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# AUTORADIOGRAPHIC DISTRIBUTION OF 5-HYDROXYTRYPTAMINE AND 5-HYDROXYTRYPTOPHAN IN THE MOUSE*

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**Abstract**—The distribution in mice of $^{14}$C-5-hydroxytryptophan and $^{14}$C-5-hydroxytryptamine was studied at various intervals after intravenous injection. With guidance from the whole body autoradiograms, a number of organs were then selected for detailed autoradiographic investigation of $^{3}$H-5-hydroxytryptophan.

The radioactivity from the injected 5-hydroxytryptophan initially accumulated in the exocrine pancreas and other organs characterized by a rapid protein synthesis. These localizations disappeared gradually during the first 4 hr and simultaneously a pattern took over which was very similar to the 5-hydroxytryptamine pattern.

This "amine pattern" which was rapidly appearing after injection of 5-hydroxytryptamine was characterized by a localization in the adrenal medulla, the thyroid, the islands of Langerhans, the bone marrow, the red pulp of the spleen and the lung. In the adrenal medulla the radioactivity accumulation could still be seen after 4 days whereas the other tissues were almost totally void of radioactive substance. The 5-hydroxytryptophan pattern was very similar to that obtained earlier with $^{14}$C-2-dihydroxyphenylalanine (DOPA). Exceptions were the higher concentrations after injection of 5-hydroxytryptophan in bone marrow, blood and spleen, which may represent uptake in platelets of 5-hydroxytryptamine. In the thyroid the radioactivity was localized in a few cells scattered between and in the follicular walls (apparently the so-called parafollicular cells).

The specific uptake observed both in the islands of Langerhans and in the thyroid may indicate the possible role of 5-hydroxytryptamine in influencing the synthesis or liberation of the corresponding hormones, thus being a possible factor in the regulation of the basal metabolism of the body.

EVER since Rapport[1] was able to identify the vasoconstrictory substance in blood platelets as 5-hydroxytryptamine (5-HT), and Erspamer and Asero[2] could show that the "enteramine" in the enterochomaffin cells in the digestive tract also was identical with 5-HT, the interest for this compound has rapidly been rising. It is now placed in the group of very important "biogenic amines" together with adrenaline, noradrenaline and dopamine, and it is assumed to take an active part in neurochemical functions.

The biochemical synthesis of 5-HT has been shown to proceed by oxidation of tryptophan to 5-hydroxytryptophan (5-HTP) which is then decarboxylated to 5-HT

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by 5-hydroxytryptophan decarboxylase (5-HTP-ase)[3]. This having been established, it has been possible to study the metabolism of 5-HT by administration of radioactively labelled precursors, tryptophan and 5-HTP[4, 5]. The normal occurrence of 5-HT in various tissues has been investigated by a number of authors (reviewed by Erspamer[6]) and the list of these data seems to be rather comprehensive.

There is, however, a striking lack of morphological correlation to the biochemical information. It was thought by the present authors that this gap could to some extent be filled by autoradiographic methods.

Since 5-HT and 5-HTP are easily diffusible within the tissues subjected to aqueous solutions[7] it was important that in the methods employed, water should either be totally avoided or the isotope and its metabolites insolubly fixed in the tissue. Several techniques were tried; one involving frozen sections,[8, 9], one freeze-drying and subsequent dry autoradiography[10] and one freeze-drying and gas fixation[11] followed by conventional wet autoradiography.

## MATERIAL AND METHODS

The following isotopes were used:

$^{14}$C-DL-5-HTP (3-(5-hydroxy-3-indolyl)alanine 3 – $^{14}$C), sp.a. 4·35 mC/mM.

$^{14}$C-5-HT (5-hydroxytryptamine-3′-$^{14}$C creatinine sulphate), sp.a. 6·25 mC/mM.

$^{3}$H-DL-5-HTP (generally labelled) sp.a. 927 mC/mM.

The isotopes were supplied by the Radiochemical Centre, Amersham, England.

The mice used for the experiments were all kept under the same conditions; room temperature 25°, water *ad lib.* and fed with a diet of coarse hard bread ("mouse bread").

