Brit. J. Pharmacol. (1963), **20**, 178–189.

UPTAKE OF 5-HYDROXY[^{14}C]TRYPTOPHAN BY RAT AND DOG BRAIN SLICES

BY

S. E. SMITH

From the Department of Pharmacology and Therapeutics, St. Thomas's Hospital Medical School, London

(*Received November* 14, 1962)

Slices of rat brain take up and concentrate 5-hydroxy[^{14}C]tryptophan when incubated at 37° C *in vitro*. Uptake is inhibited by methyldopa and L-phenylalanine in a competitive manner. Only a small proportion of the 5-hydroxy[^{14}C]tryptophan taken up is decarboxylated. This observation, and the lack of correlation between the effects of various amino-acid inhibitors on uptake and on decarboxylase, suggest that the two processes are not linked. In the cold some passive diffusion of the amino-acid occurs, as is thought to take place also at 37° C. Experiments with dog brain show that uptake varies from one part of the brain to another, being greatest in the caudate nucleus. The possible implications of the uptake mechanism in the treatment of animals or patients with large doses of amino-acids are discussed.

As far as is known most of the 5-hydroxytryptamine in the brain is formed by decarboxylation of the amino-acid 5-hydroxytryptophan. It is not known whether the latter is formed *in situ* by hydroxylation of tryptophan or whether it is picked up from the circulation. Though the hydroxylating enzyme has been demonstrated in bacteria (Mitoma, Weissbach & Udenfriend, 1956), in the toad (Udenfriend, Clark & Titus, 1953) and in mammalian intestine (Cooper & Melcer, 1961), all attempts have failed to locate the enzyme in brain. Consequently the possibility that the brain might take up 5-hydroxytryptophan assumes some importance. Uptake by the brain has been shown for other amino-acids: glutamic acid (Stern, Eggleston, Hems & Krebs, 1949), arginine and histidine (Neame, 1961a, b), leucine and lysine (Lajtha & Mela, 1961), methionine, ornithine and proline (Neame, 1961a), phenylalanine and tryptophan (Guroff & Udenfriend, 1962) and tyrosine (Chirigos, Greengard & Udenfriend, 1960; Guroff, King & Udenfriend, 1961). Entry of 5-hydroxytryptophan into the brain can occur *in vivo*, for its administration to animals increases brain concentrations of 5-hydroxytryptamine (Udenfriend, Weissbach & Bogdanski, 1957). More recently Schanberg & Giarman (1960) have demonstrated that uptake occurs *in vitro* and that this is an active process dependent on the presence of glucose and inhibited by dinitrophenol.

In the present work the uptake mechanism and the action on it of various compounds of pharmacological interest are studied. In particular the effect of methyldopa, an inhibitor of L-amino-acid decarboxylase, is examined. *In vivo*, methyldopa reduces brain concentrations of 5-hydroxytryptamine (Smith, 1960; Hess, Conna-

macher, Ozaki & Udenfriend, 1961) but fails in rat brain to induce as great an accumulation of the amino-acid (Sharman & Smith, 1962) as would be expected from a decarboxylase inhibitor. Its effect on 5-hydroxytryptophan uptake is therefore of interest. The effect of phenylalanine is also examined, for this substance may be involved in the aetiology of the mental defect associated with phenylketonuria.

METHODS

Uptake of 5-hydroxy[^{14}C]tryptophan. Female black and white hooded rats (aged between 51 and 89 days) were killed by decapitation and five or six brain slices cut coronally as described by Neame (1961a). A separate brain was used for each determination. The combined slices were weighed (200 to 300 mg) and incubated at 37° or at 0° C in 5 ml. of Krebs-Ringer-phosphate solution at *p*H 7.4, gassed with pure oxygen, and containing 5-hydroxy[β-^{14}C]-tryptophan. In most experiments the labelled isotope (Radiochemical Centre, Batch 11: 19.2 μc/mg, or Batch 12: 31.3 μc/mg) was diluted with non-radioactive DL-5-hydroxytryptophan, the mixture being made up fresh and assayed fluorimetrically on each occasion. In the majority of experiments the incubation mixture contained 10 μg/ml. of 5-hydroxytryptophan (4.55×10^{-5} M). In two experiments the concentrations were 1.0, 1.6 or 3.0 μg/ml. Other substances were added in Krebs solution. Preliminary experiments indicated that the uptake of 5-hydroxy[^{14}C]tryptophan was greater in Krebs-Ringer-phosphate solution gassed with pure oxygen than in Krebs-Ringer-bicarbonate solution gassed with 95% oxygen and 5% carbon dioxide.

After incubation the slices were removed, washed once with cold Krebs-Ringer-phosphate solution and homogenized in 5 ml. of 70% ethanol. The homogenates were shaken for 30 min and allowed to stand in the refrigerator overnight. After centrifugation 0.5-ml. aliquots of the supernatant, with and without added standards, were dried on planchettes and the radioactivity counted in a gas flow chamber. In some experiments 0.5-ml. aliquots were added to a liquid phosphor (Nuclear Enterprises NE 220, or as described by Bray (1960)) and counted in a Packard Tricarb liquid scintillation spectrometer. Recovery of standards added to the whole homogenate was quantitative. Uptake was calculated as μg of 5-hydroxytryptophan/g wet weight and expressed either as such or as the ratio of the concentration in the tissue to the final concentration in the medium. The effect of inhibitors added in different concentrations to the incubation mixtures was assessed by calculating the "active" uptakes as differences between uptakes at 0° and 37° C and plotting the logarithm of the dose of the inhibitor against the percentage inhibition of uptake. The dose needed for 50% inhibition was read off by interpolation.

