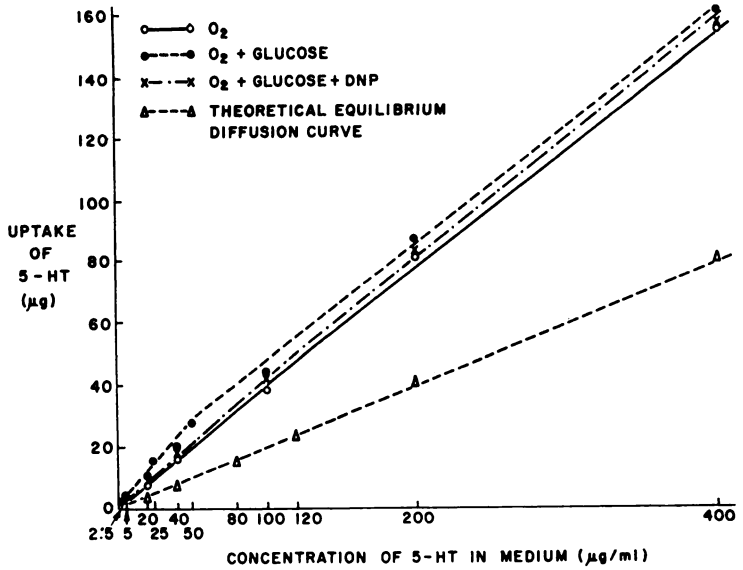

FIG. 3. Relationship of concentration of 5-HT (1-C^{14}) to its uptake by slices of rat brain. Incubation time was 60 minutes.

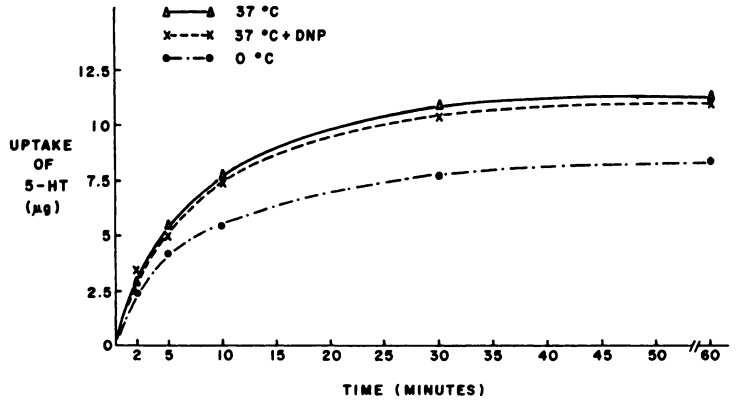

FIG. 4. Rate of uptake of 5-HT (1-C^{14}) by slices of rat brain. 20 μg 5-HT (1-C^{14}) was used and glucose (10^{-2} M) was present in all three experiments.

though the 5-HT concentration curves are somewhat higher than the theoretical diffusion curve, its uptake was not significantly increased by O_2, or decreased by DNP (figs. 3 and 4).

Uptake of 5-HTP was markedly dependent upon pH and temperature (figs. 5 and 6). Clearly, the 5-HTP curves reflect an optimal uptake in the biological range, *i.e.*, pH 7.4 and 37.5°C, while the uptake of 5-HT does not demonstrate either critical temperature or pH relationships. The Q_{10}-value (calculated from the data in fig. 5) for the uptake of 5-HTP between 17°C and 27°C at pH 7.4 was 1.6; this figure corrected for diffusion becomes 2.3; on the other hand, the Q_{10}-value for 5-HT uptake was 1.2 and when similarly corrected falls to 1.0. These values suggest a facilitated transport for 5-HTP but not for 5-HT.

The 5-HTP transport mechanism of the brain could not be saturated by 5-HTP at the levels tested (fig. 7). Owing to the relative insolubility of 5-HTP in saline solutions, higher levels of 5-HTP in the medium could not be tested.