*Whole body autoradiography*

Twelve female white mice, weighing 35 g and in advanced pregnancy were used. Six of these were injected intravenously with $^{14}$C-labelled DL-5-HTP, 5 $\mu$C each (13 mg per kg body weight) and the other six 5 $\mu$C $^{14}$C-labelled 5-HT (9 mg per kg body weight). An animal from each group was sacrificed after 2 min, 20 min, 1 hr, 4 hr, 24 hr and 4 days respectively by immersion (after ether anaesthesia) in a mixture of solid carbon dioxide and hexane at a temperature of about −70°. 20 $\mu$ thick sagittal sections from the frozen animals were cut and dried in a freeze-room (−10°) and pressed against Structurix X-ray film. The exposure time was about two months. The autoradiographic method is described in detail by Ullberg.[8, 9]

*Micro-autoradiography*

Four female mice of the same strain as above, weighing about 20 g were each given 1 mC 5-HTP-$^{3}$H (12 mg per kg body weight) intravenously. The survival periods based upon the results of the whole body autoradiographic experiments were 20 min, 45 min and 4 hr. The animals were sacrificed by decapitation. One non-injected animal was used as a control. Immediately after sacrifice specimens from pancreas, thyroid, adrenals, duodenum and skin were immersed in liquid propane and then transferred to a container cooled in a mixture of solid $CO_2$ and acetone. Thereafter they were freeze-dried for 3 days in a Glick-Malmström apparatus at a pressure of $10^{-4}$ mm mercury. After the freeze-drying was completed, the specimens were divided into two identical groups; one of these was embedded in hot paraffin *in vacuo*, the

other was rapidly transferred to a tight glass jar containing dry paraformaldehyde. The jar was then placed in an incubator at 80° for one hour, after which the specimens were removed and embedded *in vacuo* in hot paraffin. This gas fixation is the method suggested by Falck[11] for histochemical demonstration of monoamines in tissues. The tissue that had undergone a conventional freeze-drying (without paraformaldehyde treatment) was subjected to a recently described method for dry autoradiography.[10] Sections with a thickness of 4–6 $\mu$ were taken on a piece of Scotch tape and in the dark-room attached on to autoradiographic plates (Ilford G5 Nuclear Emulsion Plate) which had been pretreated with glycerin.

The specimens that had been treated with paraformaldehyde gas were subjected to conventional stripping film autoradiography.[12] Sections (2–4 $\mu$) were floated on a water surface, mounted on slides, deparaffinized in xylene and carried through alcohol to distilled water. Stripping film (Kodak AR 10) was stretched on water, and floated on to the sections. Exposure was carried out at +4° for 8–45 days in the presence of a drying agent. The films were developed for 5 min in Kodak D 19. Rinsing and straining was performed at +4°, which prevented the film from loosening from the slide.

Peritoneal smears were treated with formaldehyde gas after drying in air and covered with stripping film as described above.

## RESULTS

The distribution pictures of $^{14}$C-5-HTP and $^{14}$C-5-HT were very similar except for the initial patterns.

The radioactivity from 5-HTP was rapidly taken up in the exocrine pancreas and, although less pronounced, in some other tissues characterized by a rapid protein synthesis such as the gastrointestinal mucosa, salivary gland and hypophysis. After a peak at 20 min, the first pattern gradually disappeared and the distribution picture became very similar to the 5-HT pattern.

This "amine pattern" which was rapidly appearing after injection of 5-HT was characterized by a localization in the adrenal medulla, the thyroid, the islands of Langerhans, the bone marrow, the red pulp of the spleen and the lung. A more liberal penetration of the radioactivity to the brain and to the fetus could be observed after injection of 5-HTP than after injection of 5-HT.

After 4 days, activity was observed almost exclusively in the adrenal medulla, both in the animals injected with the amino acid and with the amine.

*Circulatory system*

The initial high concentration of both $^{14}$C-5-HTP and $^{14}$C-5-HT in the *blood*, following the intravenous injections, decreased with a slow rate and after 4 days there was still some radioactivity present in the blood.

5-HT seemed to be taken up by the *bone marrow* to a larger extent than 5-HTP. Two min after injection the 5-HT concentration was higher in the bone marrow than in the blood and this ratio remained constant throughout the investigation.

In the *spleen* 5-HT was taken up more rapidly than 5-HTP. However, the uptake of both substances was quite slow, and the radioactivity, which was mainly concentrated to the marginal sinuses, was very high after 4 days.

Immediately after injection 5-HT was concentrated in the *myocardium* but the activity decreased rapidly. The radioactivity of 5-HTP in the heart muscle never exceeded that of the blood.