^{14}C was estimated in protein precipitates of three samples. After washing five times with 70% ethanol, the precipitates were suspended in 4 ml. of 0.1 N NaOH and 0.5 ml. of this suspension added to 1 ml. of hyamine solution (M concentration in methanol). After clearing, aliquots were added to the liquid phosphor, acidified with acetic acid and counted.

In some experiments male greyhound dogs were anaesthetized with thiopentone (25 mg/kg) and exsanguinated. Slices cut from different parts of the brain were incubated, extracted and counted for ^{14}C in the same manner as the rat brain slices.

Chromatography. Aliquots of an ethanolic extract of rat brain were subjected to ascending chromatography on Whatman no. 1 paper in four solvent systems:

Butanol:acetic acid:water (12:3:5)	15 hr run
Isopropanol:ammonia:water (100:10:15)	15 hr run
Butanol:pyridine:water (60:60:60)	15 hr run
20% aqueous KCl	2 hr run

Non-radioactive 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindolylacetic acid were added as markers. Radioactivity on the strips was estimated in a gas flow chamber coupled to a recording rate-meter.

Chemical partition of ^{14}C in brain extracts. After incubation with 5-hydroxy[^{14}C]tryptophan, brain slices were homogenized in two volumes (w/v) of 0.1 N HCl and 5-OR indolyl compounds extracted as described by Ashcroft & Sharman (1962). In this method the total 5-OR indolyl compounds are measured in the supernatant obtained after removal of protein from the homogenates. Basic and acidic 5-OR indolyl compounds are measured by further extraction of this supernatant after alkalinization and acidification respectively. The concentration of neutral or amphoteric 5-OR indolyl compounds is calculated by subtracting the sum of the basic and acidic fractions from the total. In the present work ^{14}C in the final extracts was counted in the liquid scintillation spectrometer and 60 to 70% of 5-hydroxy[^{14}C]tryptophan added to homogenates was recovered in the protein-free supernatant. Preliminary experiments also showed that 15% of 5-hydroxy[^{14}C]tryptophan added to the supernatant was recovered in the basic component, and 0.4% in the acidic component. Partition of ^{14}C into the true neutral, basic and acidic fractions was therefore calculated taking these extractions into account.

Estimation of 5-hydroxytryptophan decarboxylase. The cerebellum and part of the cerebral cortex were removed from the brains of female black and white hooded rats and the remainder of the brains homogenized in six volumes (w/v) of ice-cold distilled water. The homogenates were centrifuged at 3,000 *g* for 30 min at 5° C, and the supernatant was used as the enzyme source. Reaction mixtures were made up as follows:

1. Enzyme ... ... 1.0 ml.
2. 0.2 M Phosphate buffer, *p*H 8.0 ... ... 2.5 ml.
3. Additions ... ... 1.0 ml.
4. DL-5-Hydroxytryptophan, 1.1 mg (final concentration 10^{-3} M) 0.5 ml.

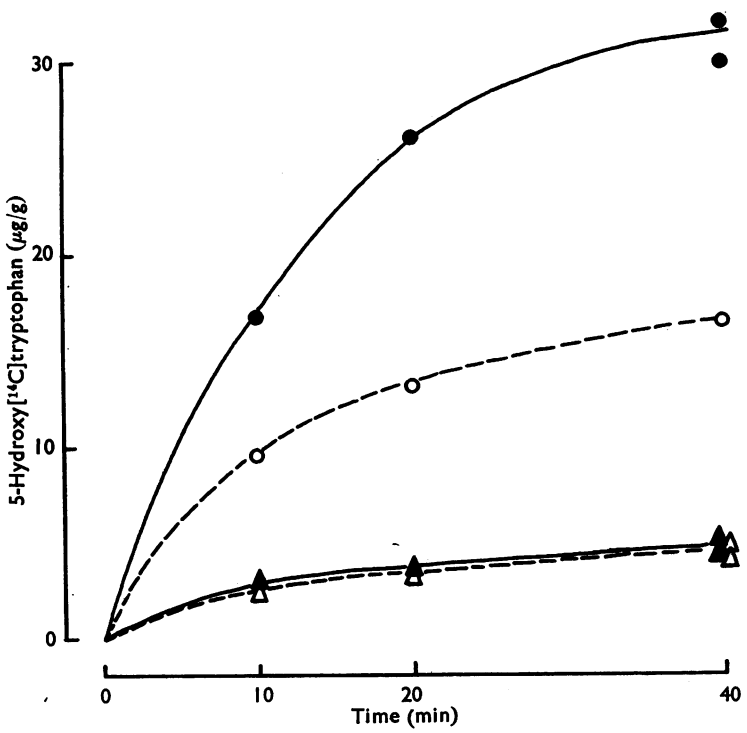

Fig. 1. Uptake of 5-hydroxy[^{14}C]tryptophan by rat brain slices. Initial concentration in medium, 10 μg/ml. Ordinate: uptake in μg/g. Abscissa: time in min. ●——● Control, 37° C. ○ - - - ○ α-Methyldopa, 10^{-2} M, 37° C. ▲——▲ Control, 0° C. △ - - - △ α-Methyldopa, 10^{-2} M, 0° C.

and incubated at 37° C, gassing with nitrogen. The substrate was added last after prior incubation for 15 min. No pyridoxal-5-phosphate was added. Aliquots, taken 3 and 33 min after addition of substrate, were assayed fluorimetrically for 5-hydroxytryptamine by the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956). Enzyme activity was calculated as μg of 5-hydroxytryptamine formed/mg of protein/hr.

