of inhibition of detoxication mechanisms nor is epinephrine release implicated since the effect is not prevented by adrenergic blocking agents. The nature of this effect and its relationship to the mechanism of action of the barbiturates is discussed.

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Turnover of 5-Hydroxytryptamine (Serotonin) in Tissues.* (23868)

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Development of sensitive and specific analytical methods for serotonin (5-hydroxytryptamine)(1,2) has made it possible to demonstrate its presence in many tissues. Thus, although the bulk of serotonin is found in mucosa of gastro-intestinal tract, significant amounts are also found in blood platelets, brain, spleen and lung. Enzymes involved in serotonin formation and destruction are present in all depots, except blood platelets, indicating that the amine is apparently made, released and destroyed for specific functions in these depots. It is hoped that these studies on turnover of serotonin in various tissues may be helpful in elucidating the nature of these functions.

Materials and methods. A solution of serotonin hydrochloride was prepared in the following manner. Serotonin creatinine sulfate was dissolved in water adjusted to pH 10 and the solution saturated with NaCl. Serotonin was extracted into n-butanol, and after addition of heptane it was reextracted into dilute HCl. The serotonin concentration was standardized colorimetrically. *DL*-tryptophan 2-C^{14} (specific activity 0.3 $\mu c/\mu M$) was obtained from Tracerlab Inc. Radioactive 5-hydroxytryptophan (5HTP) was synthesized in this laboratory following procedure outlined by Ek and Witkop(3). The amino acid was labelled in the β-carbon of the side chain and had a specific activity of 0.1 μc μM. Radioactivity measurements were made with Robinson gas flow proportional counter having background of 2 counts/minute. Counts were corrected to constant weight, based on experimentally determined self-absorption curve, and expressed as counts/minute/μmole of compound. Serotonin in tissues was isolated by previously

* The authors wish to express their thanks to Mrs. Doris Titus for carrying out synthesis of 5HTP-C^{14} and to Mrs. Betty G. Redfield for studies with harmaline.

TABLE I. Labelling of Tissue Serotonin following Tryptophan-C^{14}.

Days following last inj. of tryptophan-C^{14}	Tissue	Serotonin, cpm/μMole
4	Platelets	1239
9	"	285
9	Spleen	264
9	Stomach	103
9	Intestine	97

described procedure employing butanol extraction followed by re-extraction with dilute acid(1). One aliquot of final acid extract was assayed for serotonin spectrophotofluorometrically(4) and 10 mg of serotonin hydrochloride were added to the remainder of extract. The extract, containing carrier, was evaporated under nitrogen to 0.2-0.3 ml and the amine was precipitated by dropwise addition of a saturated aqueous solution of picric acid. The picrate salt was then recrystallized to constant specific activity. Results obtained by this procedure were frequently checked by procedure employing chromatographic isolation on Whatman #1 paper (propanol-1 N NH_3, 5:1) of the carrier-free serotonin in the final acid extract. The 2 methods were always in excellent agreement. Radioactivity studies were carried out on adult rabbits. Tryptophan-C^{14} was administered intraperitoneally every day for 7 days, 2.7 mg, containing 4 μc/day. 5HTP-C^{14} was given in single intraperitoneal doses of 9.6 mg. Harmaline† was administered intraperitoneally to rats in doses of 20 mg/kg and the studies of effect on brain serotonin levels were carried out as described previously (5). Blood platelets were isolated according to procedure of Dillard *et al.*(6).

Results. Following administration of its precursors in radioactive form the various body pools of serotonin became labelled. Disappearance of radioactivity from serotonin in these depots can be taken as a measure of turnover of this amine in the particular tissue. Both tryptophan-C^{14} and 5-hydroxytryptophan-C^{14} were administered to rabbits for such studies. The data obtained in various tissues are summarized in Table I and Fig. 1.

† Harmaline (free base) was generously supplied by Pfizer and Co.