*Excretory organs*

After an initial high concentration in the *kidney* and *urinary bladder*, following the intravenous injection of both 5-HT and 5-HTP, a persisting radioactivity was seen in the cortical zone of the kidney.

*Skin*

5-HTP rapidly appeared in the skin, partly on the surface of the corneal layer and partly in the dense connective tissue of the dermis. Mast cells were localized after staining in toluidine blue but neither these nor other connective tissue cells stood out against the activity of the dermal collagen.

*Peritoneal smears*

In peritoneal smears $^{3}$H-5-HTP-radioactivity was localized to mast cells, which could easily be recognized by their metachromatic staining in toluidine blue. However, there was a wide variation in the amount of radioactivity between different mast cells. Other peritoneal cells did not show any radioactivity whatsoever.

*Digestive tract*

In the gastric and intestinal mucosa 5-HTP-activity was found to accumulate initially. Later the intestinal contents showed a high amount of radioactivity. Four hours after $^{3}$H-5-HTP-injection microautoradiography showed accumulation in a few scattered cells which were yellowishly fluorescent after formaldehyde treatment. After injection of $^{14}$C-5-HT the activity was low in the mucosa and did not appear in the intestinal contents in the whole body autoradiograms.

The exocrine *pancreas* showed a high concentration of radioactive material during the first 4 hr after injection of 5-HTP.

Twenty and 45 min after injection all the exocrine cells had a high amount of radioactivity. After four hours the remaining activity was localized near or in the acinar lumina and in the excretory pathways.

On the contrary, only a low activity of short persistence was seen in the exocrine pancreas after 5-HT administration.

*Endocrine organs*

5-HT and 5-HTP-radioactivity was found to appear in the *thyroid* 20 min after injection and persisted after 24 hr. Microautoradiography with $^{3}$H 5-HTP revealed that this activity was situated not in the follicular cells proper or in the colloid, but in rather few, scattered cells, situated in or between the follicular walls (Fig. 5). In a hematoxylin-eosin stain these cells were found to have a comparatively large, lightly staining cytoplasm. Cytoplastic details were difficult to examine under the autoradiographic grains, but as far as could be seen, there were no granular inclusions. The nucleus was pale staining, round or oval, and contained one or more nucleoli. The distribution of these cells within the gland was highly variable; some parts being totally devoid of them, others showing fairly large numbers. The follicular cells and the colloid showed no activity. The labelled cells did not show metachromasia after toluidine blue staining.

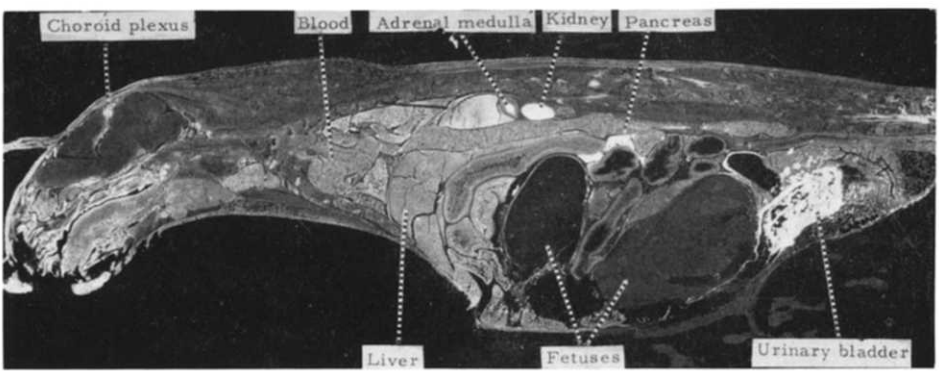


FIG. 1. Autoradiogram of a mouse 20 min after i.v. injection of $^{14}$C-5-hydroxytryptophan. Note the accumulation of radioactivity in the adrenal medulla and the pancreatic tissue.

*facing page 316*—B.P.