The uptake of 5-HTP under anaerobic conditions, at two concentrations (10 and 20 μg/ml), was approximately 50% more effective in the presence of glucose than in its absence or when NaF was added simultaneously (table 1). The

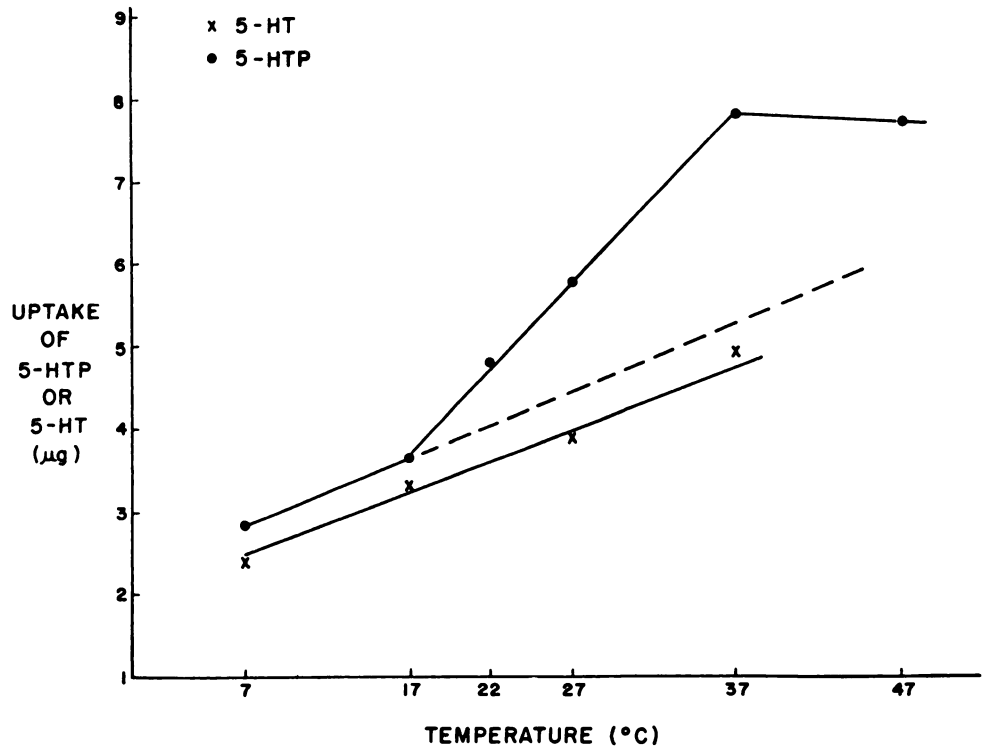

FIG. 5. Effect of temperature on the uptake of 5-HTP (1-C^{14}) and 5-HT (1-C^{14}) (20 µg/ml) at initial rates of reaction (8 min).
The Q_{10}-values for 5-HTP and 5-HT are 1.6 and 1.2, respectively; when corrected for diffusion they are 2.3 and 1.0, respectively. Each point represents 4 determinations.

data presented in figure 8 show that a low intracellular/extracellular (In/Ex) potassium concentration-ratio led to an inhibition of the uptake of 5-HTP, while on the other hand, a high In/Ex potassium ratio increased the transport of 5-HTP into the slice.

A comparative study of the uptake of radioactive 5-HT and 5-HTP by a number of selected tissues was also undertaken (table 2). The greatest uptake of 5-HTP occurs in those tissues that have a high 5-HTP decarboxylase activity and are known to store 5-HT (*e.g.*, mast cells and brain). In these tissues there is approximately a 20- and a 4-fold increase, respectively, over the theoretical equilibrium diffusion values. These increases were considerably lowered by the action of DNP. It is of interest that a tissue like spleen, which has no decarboxylase activity, but stores large quantities of 5-HT (presumably obtained from the destruction of platelets), has a low uptake of 5-HTP, almost equivalent to the amount that theoretically enters by diffusion. The slightly increased uptake of 5-HTP by liver and spleen caused by DNP is probably due to increased permeability of the cell membranes. Green and Day (1961) first demonstrated the concentrating capacity of neoplastic mast cells for 5-HT, and it may be seen in table 2 that these cells alone, in contrast to other tissues studied, concentrated the largest amount of 5-HT, a phenomenon which was significantly inhibited by DNP.