Platelets. Data on platelet serotonin turnover are summarized in Table II. Following administration of tryptophan-C^{14}, 3-4 days were permitted to elapse before drawing first blood sample. By this time the specific activity of free plasma tryptophan was much lower than that of platelet serotonin. Following 5HTP, the amino acid persisted for several hours in the tissues. The initial level for platelet serotonin was taken 5 hours after 5HTP injection. In both cases the fall in specific activity of platelet serotonin can be taken as index of turnover of serotonin in this tissue. Experiments with tryptophan-C^{14} and 5HTP-C^{14} yielded half-lives of 48 and 33 hours respectively. A half-life of this order of magnitude is not inconsistent with reported values of platelet survival time(7,8). Studies with blood platelets indicate that this tissue contains little, if any catalysts involved in serotonin formation or destruction and that the amine is taken up by the platelet from surrounding fluids(9-11). The resulting binding by the platelet is such that serotonin appears to be liberated mainly as a result of platelet breakdown.

Spleen. It is apparent from data in Fig. 1 that the turnover of serotonin in spleen parallels that of platelet serotonin. Nine days after administering tryptophan-C^{14} similar specific activities were also obtained for platelet and spleen serotonin (Table I). It would appear that the bulk of serotonin in spleen may be there in platelets stored in this organ.

Gastro-intestinal tract. The mucosa of gastro-intestinal tract comprises the major de-

TABLE II. Turnover of Serotonin in Platelets.

	Tryptophan-C^{14}		5HTP-C^{14}	
Time	cpm/μM	Fraction remaining	cpm/μM	Fraction remaining
Initial	1239	1.00	32,800	1.00
24			19,350	.59
48	900	.73	14,800	.45
72			7,960	.24
96	390	.31		
120	285	.23		
Calculated half-life	48 hr		33 hr	

Each value represents avg obtained on 2 animals from which blood samples were obtained at times indicated. Animals used in tryptophan-C^{14} studies weighed 2.5-3.0 kg. Those used for 5HTP-C^{14} studies weighed 1.5-2.0 kg.

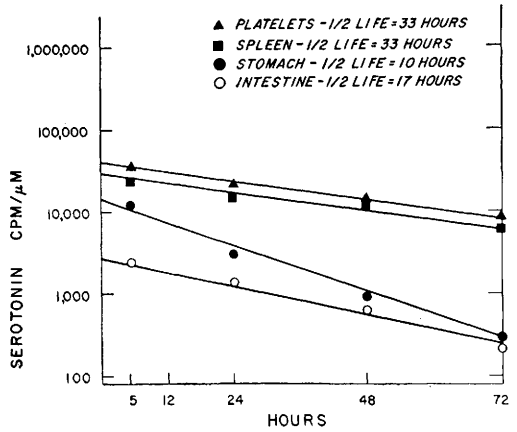

FIG. 1. Half-lives of serotonin in rabbit tissues following administration of 5HTP-C^{14}.

pot of serotonin. It is apparent from data in Fig. 1 that serotonin in these tissues turns over more rapidly than does platelet serotonin. That serotonin in stomach and intestine have similar turnovers is further suggested by data in Table I. Stomach and intestine, in the rabbit, contain approximately 10-20 μg of serotonin/g of tissue. Half lives of 10-17 hours indicate that large quantities of this substance are released daily from these tissues. Release of such quantities of serotonin is consistent with suggestions that serotonin has a regulatory function in the gastro-intestinal tract(12) perhaps as a regulator of peristalsis(13).

In contrast to the rapid turnover of serotonin in rabbit gastro-intestinal mucosa are the findings of a fairly slow serotonin turnover (half-life about 5 days) in malignant carcinoid tumors in man(14). Since serotonin in both gastro-intestinal mucosa, and carcinoid tumors is associated with enterochromaffin tissue, the difference in serotonin turnover is surprising. It will be important to determine whether this represents a species difference or a difference between normal and neoplastic tissue.