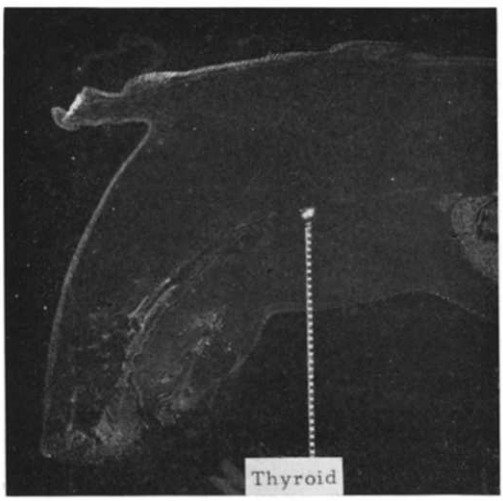


FIG. 2. Autoradiogram of the head of a mouse, which 24 hours after sacrifice had been injected with $^{14}$-C-5-hydroxytryptophan. Note the accumulation of radioactivity in the thyroid gland.

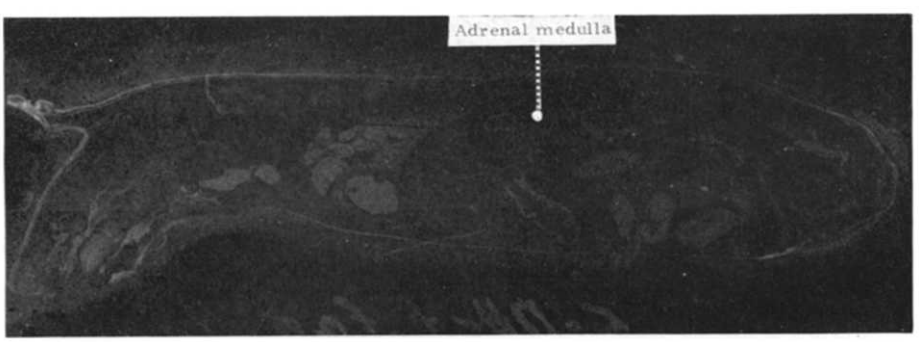


FIG. 3. Autoradiogram of a mouse, sacrificed 4 days after i.v. injection with $^{14}$C-5- hydroxytryptophan. Note the persisting radioactivity in the adrenal medulla.

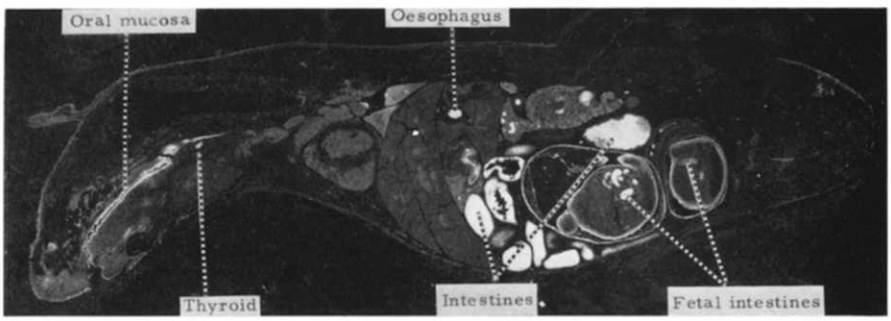


FIG. 4a. Autoradiogram of a mouse which 4 hours before sacrifice had been injected with $^{14}C$-5-hydroxytryptophan. Note the radioactivity in the thyroid gland, the adrenal medulla, the spleen, and in the islets and the excretory ducts of the pancreas.

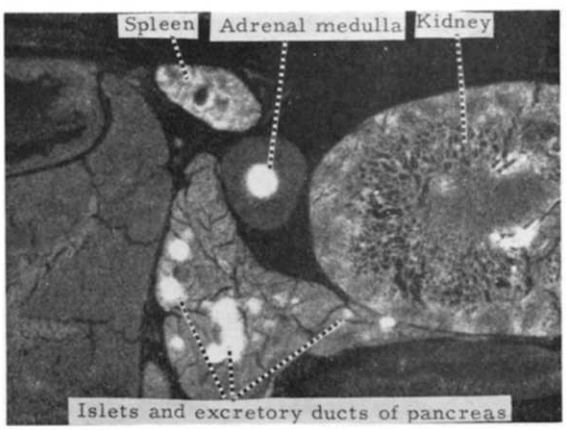


FIG. 4b. Detail of Fig. 4a.