The influence of various substances on the uptake of 5-HTP by brain tissue. A wide variety of drugs that are capable of modifying the function of brain or of influencing the metabolism of 5-HT were tested in concentrations known to alter pertinent enzyme systems or the general behavior of rats; these included reserpine, 5-HT (in concentrations up to 10 times the equimolar concentration of 5-HTP), LSD-25, chlorpromazine, imipramine, iproniazid, α-methyl - dihydroxyphenylalanine, morphine and phenobarbital. None of these agents changed the uptake of 5-HTP (table 3).

The stability of the 5-HTP-transport system was further shown by the inability of other "neu-

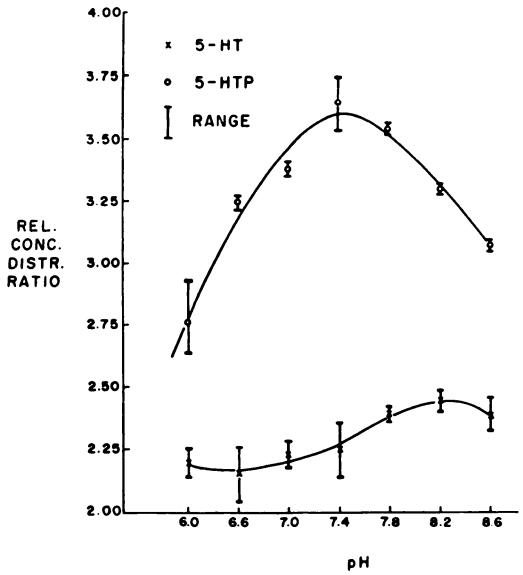

FIG. 6. The effect of pH on the relative concentration-distribution ratio of 5-HT (1-C^{14}) and 5-HTP (1-C^{14}).

Each point represents at least 4 separate determinations. The relative concentration-distribution ratio refers to the ratio of the substrate concentration found in the slice to that in the medium.

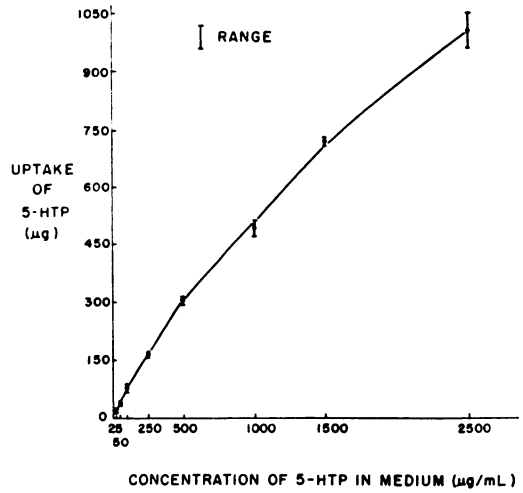

FIG. 7. The relationship between the concentration of 5-HTP (1-C^{14}) in the medium and its uptake by rat brain slices.

Each point represents 6 individual determinations.

roamines," such as histamine, norepinephrine, epinephrine and *gamma*-aminobutyric acid, to influence significantly the uptake of the amino acid (table 4).

TABLE 1

Uptake of 5-hydroxytryptophan by brain slices under anaerobic conditions[a]

Conditions	Mean Uptake of 5-HTP (µg/200 mg/60 min)	
	Added conc. 20 µg/ml	Added conc. 10 µg/ml
Oxygen + Glucose	14.28	7.76
Nitrogen + Glucose	8.94	5.80
Nitrogen	6.23	4.52
Nitrogen + Glucose + Sodium fluoride	5.97	4.14

[a] Warburg flasks flushed with pure nitrogen. Each value represents 3 values.

In contrast to these inactive agents, several amino acids significantly inhibited the uptake of 5-HTP by rat brain slices (table 5). Thus, L-phenylalanine inhibited the accumulation of 5-HTP in the slices to the extent of 45% of that seen in the untreated control slices. It is important to realize that the total accumulation of radioactive compounds measured in the slice is a reflection of the sum of the 5-HTP that has entered by diffusion as well as by facilitated transport mechanisms. Accordingly, one can assume that an inhibition of 45% actually reflects an even greater inhibition of the facilitated transport system. The findings with L-tyrosine and L-dihydroxyphenylalanine show that the addition of phenolic hydroxyl groups to phenylalanine causes a decrease in inhibitory activity. Furthermore, the addition of an α-methyl group to an active compound (dopa) causes an almost complete loss of inhibitory activity. This reduction in activity by α-methylation was observed also in the tryptophan series. Whereas tryptophan is a powerful inhibitor of the 5-HTP transport system, the α-methyl derivatives of tryptophan and 5-hydroxytryptophan are relatively inactive. The addition of a benzyloxy group on the 5-position