Uptake of inulin. Rat brain slices were incubated at 37° C in Krebs-Ringer solution containing 0.5% inulin. After incubation the slices were washed and homogenized in 2.5% trichloroacetic acid. The homogenates were centrifuged and the inulin in the supernatant estimated by the method of Hubbard & Loomis (1942).

RESULTS

Effect of incubation time and temperature on 5-hydroxy[^{14}C]tryptophan uptake. Uptake of 5-hydroxy[^{14}C]tryptophan by brain slices was measured with incubation at 37° and 0° C. The results are illustrated in Fig. 1. Uptake at 37° C increased with prolonged incubation but was not linear with time, the fastest rate of uptake occurring initially. After incubation for 40 min the mean uptake of fifteen determinations was 27.7 μg/g (s.e. of mean, $\pm$0.8), giving a final tissue/medium ratio of 3.29$\pm$0.10. At 0° C uptake was considerably slower, approximately 12% of that at 37° C. The mean value of four determinations of 40 min of uptake was 4.0$\pm$0.1 μg/g, giving a final tissue/medium ratio of 0.41$\pm$0.02.

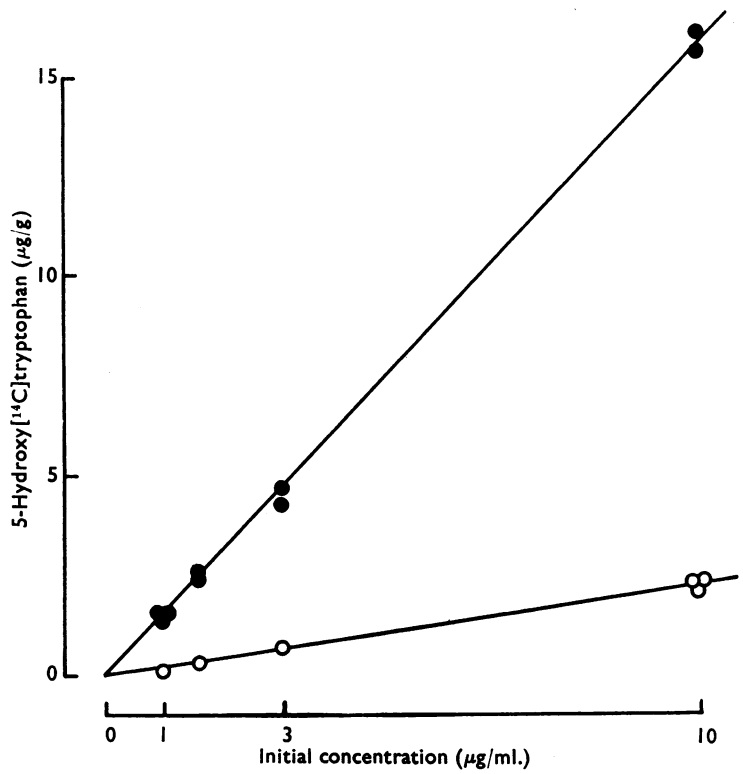

Fig. 2. Uptake of 5-hydroxy[^{14}C]tryptophan by rat brain slices from media containing different concentrations of substrate. 10 min incubation. Ordinate: uptake in μg/g. Abscissa: initial concentration in medium in μg/ml. ● —— ● At 37° C; ○ —— ○ at 0° C.

No ^{14}C was found in the protein precipitates obtained in three experiments, indicating that 5-hydroxytryptophan is not taken up into protein.

Effect of substrate concentration on 5-hydroxy[^{14}C]tryptophan uptake. Brain slices were incubated for 10 min in the presence of 1.0, 1.6, 3.0 or 10 μg of 5-hydroxy-[^{14}C]tryptophan/ml. The resulting uptake was proportional to the initial concentration in the medium, both at 37° and at 0° C (Fig. 2).

Radiochromatography of brain extracts. In chromatograms of extracts of brain slices incubated for 40 min with 5-hydroxy[^{14}C]tryptophan (10 μg/ml.) most of the ^{14}C was recovered as 5-hydroxy[^{14}C]tryptophan, only a small proportion as 5-hydroxy[^{14}C]tryptamine and none as 5-hydroxy[^{14}C]indolylacetic acid. Tracings of the output from the rate-meter are shown in Fig. 3.