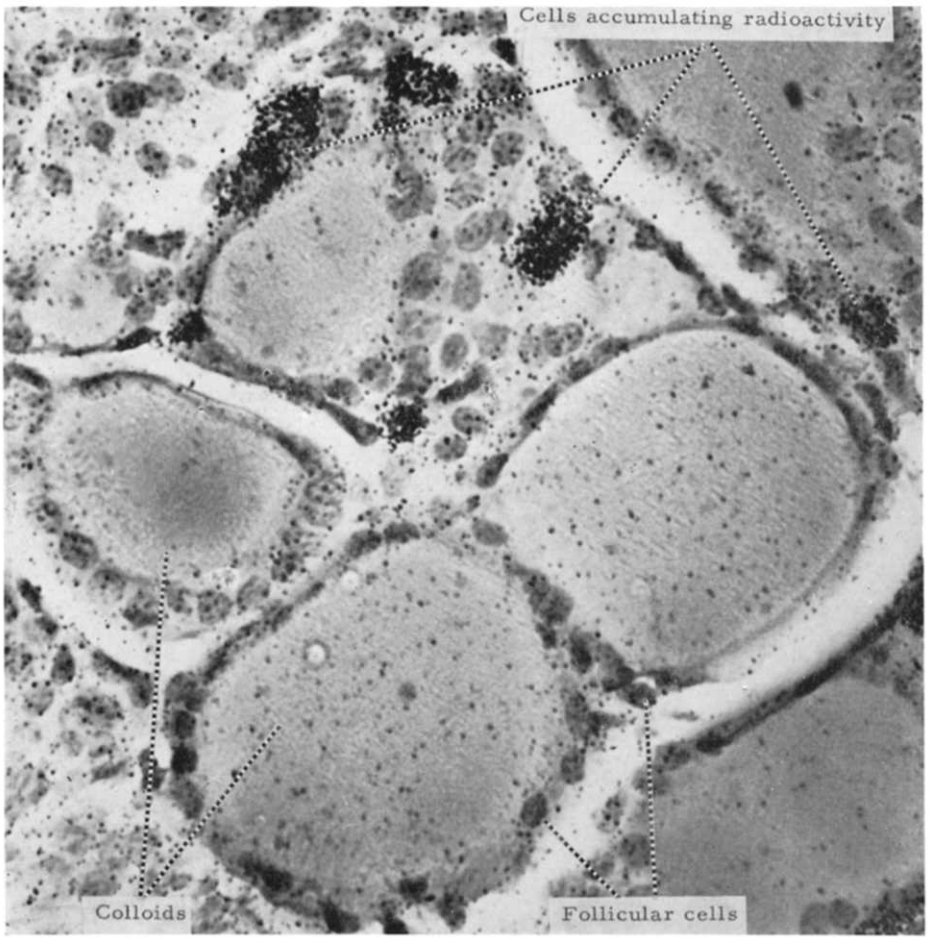


FIG. 5. Microautoradiogram of a mouse thyroid 45 min after i.v. injection of $^{3}$H-5-hydroxytryptophan. The radioactivity is concentrated in a few scattered cells, probably the parafollicular cells. Haematoxylin-eosin stain (× 300)