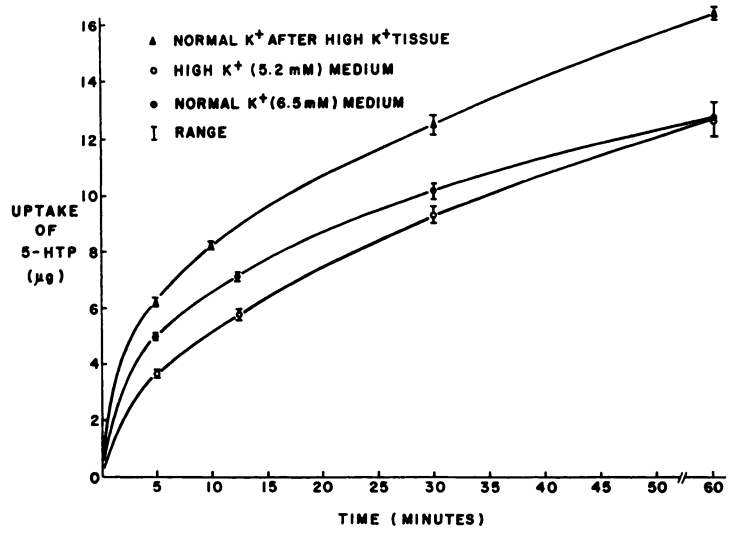

FIG. 8. The effect of K^+ on the uptake of 5-HTP by slices of rat brain.
High intracellular tissue levels of K^+ were obtained by preincubating brain slices for 60 minutes in a high K^+ medium (52 mM), in which the Na^+ had been reduced from 134 mM to 82 mM. These slices were then incubated for varying periods of time in the normal medium containing the radioactive 5-HTP.

TABLE 2

Comparative uptake of 5-hydroxytryptophan and 5-HT by several selected tissues

Tissue	Species	5-Hydroxytryptophan Decarboxylase Activity[a]	Serotonin Storage[b]	Uptake of 5-HTP (µg/200 mg)[c]	Uptake of 5-HTP (µg/200 mg) + DNP	Uptake of 5-HT (µg/200 mg)[c]	Uptake of 5-HT (µg/200 mg) + DNP
Neoplastic mast cells	Mouse	+	+	68.56	30.9	32.20	9.30
Brain	Rat	+	+	15.09	10.15	9.20	7.86
Kidney	Rat	+	−	8.74	8.12	11.22	8.09
Liver	Rat	+	−	5.80	6.28	10.85	9.57
Spleen	Rat	−	+	4.39	5.43	8.22	6.60
Ehrlich ascites cells	Mouse	−	−	4.36	3.69	2.72	3.20

[a] Plus indicates activity, minus indicates no activity (Gaddum and Giarman, 1956; Giarman, unpublished observations).

[b] Plus indicates storage, minus indicates no storage (Erspamer, 1954; Giarman, unpublished observations).

[c] The theoretical equilibrium diffusion value is 4.00. (Calculated on the assumption that the tissue is 100% water, and, therefore, represents 0.2 ml.)

of 5-HTP, however, did not abolish inhibitory activity.

The inhibitory action of tryptophan resides only in the L-isomer; D-tryptophan is completely inactive (table 6).

Other amino acids, such as leucine, isoleucine, and proline can also exert a potent inhibition on the uptake of 5-HTP (table 7).