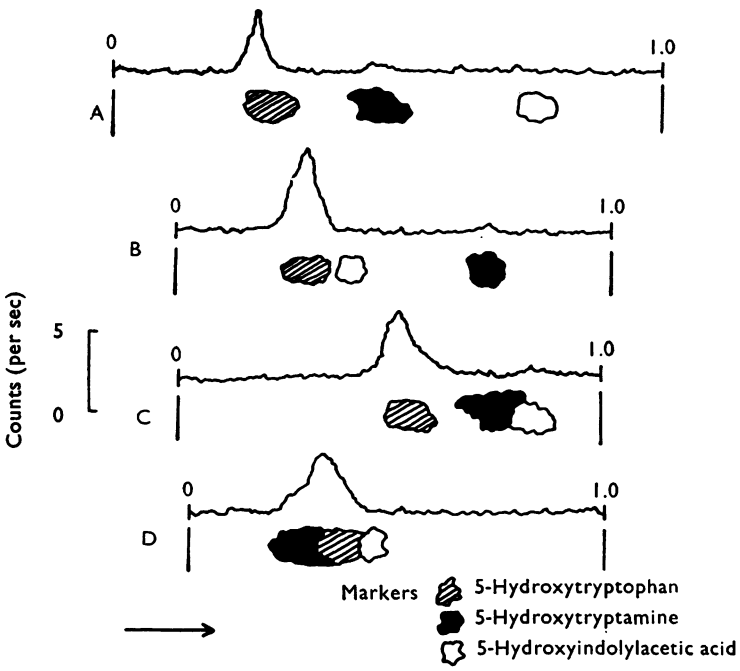

Fig. 3. Outline tracings of radiochromatograms of extracts of rat brain slices incubated with 5-hydroxy[^{14}C]tryptophan. Solvent systems: A, butanol: acetic acid: water; B, isopropanol: ammonia: water; C, butanol: pyridine: water; D, aqueous KCl.

Chemical partition of brain extracts. Incubation of rat brain slices for 40 min with 5-hydroxy[^{14}C]tryptophan and subjection to chemical partition showed that 83.5% of the ^{14}C appeared in the neutral or amphoteric fraction, representing presumably unchanged 5-hydroxy[^{14}C]tryptophan, some 14.5% in the basic fraction and 2% in the acidic fraction. The results are illustrated in Fig. 4.

In two similar experiments with dog caudate nuclei, 58.3 and 62.2% of the isotope were recovered in the neutral or amphoteric fractions, 38.9 and 35.4% in the basic fractions and 2.8 and 2.4% in the acidic fractions respectively. The results indicate

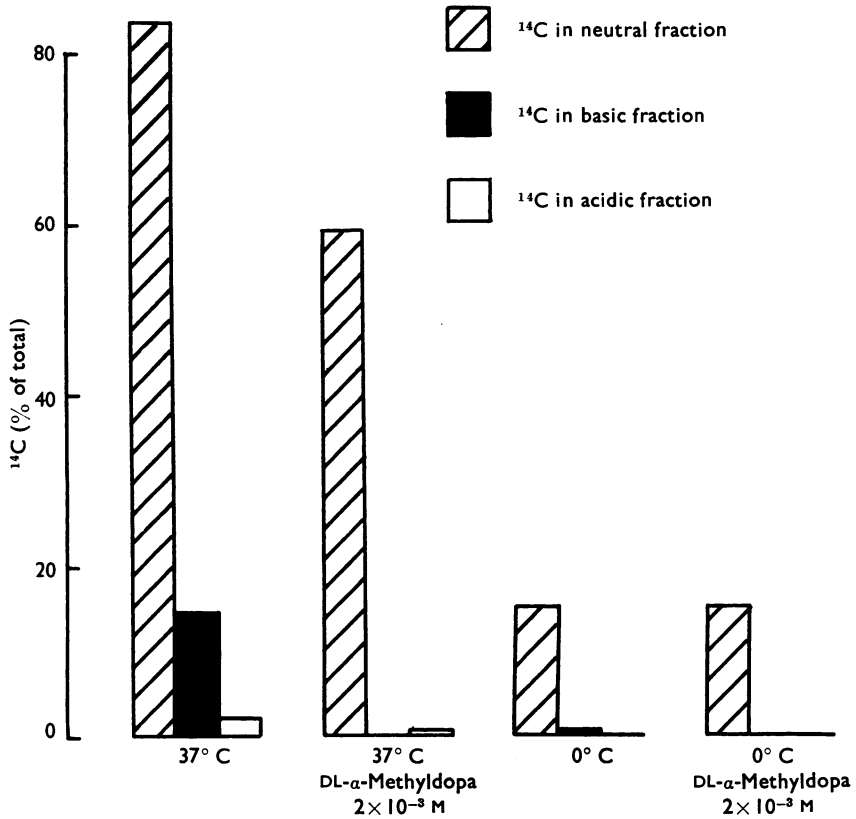

Fig. 4. Partition of extracts of rat brain slices incubated with 5-hydroxy[^{14}C]tryptophan (10 μg/ml.). Histograms represent ^{14}C contained in three fractions expressed as % of the total ^{14}C of the control slices at 37° C.

that these slices decarboxylated a larger proportion of the 5-hydroxy[^{14}C]tryptophan taken up than did rat brain slices.

Action of methyldopa and other amino-acids on uptake of 5-hydroxy[^{14}*C*]*-tryptophan and on 5-hydroxytryptophan decarboxylase.* Methyldopa inhibited the uptake of 5-hydroxy[^{14}C]tryptophan at 37° C, but had no effect at 0° C. The inhibition by a concentration of 10^{-2} M is shown in Fig. 1. Other amino-acids also affected uptake. The molar concentrations that induced 50% inhibition with 40 min incubation, calculated from log. dose/response lines for each amino-acid, are shown in Table 1. Of the substances tested the most potent was L-phenylalanine. L-Tryptophan, DL-dopa, DL-α-methylmetatyrosine (*m*-hydroxyphenyl-α-methyl-DL-alanine), L-histidine, L-glutamic acid and L-tyrosine all had some inhibitory activity. L-Tyrosine (15×10^{-4} M, nearly a saturated solution) produced 44% inhibition. Dose/response lines for four of the amino-acids are shown in Fig. 5.