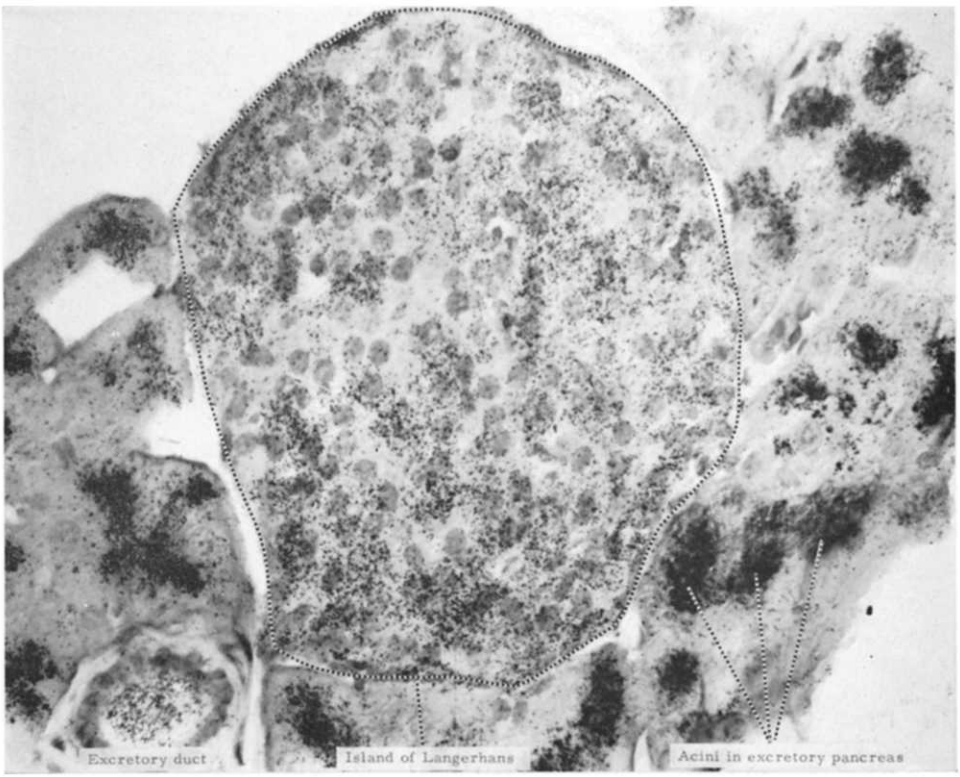


FIG. 6. Microautoradiogram of the pancreas with an island of Langerhans 4 hr after injection of $^{3}$H-5-hydroxytryptophan. In some of the islet cells the radioactivity is high, while other cells show little activity. The radioactivity is also concentrated in the acini and excretory ducts of the pancreas. (× 150)

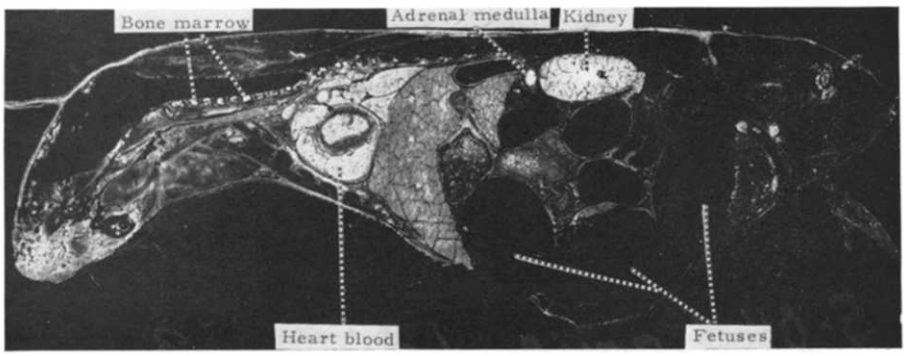


FIG. 7. Autoradiogram of a mouse which 1 hr before sacrifice had been injected with $^{14}$C-5-hydroxytryptamine. Note the high radioactivity in the adrenal medulla, bone marrow and blood. No radioactivity seems to have passed into the brain or the fetuses.

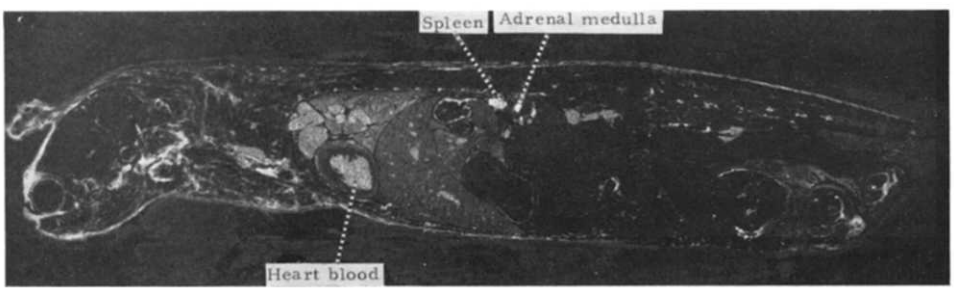


FIG. 8. Autoradiogram of a mouse injected 4 days before sacrifice with $^{14}$C-5-hydroxytryptamine. Radioactivity is persisting in the adrenal medulla, the spleen, and in the blood.

This picture was principally the same 20 min, 45 min and 4 hr after the injection; in the follicular cells proper and in the colloid the radioactivity was low.

In the sections of freeze-dried but not formaldehyde treated thyroid tissue, which were deparaffinized and passed through alcohol to distilled water, before the application of photographic film, there was a random activity spread out all over the tissue, without any sign of localization to specific cells.