The uptake of 5-HTP-(1-C^{14}) by various areas of bovine and rabbit brains. It was not possible to obtain adequate amounts of the smaller regions of rabbit brain for study; but when comparable areas from both rabbit and bovine brain were available, it was found that they showed similar regional rank-order patterns. Those areas known to be rich in endogenous serotonin and in 5-hydroxytryptophan decarboxylase activity had the highest capacity for concentrating 5-HTP-1-C^{14} from the medium, *e.g.* (by decreasing capacity), the caudate nucleus, the amygdala, the hippo-

TABLE 3

The influence of certain drugs on the uptake of 5-HTP-C^{14}[a] by rat brain slices (200 mg wet weight) as percent of control[b]

Drug Added	Conc. of Drug Added to Medium or Administered Intraperitoneally	Duration of Incubation	
		6 min	12 min
1. Phenobarbital	125 mg/kg—2.5 hr	93.7	97.7
2. Reserpine	5 mg/kg—5 hr	102.0	98.0
3. Reserpine	5 mg/kg—48 hr	101.4	108.5
4. Chlorpromazine	25 mg/kg—2 hr	85.1	90.5
5. LSD-25	500 μg/kg—30 min	97.0	92.9
6. Iproniazid	100 mg/kg—15 hr	104.1	93.6
7. Imipramine	15 mg/kg—1 hr	94.0	93.1
8. Morphine sulfate	60 mg/kg—2 hr	104.0	99.8
9. Carbachol	1 μg/ml	108.0	112.4
10. Physostigmine sulfate	200 μg/ml	116.9	86.9
11. 5-Hydroxyindole acetic acid (5-HIAA)	200 μg/ml	102.1	94.8
12. Pyridoxal phosphate	247 μg/ml (1 mM)	97.4	99.6
13. Dinitrophenol (DNP)	18.4 μμg/ml (10^{-4} M)	74.0	76.6

[a] Concentration in medium: 20 μg of 5-HTP-C^{14} per ml.
[b] Each value represents the average of at least 4 experiments.

TABLE 4

The influence of certain amines and amino acids on the uptake of 5-HTP-C^{14} by rat brain slices (200 mg wet weight) as percent of control[a]

Compound Added	Conc. of Compound Added to Medium or Administered Intraperitoneally	Duration of Incubation	
		6 min	12 min
1. α-Ethyltryptamine	200 μg/ml	95.6	96.8
2. 5-HT	200 μg/ml	90.5	93.6
3. Histamine	100 μg/ml	111.0	97.1
4. L-Histidine	200 μg/ml	81.4	92.7
5. γ-Aminobutyric acid (GABA)	5.2 mg/ml	98.2	98.8
6. Glutamic acid	25 mg/ml	108.9	99.8
7. Norepinephrine	200 μg/ml	—	104.5
8. Norepinephrine	1.7 mg/ml	92.2	81.7
9. Epinephrine	200 μg/ml	—	96.6
10. Epinephrine	1.1 mg/ml	102.3	88.6

[a] Each value represents the average of at least 2 experiments.

campus and the thalamus. Two structures stood out as exceptions to this: namely, the medulla and the pineal body. The latter is especially interesting in view of the relatively high levels of 5-HT reported by Giarman *et al.* (1960) to be present in the pineal tissue of a number of species, including the cow. The possibility that 5-HT is actively transported into the pineal body, in contrast to other regions of the brain, is now being investigated.

Other brain regions tested are also listed with respect to decreasing capacity to transport 5-HTP, the cortex (gray), cerebellum, hypothalamus, tectum, medulla and pineal body.

DISCUSSION. The data from the studies of the uptake of 5-HTP-(1-C^{14}) and 5-HT-(1-C^{14}) have indicated that 5-HT is not transported in the same manner as its precursor by brain slices, nor do brain slices behave like platelets (Born and Bricknell, 1959) or mast cells (table 2) in concentrating 5-HT. The difference in cerebral transport of 5-HT is evident from the following: (1) the fact that the uptake of 5-HT is not significantly increased by oxygen or glucose, (2) the failure of pH and temperature variation to alter significantly the uptake of 5-HT, (3) a calculated

TABLE 5

The influence of certain aromatic amino acids on the uptake of 5-HTP-C^{14}[a] by rat brain slices (values expressed as percent of control[b])