Brain. Several attempts were made to determine half-life of brain serotonin using 5HTP-C^{14}. However, it was apparent that entrapment of traces of blood, containing approximately 4 μg/ml of serotonin with a relatively long half-life, would seriously influence brain serotonin, 0.5 μg/g. Although values of less than 10 hours were obtained by the isotopic method, they were not considered to be even an index of maximal half-life values.

In a previous report it had been shown that following administration of the monoamine oxidase inhibitor, isopropyl-isonicotinyl hydrazine (marsilid) there was a rapid rise of brain serotonin. In both rabbits and rats marsilid produced large increases in brain serotonin, the serotonin content doubling in 2-3 hours (5). This rapid increase following marsilid suggested a rapid rate of serotonin turnover in brain. It was considered that the turnover was even faster than was apparent from the rise in serotonin since as *in vitro*, marsilid may require some time to become an effective inhibitor of serotonin metabolism. More recently it has been possible to find more potent inhibitors of monoamine oxidase(15,16). An analogue of one of these, harmaline, has been studied as a monoamine oxidase inhibitor both *in vitro* and *in vivo* (to be published). When harmaline was administered to rats there occurred an extremely rapid increase in brain serotonin levels. A typical experiment, Table III, indicates that doubling of brain serotonin occurs within minutes. This is much more rapid than was obtained with marsilid. If this is now taken as the index of serotonin turnover in brain then it would certainly suggest rapid formation and release of this amine in the central nervous system.

Other tissues. Serotonin is also found in the lung of many animal species and in skin of rats and mice. However, these studies were carried out before serotonin localization in these tissues was known.

Discussion. It is obvious that the various

TABLE III. Increase in Brain Serotonin following Administration of Harmaline.

Time, min.	Brain serotonin, μg/g
0 (controls)	.40
10	.60
20	.73
30	.80
60	.87

Harmaline, 5 mg/kg, was administered intraper. to adult rats following which the animals were sacrificed at indicated intervals. Each value comprises pooled brain from at least 3 animals, except for controls which are pooled averages of 9 animals.

depots of serotonin differ from one another in many respects. Although total amount of serotonin in brain is small compared to that in the gastro-intestinal tract it is apparent that synthesis and metabolism of serotonin occurs much more rapidly in the central nervous system than it does peripherally. This may also be true for norepinephrine since levels in the brain also rise rapidly following administration of monoamine oxidase inhibitors(15). However, with catecholamines too, peripheral depots, such as those in adrenal gland are known to turn over very slowly(17). The rapid turnover of these amines in the central nervous system is indicative of an important function.

Serotonin in blood platelets is quite distinct from that in other tissues. Its turnover does not reflect synthesis and metabolism by platelets. The amounts of serotonin in this tissue depend on amounts released from other depots most probably the gastro-intestinal tract. This would explain increased platelet levels in malignant carcinoid. The turnover in platelets is, therefore, a function of uptake and platelet survival.

Summary. Using tryptophan-C^{14} and 5-hydroxytryptophan-C^{14} it has been possible to measure turnover of serotonin in a number of tissues in the rabbit. The measured half lives are: platelets and spleen, 33-48 hours; stomach, 17 hours; intestine, 11 hours. Although amounts of serotonin in brain were too small to permit isotopic measurement it was possible to estimate its turnover from the increase following administration of harmaline, an inhibitor of serotonin metabolism. Such estimates indicate a half-life in brain of the order of minutes.

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Effect of N-Acetyl-para-Aminophenol on Plasma Levels of 17-Hydroxycorticosteroids.* (23869)

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Hench(1) observed that rheumatoid arthritis patients experienced marked or complete temporary remissions of arthritis during bouts of spontaneous intercurrent jaundice. Although there was some correlation between extent of remission and serum bilirubin concentration, Hench was of the opinion that bilirubin was not responsible for remission of

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