After the vanishing of the 5-HTP in the excretory parts of the pancreas, spots of strong activity were revealed. Microautoradiography with $^{3}$H-5-HTP showed a high uptake in the *islands of Langerhans* which could be seen as early as 20 min after injection. However, at that time it was partly masked by the high activity in the exocrine parts. After 4 hr, the blackening of the film was more intense in the islets than in the excretory cells, but lower than in the acinar lumina and excretory ducts. Within an island the labelled isotope seemed to be irregularly distributed in the different cells. No attempt has been made to differ between A- and B-cells, but some cells in the hematoxylin stained sections lack labelling almost completely while others show a high uptake (Fig. 6). In the blood vessels in the neighbourhood of the islands the activity after 4 hours was relatively low.

In the freeze-dried, unfixed sections, that had passed through water before the exposure, the picture of the exocrine part did not differ from the one described above. In the islands, however, the activity that was normally unevenly distributed between the different cells was diffusely spread throughout the islands.

Two minutes after the injection of $^{14}$C-5-HT the *adrenal medulla* already showed a very high radioactivity. Twenty min after injection the activity was high also for 5-HTP. The high activity then persisted throughout the investigation period and was after one and four days by far the highest activity noted in the body. At 20 min after the injection of $^{3}$H-5-HTP the peripherial parts of the medulla seemed to show the highest amount of radioactivity. No further difference between the individual cells could be seen.

*CNS*

Only 5-HTP seemed to cross the blood-brain barrier. The concentration was high in the choroid plexus. In the brain tissue proper there was slight activity mainly localized in the grey matter.

*Fetus*

5-HTP was not concentrated in the placenta, but 20 min after the injection radioactivity was detectable in the fetus. The highest concentration was found in the fetal adrenal medulla and in the intestines and skin. Most of the activity disappeared after one day. The placental passage of $^{14}$C-5-HT was almost totally blocked.

*Control animals*

In none of the tissues from the untreated animals was there any reduction of silver grains that could be seen on the film.

## DISCUSSION

In the technique used for whole body autoradiography, the tissues never come in contact with any solvent that might cause diffusion of the isotope. The micro-autoradiograms obtained after either freeze-drying followed by gas fixation and subsequent

wet autoradiography or dry microautoradiography of unfixed tissue were identical. However, a strong diffusion of the radioactivity was observed in the freeze-dried but unfixed thyroid and pancreatic islands if they had been in contact with water before exposure. This showed that the radioactivity still was in a water soluble form.

The great similarity between the pictures obtained with 5-HT and 5-HTP especially after long survival periods indicates that 5-HTP may be rapidly decarboxylated to 5-HT and stored as such, or that 5-HTP can be stored at the same sites as 5-HT. Other metabolites of 5-HTP are not considered to remain in the body for any length of time.[6]

It has been shown that the decarboxylation of administered 5-HTP to 5-HT is a fast reaction and that the 5-HT content of the tissues will rise rapidly after 5-HTP administration.[13]

Udenfriend, Weissbach and Bogdanski[13] found that after the injection of $^{14}$C-5-HTP to rabbits, all the body depots of 5-HT were highly labelled. However, after larger doses even tissues that normally did not contain measurable amounts of 5-HT had high 5-HT values.

The administered doses of 5-HT and 5-HTP in the present investigation (9 and 12–13 mg per kilogram body weight respectively) are in the same range as the lowest doses used by Udenfriend *et al.*[13]

When dl-5-HTP is administered the d-isomer is probably rapidly excreted through the urine, unchanged.[3]

There are great similarities between the picture obtained with 5-HT and with 5-HTP. A few differences are, however, noted. Most evident is the pronounced accumulation of 5-HTP, but not of 5-HT, in protein synthesizing tissues, such as exocrine pancreas and intestinal mucosa. 5-HTP is evidently treated by these tissues as a protein-forming amino acid (see below). Winter and Street[45] also found that 5-HTP may participate in peptide formation.

*Circulatory system*

5-HT is very easily taken up by *blood platelets*,[14] but they are unable to synthesize 5-HT from 5-HTP.[15] The half-life of 5-HT in blood has been calculated to 24–48 hr[15] which explains the high blood activity found in the present investigation after $^{14}$C-5-HT administration even after long survival periods.

Radioactivity is gradually increased in the *spleen*, the site where most of the platelets finally leave the circulation. The spleen is also known to be richly supplied with adrenergic nerve endings, which possibly might accumulate 5-HT.