Compound Added	Conc. of Amino Acid Added to Medium or Administered Intraperitoneally	Duration of Incubation	
		6 min	12 min
1. L-Phenylalanine	200 μg/ml	59.9	54.0
2. L-Tyrosine	200 μg/ml	73.7	68.6
3. DL-Dihydroxyphenylalanine (dopa)	200 μg/ml	75.6	76.7
4. L-Dopa	200 μg/ml	68.7	66.0
5. α-Methyldopa	200 μg/ml	96.4	92.2
6. α-Methyldopa	400 mg/kg[c]	96.3	95.8
7. α-Methyldopa	400 mg/kg[c] and 21 μg/ml (0.1 μM)	99.6	97.2
8. Tryptophan	200 μg/ml	—	56.7
9. α-Methyl-5-HTP	200 μg/ml	116.1	86.9
10. α-Methyltryptophan	200 μg/ml	89.5	84.6
11. 5-Benzyloxytryptophan	200 μg/ml	66.7	58.1

[a] Concentration in medium: 20 μg of 5-HTP-C^{14} per ml.
[b] Each value represents the average of at least 4 experiments.
[c] Animals sacrificed 45 minutes after drug.

TABLE 6

The effect of various concentrations of D- and L-tryptophan on the uptake of 5-HTP-C^{14} by rat brain slices[a] (values reported as percent of control)

5-HTP (μg/ml)	L-Tryptophan 20 μg/ml	L-Tryptophan 200 μg/ml	D-Tryptophan 20 μg/ml	D-Tryptophan 200 μg/ml
10	77.0	61.0	100.8	85.8
20	80.0	56.7	105.0	91.2
40	81.6	53.3	96.0	97.7

[a] Incubation time: 12 minutes.

TABLE 7

The influence of certain nonaromatic amino acids on the uptake of 5-HTP-C^{14}[a] by rat brain slices (values expressed as percent of control[b])

Compound Added	Conc. of Compound Added to Medium (μg/ml)	Duration of Incubation	
		6 min	12 min
1. L-Leucine	200	63	53
2. L-Isoleucine	200	62	57
3. L-Proline	200	80	61
4. L-Glycine	200	110	106
5. L-Arginine	200	110	101
6. L-Lysine	200	107	—
7. L-Alanine	200	80	86
8. DL-Valine	400	—	109

[a] Concentration in medium: 20 μg of 5-HTP-C^{14} per ml.

[b] Each value represents the average of at least 4 experiments.

Q_{10}-value (1.2) that suggests a nonenzymatic mechanism, and (4) the inability of respiratory inhibitors like dinitrophenol to inhibit the uptake of 5-HT by the brain slice.

The finding that 5-HTP but not 5-HT is actually transported by brain tissue may explain some of the results reported by Glasser and Mantegazzini (1960). These investigators found that the electroencephalographic pattern of midpontine pretrigeminal preparations of cats was influenced by the intracarotid injection of 5-HTP only and not by a similar injection of 5-HT.

The finding that the 5-HT concentration curves are somewhat higher than the theoretical diffusion curve, possibly can be explained by the fact that the particulate fraction of brain homogenates adsorbs exogenously added 5-HT at 0°C, as well as at 37°C (Giarman and Schanberg, 1958). It is likely that as the 5-HT diffuses into the slice, it similarly becomes adsorbed to some of the particulate structures of the cell, effectively shifting the diffusion equilibrium towards the accumulation of more 5-HT in the cells than would theoretically occur on the basis of a simple diffusion process.

The demonstration (table 1) that the energy of glycolysis, even under strictly anaerobic conditions, enables brain cells to concentrate 5-HTP actively is similar to the findings of Negelein (1952) and Johnstone (1959), which indicated that Ehrlich ascites neoplastic cells under similar conditions take up certain amino acids against a concentration gradient. It was also reported by Hughes and Brodie (1959) that the uptake of 5-HT by platelets can occur as readily anaerobically as aerobically. However, it has been reported that rat brain slices incubated under 95% N_2 and 5% CO_2 did not concentrate L-tyrosine (Guroff *et al.*, 1961).