Chemical partition of extracts of brain slices incubated in the presence of methyldopa (2×10^{-3} M) showed that all the ^{14}C remained as 5-hydroxy[^{14}C]tryptophan (Fig. 4).

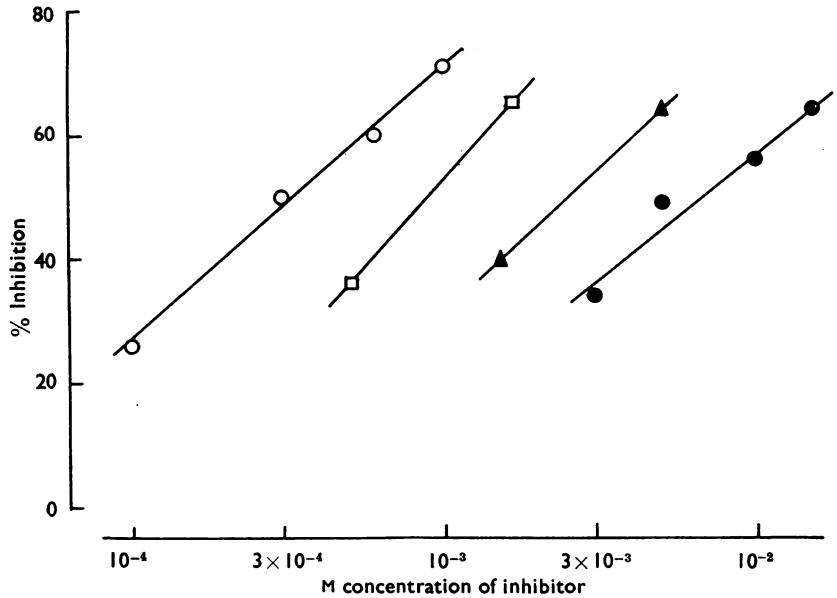

Fig. 5. Inhibition of uptake of 5-hydroxy[^{14}C]tryptophan by rat brain slices induced by four amino-acids. Ordinate: % inhibition. Abscissa: concentration of inhibitor (M). ○——○ L-Phenylalanine. □——□ L-Tryptophan. ▲——▲ DL-Dopa. ●——● DL-α-Methyldopa.

Decarboxylation of 5-hydroxytryptophan was inhibited by methyldopa, DL-α-methylmetatyrosine and DL-dopa. L-Phenylalanine, L-tryptophan, L-histidine and L-glutamic acid had only slight inhibitory activity; L-tyrosine had no action. There was thus no correlation between the ability of the amino-acids to inhibit uptake and their ability to inhibit decarboxylation.

Action of other substances. Three potent inhibitors of decarboxylase, *m*-hydroxybenzyloxyamine (NSD 1024), 4-bromo-3-hydroxybenzyloxyamine (NSD 1055) and *N*-*o*-hydroxybenzyl-*N*-methylhydrazine (NSD 1039) were tested for inhibitory activity against 5-hydroxy[^{14}C]tryptophan uptake. The results are shown in Table 1; NSD 1024 had no effect, NSD 1055 precipitated out in the Krebs-Ringer solution, and NSD 1039 (10^{-2} M) produced only slight inhibition.

Phenylpyruvic acid inhibited decarboxylase only slightly (31% at 10^{-2} M). Phenylacetic acid and phenyl-lactic acid had no action. None of these three substances had any effect on 5-hydroxy[^{14}C]tryptophan uptake (Table 1).

Analysis of inhibition of 5-hydroxy[^{14}C]*tryptophan uptake by methyldopa and phenylalanine.* Uptake from media containing different substrate concentrations was measured at 37° and at 0° C in the presence of DL-α-methyldopa (5×10^{-3} M) or L-phenylalanine (10^{-3} M). Estimates of the initial rates of uptake were obtained by incubating for 10 min, and the active uptake was taken as the difference in uptakes at the two temperatures. The results were subjected to reciprocal analysis by the method of Lineweaver & Burk (1934) (Fig. 6). Regression lines calculated for the three sets of conditions give intercepts on the y axis which are close (control,

TABLE 1

INHIBITION OF 5-HYDROXY[^{14}C]TRYPTOPHAN UPTAKE AND OF 5-HYDROXYTRYPTOPHAN DECARBOXYLASE

Concentrations are those producing 50% inhibition. > indicates less than 50% inhibition by the highest concentration tried. ∞ indicates no inhibitory activity

Substance	Uptake	Decarboxylase
L-Phenylalanine	$3{\cdot}3\times10^{-4}$ M	$>3{\cdot}0\times10^{-3}$ M
L-Tryptophan	$8{\cdot}6\times10^{-4}$ M	$>3{\cdot}0\times10^{-3}$ M
L-Glutamic acid	$>10\times10^{-4}$ M	$>1{\cdot}0\times10^{-2}$ M
L-Tyrosine	$>15\times10^{-4}$ M	∞
DL-Dopa	24×10^{-4} M	$1{\cdot}6\times10^{-4}$ M
DL-α-Methylmetatyrosine	55×10^{-4} M	$1{\cdot}5\times10^{-7}$ M
DL-α-Methyldopa	67×10^{-4} M	$5{\cdot}6\times10^{-7}$ M
L-Histidine	69×10^{-4} M	$>1{\cdot}0\times10^{-2}$ M
NSD 1039	$>1{\cdot}0\times10^{-2}$ M	$1{\cdot}1\times10^{-7}$ M
NSD 1024	∞	$1{\cdot}8\times10^{-8}$ M
NSD 1055	—	$4{\cdot}5\times10^{-9}$ M
Phenylacetic acid	∞	∞
Phenylpyruvic acid	∞	$>1{\cdot}0\times10^{-2}$ M
Phenyl-lactic acid	∞	∞
(Substrate concentration	$4{\cdot}55\times10^{-5}$ M	$1{\cdot}0\times10^{-3}$ M)

0 ; methyldopa, 0.01 ; phenylalanine, 0.03) and within the error of the method. This indicates that inhibition induced by each substance is competitive.