It has been shown that a high concentration of potassium ions leads to an inhibition of amino acid transport by Ehrlich ascites cells (Christensen and Riggs, 1952) and of the uptake of L-tyrosine in rat brain slices (Guroff *et al.*, 1961). Christensen and Riggs (1952) observed that the uptake of neutral amino acids produced a somewhat less than stoichiometric loss of potassium from cells, with replacement by sodium ions; the accumulated amino acids were in turn displaced when the extracellular K^+ level was again elevated (Christensen *et al.*, 1952). The data in figure 8 indicate that 5-HTP transport in the brain is similarly dependent upon potassium, the uptake of 5-HTP being increased apparently by an efflux of K^+ from brain slices made high in K^+ and decreased by an influx of K^+ from a high K^+ medium (52 mM) into normal brain slices.

The findings that 5-HTP, and not 5-HT, is actively transported by brain tissue and that 5-HT in brain is associated with the particulate fraction (Giarman and Schanberg, 1958) strongly support the concept that 5-HTP is actively transported *in vivo* into the brain cell where it is decarboxylated to 5-HT, which is subsequently bound and stored, or metabolized further. This differs from the proposed mechanism of 5-HT storage in platelets, where the high concentration of 5-HT is maintained entirely by an active transport mechanism for this amine (Hughes and Brodie, 1959).

It was found that L-tryptophan, L-phenylalanine, L-leucine and L-isoleucine were the most potent of the inhibitors tested. Interestingly, Christensen *et al.* (1952) have shown that these amino acids competed *in vitro* for the transport of one another in Ehrlich ascites cells. These investigators divide the amino acids into three groups: (1) neutral (dipolar), (2) cationic, and (3) anionic. They found, in general, that the transport of a neutral amino acid was inhibited by dipolar and stimulated by cationic amino acids. The anionic group had no significant effect upon

the uptake of neutral amino acids. From the data previously described, it can be seen that 5-HTP fits into the classification of a neutral amino acid. It is of considerable interest that the uptake of 5-HTP-(1-C^{14}) by rat brain *in vivo* has been shown recently to be inhibited by a number of amino acids similar to those reported here to be inhibitory *in vitro* (McKean *et al.*, 1962).

Chirigos *et al.* (1960), studying the uptake of L-tyrosine-U-C^{14} by rat brain *in vivo*, and Guroff *et al.* (1961), using techniques *in vitro* found that the concentration of L-tyrosine was markedly diminished by other amino acids. This action was limited to other aromatic amino acids, and to leucine, isoleucine, valine, histidine and cystine. Similar findings have been reported recently by Neame (1961). It is of interest that the data from these studies correlate so closely with the results of the experiments reported in this work. It is surprising, however, that L-histidine and DL-valine had little or no effect upon the uptake of 5-HTP, because these amino acids were also found by Christensen *et al.* (1952) and Chirigos *et al.* (1960) to be inhibitory to the uptake of amino acids in this category.

The fact that L- and not D-tryptophan caused an inhibition of the uptake of 5-HTP suggests that L- and not D-5-HTP is actively transported by the brain slices. Because DL-5-HTP was used throughout these experiments one may conclude that the intracellular to extracellular ratio (relative concentration ratio) is actually twice as high as reported.

Despite the relationship shown in table 2 between the uptake of 5-HTP and the presence of the decarboxylating enzyme in the tissue, our finding that α-methyldopa [a potent inhibitor of 5-HTP decarboxylase (Smith, 1960)] had no effect upon the uptake of 5-HTP suggested that the presence of both 5-HTP decarboxylase activity and the 5-HTP transport system in the same tissues only reflects the fact that these specific tissues are associated with the physiological disposition of 5-HT.

SUMMARY

The uptake of DL-5-HTP-1-C^{14} and 5-HT-1-C^{14} by slices of rat brain has been studied. A facilitated transport of 5-HTP by these slices was demonstrated by (1) enhancement of the relative concentration ratio (slice/medium) by oxygen and glucose, (2) interference with this enhancement by dinitrophenol, and (3) pH and temperature curves showing optimal concentrating conditions to be pH 7.4 and 37.5°C. Under similar conditions, brain slices did not demonstrate a facilitated transport of 5-HT, thus differing from other tissues such as platelets and neoplastic mast cells. The 5-HTP transport system in brain tissue does not seem to be associated with the activity of 5-hydroxytryptophan decarboxylase, as evidenced by the inability of the potent 5-HTP-decarboxylase inhibitor, α-methyldopa, to influence the uptake of 5-HTP. It was also found that the uptake of 5-HTP was accelerated, apparently, by an efflux of K^+ from the slice and decreased by an influx of K^+ into the brain tissue.

In contrast to their ability to depress respiration of brain tissue or influence the metabolism of 5-HT, such drugs as phenobarbital, morphine, reserpine, chlorpromazine, LSD-25, imipramine, and iproniazid, in concentrations which alter behavior, did not significantly influence the facilitated transport of 5-HTP. On the other hand, the naturally occurring amino acids L-tryptophan (but not the D-isomer), L-phenylalanine, L-tyrosine, L-dihydroxyphenylalanine, L-leucine, L-isoleucine and L-proline all cause a significant decrease in the uptake of 5-HTP by brain slices.

ACKNOWLEDGMENTS. The author wishes to acknowledge the generosity of the following sources of certain of the drugs used in this work.

Ciba Pharmaceutical Co. for reserpine, Merck Sharp & Dohme for α-methyldopa, the Geigy Co. for imipramine, the Upjohn Co. for α-ethyltryptamine. The competent technical assistance of Mrs. S. Scholsohn and Mr. Carl McIlroy is also gratefully acknowledged.

He also wishes to express his sincere gratitude to Professor Nicholas J. Giarman for many invaluable suggestions throughout the course of these studies, as well as in the preparation and writing of this paper.

REFERENCES

BORN, G. V. R. AND BRICKNELL, J.: J. Physiol. **147:** 153, 1959.

CHIRIGOS, M. A., GREENGARD, P. AND UDENFRIEND, S.: J. biol. Chem. **235:** 2075, 1960.

CHRISTENSEN, H. N. AND RIGGS, T. R.: J. biol. Chem. **194:** 57, 1952.

CHRISTENSEN, H. N., RIGGS, T. R., FISCHER, H. AND PALATINE, I. M.: J. biol. Chem. **198:** 1, 1952.

COOPER, J. R. AND MEICER, I.: J. Pharmacol. **132:** 265, 1961.

CROSSLAND, M. A.: J. Pharm., Lond. **12:** 1, 1960.
DALGLIESH, C. E.: in Advances in Clinical Chemistry, ed. by H. Sobotka and C. P. Stewart, vol. 1, p. 193, Academic Press, New York, 1958.
ERSPAMER, V.: Rendic. Scient. Farm. **1:** 1, 1954.
FREEDLAND, R. A., WADZINSKI, I. M. AND WAISMAN, H. A.: Biochem. biophys. res. Comm. **6:** 227, 1961.
GADDUM, J. H. AND GIARMAN, N. J.: Brit. J. Pharmacol. **11:** 88, 1956.
GIARMAN, N. J., FREEDMAN, D. X. AND PICARD-AMI, L.: Nature, Lond. **186:** 480, 1960.
GIARMAN, N. J. AND SCHANBERG, S. M.: Biochem. Pharmacol. **1:** 301, 1958.
GLASSER, A. AND MANTEGAZZINI, P.: Experientia **16:** 213, 1960.
GREEN, J. P. AND DAY, M.: Fed. Proc. **20:** 136, 1961.
GUROFF, G., KING, W. AND UDENFRIEND, S.: J. biol. Chem. **236:** 1773, 1961.
HUGHES, F. B. AND BRODIE, B. B.: J. Pharmacol. **127:** 96, 1959.
JOHNSTONE, R. M.: Canad. J. Biochem. Physiol. **37:** 589, 1959.
MCKEAN, C., SCHANBERG, S. M. AND GIARMAN, N. J.: Science **137:** 604, 1962.
NEAME, K. D.: Nature, Lond. **192:** 173, 1961.
NEGELEIN, E.: Biochem. Z. **323:** 214, 1952.
RODNIGHT, R. AND MCILWAIN, H.: Biochem. J. **57:** 649, 1954.
SCHANBERG, S. M. AND GIARMAN, N. J.: Biochim. biophys. Acta **41:** 556, 1960.
SCHANBERG, S. M. AND GIARMAN, N. J.: Biochem. Pharmacol. **2:** 187, 1962.
SMITH, S. E.: Brit. J. Pharmacol. **15:** 319, 1960.