Effect of prior treatment with reserpine or iproniazid on 5-hydroxy[^{14}C]*tryptophan uptake.* Three rats were treated with reserpine (5 mg/kg intraperitoneally) and killed 22 hr later. Brain slices from these animals took up the amino-acid to the same

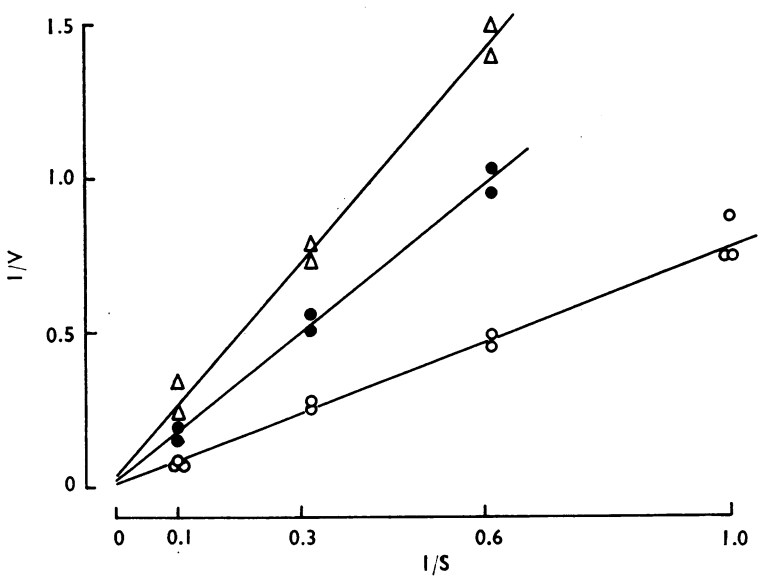

Fig. 6. Reciprocal analysis of 5-hydroxy[^{14}C]tryptophan uptake during 10 min incubation. Ordinate: 1/V, where V = difference between uptake at 37° C and mean uptake at 0° C (in μg/g). Abscissa: 1/S, where S = initial medium substrate concentration (in μg/ml.). ○ —— ○ Controls. ● —— ● DL-Methyldopa, 5×10^{-3} M. △ —— △ L-Phenylalanine, 10^{-3} M.

extent as slices from three animals treated with the reserpine solvent only. Three rats were treated with iproniazid (100 mg/kg intraperitoneally) 24 and 2 hr before being killed. Brain slices from these animals took up amounts similar to those taken up by slices from saline-treated control rats. The results obtained with rats previously treated with reserpine or iproniazid are shown in Table 2.

Inulin uptake. Rat brain slices incubated at 37° C took up inulin increasingly with incubation times up to 160 min. Between 120 and 160 min, tissue/medium ratios

TABLE 2

UPTAKE OF 5-HYDROXY[^{14}C]TRYPTOPHAN BY BRAIN SLICES OF RATS TREATED WITH RESERPINE (5 MG/KG) 22 HR EARLIER OR WITH IPRONIAZID (100 MG/KG) 24 AND 2 HR EARLIER

	Tissue/medium ratio	
Treatment	Controls	Treated
Reserpine	3·33, 2·93, 2·96	3·00, 3·08, 3·18
Iproniazid	3·07, 3·07, 2·87	3·16, 3·30, 3·18

approached 0.2. This was about half the ratio observed with 5-hydroxy[^{14}C]-tryptophan in the cold.

5-Hydroxy[^{14}C]tryptophan uptake by different parts of dog brain. Slices of various regions of dog brain incubated at 37° C took up the amino-acid to different extents. As shown in Table 3, the caudate nucleus took up the largest amounts; the hypothalamus, hippocampus, and cerebral and cerebellar cortices took up less, and the uptake by the thalamus, pons and medulla was least. At 0° C uptake was greatly reduced and varied only slightly from one part of the brain to another.

TABLE 3

5-HYDROXY[^{14}C]TRYPTOPHAN UPTAKE BY DOG BRAIN SLICES

20 min incubation. Substrate concentration, 10 μg/ml.

	Tissue/medium ratio	
Part of brain	At 37° C	At 0° C
Caudate nucleus	2·85, 4·17, 3·74	0·36
Hypothalamus	2·10	0·33
Thalamus (lateral nucleus)	1·18	0·25
Hippocampus	1·92	0·50
Cerebral cortex	1·96, 2·10	0·35
Cerebellar cortex	1·94	0·32
Pons	0·67	0·40
Medulla	0·71	0·28

DISCUSSION

The results confirm Schanberg & Giarman (1960) that brain slices actively take up 5-hydroxytryptophan. The experiments on dog brain indicate that this uptake varies from one part of the brain to another. Some areas must take up 5-hydroxy-tryptophan at very fast rates indeed to account for the initial rates illustrated in Fig. 1. This would seem surprising, for 5-hydroxytryptophan is probably used only as a precursor of 5-hydroxytryptamine, the half-life of which is thought to be about

10 min (Udenfriend & Weissbach, 1958), and the result indicates that the brain can take up the amino-acid much faster than it utilizes it.

Uptake occurs also in the cold. Some of this uptake must be intracellular, for it amounts to double that of inulin, and presumably the transfer results from passive diffusion across the cell membrane. It has been assumed that this passive diffusion occurs also at 37° C and that the difference between the measured uptakes at the two temperatures represents active transfer which must be dependent on enzymic activity.

Prior treatment with reserpine did not affect uptake; this is of some interest. Such treatment reduces the uptake of catechol amines by brain slices (Brodie, Dengler, Titus & Wilson, 1960), which suggests that different uptake mechanisms are involved.

The competitive nature of the inhibition of uptake by other amino-acids suggests that one and the same mechanism operates for 5-hydroxytryptophan, tyrosine and a number of other amino-acids. The fast uptake of 5-hydroxytryptophan may indicate therefore a relatively unspecific mechanism. The question arises as to whether this mechanism plays an important part *in vivo* for transfer of amino-acids from plasma to brain. Guroff *et al.* (1961) have pointed out that two barriers exist between plasma and the cell interior, the " blood-brain barrier," which is a property of the vascular capillary wall, and the brain cell membrane itself. Experiments *in vitro* investigate only the latter, and it is important to realize that *in vivo* limitations may be imposed on this mechanism by the permeability of the vascular component. However, if the cell membrane component is of physiological importance then there is the outstanding implication that a high concentration of any amino-acid in the plasma would reduce entry of other amino-acids into the brain. This could apply to human subjects or experimental animals treated with amino-acids, or to subjects with errors of amino-acid metabolism such as are associated with phenylketonuria, maple syrup urine disease (leucinosis) or hepatic failure.

Treating rats with methyldopa not only reduces brain 5-hydroxytryptamine concentrations but also fails to induce an accumulation of 5-hydroxytryptophan such as would be expected from inhibition of L-amino-acid decarboxylase (Sharman & Smith, 1962); it was suggested that methyldopa might reduce the availability of the amino-acid. The present experiments provide evidence for such an effect, though it is possible that it works on tryptophan transfer. In 1959, Bertler & Rosengren found that treatment of rabbits with dopa reduced brain 5-hydroxytryptamine levels by about 50%. At the time the most likely explanation was thought to be inhibition of decarboxylation of 5-hydroxytryptophan. However, methyldopa, which is over a thousand times more potent than dopa as an inhibitor of this enzyme, is needed in the same order of dosage (600 mg/kg) to produce the same effect. Possibly dopa acts as an inhibitor of tryptophan or of 5-hydroxytryptophan transfer. Feeding rats with diets enriched with L-phenylalanine lowers brain 5-hydroxytryptamine (Green, Greenberg, Erickson, Sawyer & Ellison, 1962; Wang, Harwalker & Waisman, 1962) and reduces entry of 5-hydroxy[^{14}C]tryptophan into the brain in spite of plasma concentrations greater than those in control animals (McKean, Schanberg & Giarman, 1962).

These observations have been used to explain the hydroxyindole defect of phenylketonuria (Pare, Sandler & Stacey, 1957), though whether such a defect occurs in the brain is not yet known. However, in this abnormality plasma levels of phenylalanine are well within the range of concentrations which were found to cause inhibition in the present studies. Davison & Sandler (1958) showed that phenylpyruvic, phenylacetic and phenyl-lactic acids inhibited guinea-pig kidney decarboxylase, and they ascribed the hydroxyindole defect of phenylketonuria to this inhibition. In the present experiments on rat brain enzyme these compounds showed little or no inhibition. Therefore inhibition of amino-acid uptake would appear to be a more likely mechanism than inhibition of decarboxylase.

Radiochromatography and chemical partition showed that only a small proportion of the 5-hydroxy[^{14}C]tryptophan taken up by rat brain is decarboxylated to form the amine. Yet under optimal conditions *in vitro* homogenates will decarboxylate about 100 μg of 5-hydroxytryptophan/g/hr (unpublished observations), which amount is about twenty times more than that decarboxylated in the slices. One must conclude that in the slices either the enzyme is not fully active or the substrate does not reach the enzyme site. This would seem surprising, for the enzyme is soluble and is thought to occur free in the cell cytoplasm. It is possible that amino-acid uptake occurs not into the cytoplasm but on to the cell membrane or a binding site. Alternatively, and more likely, the uptake may be largely into cells which contain no decarboxylase. This latter possibility is supported by the finding that the 5-hydroxy[^{14}C]tryptophan taken up by dog caudate nucleus, which contains large amounts of the enzyme, is decarboxylated to a much greater extent. This observation and the effects of various inhibitors on the two systems make it clear that the mechanism of uptake and the decarboxylating enzyme are not linked.

The author is grateful to Professor R. S. Stacey for his helpful advice and criticism. Methyldopa and α-methylmetatyrosine were very kindly supplied by Dr K. Pfister of Merck, Sharp & Dohme, Rahway, New Jersey, iproniazid and 5-hydroxytryptamine creatinine sulphate by Roche Products, reserpine by Ciba Laboratories and NSD 1024, NSD 1055 and NSD 1039 by Dr D. Drain of Smith & Nephew.

REFERENCES

ASHCROFT, G. W. & SHARMAN, D. F. (1962). Drug-induced changes in the concentration of 5-OR indolyl compounds in cerebrospinal fluid and caudate nucleus. *Brit. J. Pharmacol.*, **19**, 153–160.

BERTLER, Å. & ROSENGREN, E. (1959). On the distribution in brain of monoamines and of enzymes responsible for their formation. *Experientia*, **15**, 382–388.

BOGDANSKI, D. F., PLETSCHER, A., BRODIE, B. B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharmacol. exp. Ther.*, **117**, 82–88.

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.*, **1**, 279–285.

BRODIE, B. B., DENGLER, H. J., TITUS, E. & WILSON, C. W. M. (1960). Inhibition of the uptake of catechol amines in brain tissues by reserpine. *J. Physiol. (Lond.)*, **154**, 37–38*P*.

CHIRIGOS, M. A., GREENGARD, P. & UDENFRIEND, S. (1960). Uptake of tyrosine by rat brain *in vivo*. *J. biol. Chem.*, **235**, 2075–2079.

COOPER, J. R. & MELCER, I. (1961). The enzymic oxidation of tryptophan to 5-hydroxytryptophan in the biosynthesis of serotonin. *J. Pharmacol. exp. Ther.*, **132**, 265–268.

DAVISON, A. N. & SANDLER, M. (1958). Inhibition of 5-hydroxytryptophan decarboxylase by phenylalanine metabolites. *Nature (Lond.)*, **181**, 186–187.

GREEN, H., GREENBERG, S. M., ERICKSON, R. W., SAWYER, J. L. & ELLISON, T. (1962). Effect of dietary phenylalanine and tryptophan upon rat brain amine levels. *J. Pharmacol. exp. Ther.*, **136**, 174–178.

GUROFF, G., KING, W. & UDENFRIEND, S. (1961). The uptake of tyrosine by rat brain *in vitro*. *J. biol. Chem.*, **236**, 1773–1777.

GUROFF, G. & UDENFRIEND, S. (1962). Studies on aromatic amino acid uptake by rat brain *in vivo*. Uptake of phenylalanine and of tryptophan; inhibition and stereoselectivity in the uptake of tyrosine by brain and muscle. *J. biol. Chem.*, **237**, 803–806.

HESS, S. M., CONNAMACHER, R. H., OZAKI, M. & UDENFRIEND, S. (1961). The effects of α-methyldopa and α-methyl-meta-tyrosine on the metabolism of norepinephrine and serotonin *in vivo*. *J. Pharmacol. exp. Ther.*, **134**, 129–138.

HUBBARD, R. S. & LOOMIS, T. A. (1942). The determination of inulin. *J. biol. Chem.*, **145**, 641–645.

LAJTHA, A. & MELA, P. (1961). The brain barrier system—I. The exchange of free amino acids between plasma and brain. *J. Neurochem.*, **7**, 210–217.

LINEWEAVER, H. & BURK, D. (1934). The determination of enzyme dissociation constants. *J. Amer. chem. Soc.*, **56**, 658–666.

MCKEAN, C. M., SCHANBERG, S. M. & GIARMAN, N. J. (1962). A mechanism of the indole defect in experimental phenylketonuria. *Science*, **137**, 604–605.

MITOMA, C., WEISSBACH, H. & UDENFRIEND, S. (1956). 5-Hydroxytryptophan formation and tryptophan metabolism in *Chromobacterium violaceum*. *Arch. Biochem.*, **63**, 122–130.

NEAME, K. D. (1961a). Uptake of amino acids by mouse brain slices. *J. Neurochem.*, **6**, 358–366.

NEAME, K. D. (1961b). Phenylalanine as inhibitor of transport of amino acids in brain. *Nature (Lond.)*, **192**, 173–174.

PARE, C. M. B., SANDLER, M. & STACEY, R. S. (1957). 5-Hydroxytryptamine deficiency in phenylketonuria. *Lancet*, **i**, 551–553.

SCHANBERG, S. M. & GIARMAN, N. J. (1960). Uptake of 5-hydroxytryptophan by rat brain. *Biochim. biophys. Acta (Amst.)*, **41**, 556–558.

SHARMAN, D. F. & SMITH, S. E. (1962). The effect of α-methyldopa on the metabolism of 5-hydroxytryptamine in rat brain. *J. Neurochem.*, **9**, 403–406.

SMITH, S. E. (1960). The pharmacological actions of 3,4-dihydroxyphenyl-α-methylalanine (α-methyldopa), an inhibitor of 5-hydroxytryptophan decarboxylase. *Brit. J. Pharmacol.*, **15**, 319–327.

STERN, J. R., EGGLESTON, L. V., HEMS, R. & KREBS, H. A. (1949). Accumulation of glutamic acid in isolated brain tissue. *Biochem. J.*, **44**, 410–418.

UDENFRIEND, S., CLARK, C. T. & TITUS, E. (1953). Hydroxylation of the 5-position of tryptophan as first step in its metabolic conversion to 5-hydroxytryptamine (serotonin). *Fed. Proc.*, **12**, 282.

UDENFRIEND, S. & WEISSBACH, H. (1958). Turnover of 5-hydroxytryptamine (serotonin) in tissues. *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 748–751.

UDENFRIEND, S., WEISSBACH, H. & BOGDANSKI, D. F. (1957). Increase of tissue serotonin following administration of its precursor 5-hydroxytryptophan. *J. biol. Chem.*, **224**, 803–810.

WANG, H. L., HARWALKER, V. H. & WAISMAN, H. A. (1962). Effect of dietary phenylalanine and tryptophan on brain serotonin. *Arch. Biochem.*, **96**, 181–184.