The radioactivity of the *bone marrow* rapidly exceeds that of the blood, indicating that some structure specifically absorbs and binds 5-HT and possibly 5-HTP. Udenfriend and Weissbach[15] assume that the platelet 5-HT is synthesized and incorporated at the site of platelet formation, but Gaddum and Giarman[16] were unable to find 5-HTP-decarboxylase activity in the bone marrow. Further investigations on bone marrow and 5-HT are in progress.

*Mast cells*

Several authors have confirmed the initial finding by Benditt *et al.*[17] that mast cells from the rat contain 5-HT. Parrat and West[18] investigated a number of species, but found 5-HT in rat and mouse mast cells only. These cells have also been shown to

have the enzymatic facilities to decarboxylate 5-HTP to 5-HT,[19] and rat peritoneal mast cells[20] and tissue mast cells[21] are capable of taking up administered $^{14}$C-5-HT. It was therefore not surprising to find that in the present investigation peritoneal mast cells were labelled by $^{3}$H-5-HTP. The labelling was, however, very irregular, which may be an expression of varying maturity or different functional stages. Compared to the radioactivity of the adrenal medullary cells, cells in the pancreatic islands and the thyroid, that of the mast cells was relatively weak. This could be the reason why no activity was observed in the mast cells of the skin, where the high activity of the dermal collagen may have masked that of the mast cells. Adams-Ray *et al.*,[22] however, have shown that mast cells in different body regions often show varying capacities to take up administered monoamines.

The great affinity for 5-HT and 5-HTP by collagen is of some interest, since it is known that 5-HT stimulates fibrosis when repeatedly injected into the skin[23] and gives rise to damages of the heart valves when present in high concentrations, as is the case in carcinoidosis.[24]

The initial radioactivity in the gastrointestinal mucosa seen in the whole body autoradiograms after $^{14}$C-5-HTP administration probably represents incorporation of $^{14}$C-5-HTP or a labelled metabolite into gastrointestinal enzymes.

The few scattered cells in the gastric and duodenal mucosa accumulating radioactivity probably represents the enterochromaffin cells as indicated by their fluorescence. The incorporation into enterochromaffin cells was expected, being the up to now most recognized 5-HT-synthesizing cells of the body. More detailed studies of this incorporation using a combination of autoradiography and fluorescence microscopy are under investigation.

*Thyroid*

It has been known for some time that 5-HT exerts a strong effect on the thyroid metabolism[25, 26, 27] and in a few species (rat, sheep) it has been found to be normally present in high concentrations.[28] In the rat, this has been considered to be due to a large amount of 5-HT-containing mast cells. In the sheep, however, this is unlikely, since as far as it is known rat and mouse are the only species that normally have 5-HT in their mast cells.[18, 22] In the present case, the mast cells were scarce in and around the thyroid gland, and in no case did the cells containing radioactivity show metachromatic properties after toluidine blue staining.

The distribution of the radioactive cells in the thyroid and their morphology strongly resembles that of the so called parafollicular cells[29, 30, 31, 32]. There has been much discussion as to the nature and function of these cells, and they are given various names such as macrothyreocytes, clear cells, and parafollicular cells. Sunder-Plassmann[33] found them to be richly supplied with nerve endings (presumably vagal fibers) and postulated that they had a secretory activity (neurohormonal cells). Most of the recent work in this field[34, 35, 36] seems to indicate that the parafollicular cells form an integrated part of the hormone producing system of the thyroid gland.

It is well known that 5-HT can block the iodine uptake of the thyroid[25, 27, 37] both *in vivo* and *in vitro*. The autoradiographic findings show that certain cells in the mouse thyroid, probably the so-called parafollicular cells, have the capacity to take up and bind administered 5-HTP or 5-HT. This in turn makes it probable that the normally

occurring 5-HT in the sheep thyroid is localized to similar cells, and that the 5-HT action on thyroid metabolism is mediated by these cells.

*Pancreas*

5-HTP is evidently taken up and distributed by the exocrine pancreas as a protein forming amino acid. The time lapse from the administration of the isotope to the uptake in the exocrine cells, secretion into the excretory ducts and subsequent disappearance is similar to the one found with $^{35}$S-methionine, $^{35}$S-cystine and $^{14}$C-phenylalanine.[38]

The radioactivity appearing in the islet tissue after injection of $^{14}$C-5-HTP and $^{3}$H-5-HTP is not likely to represent insulin, since neither 5-HTP nor tryptophan is known to be a normal constituent of this hormone.

Falck and Hellman[39] have recently shown, by means of a histochemical technique[11] that some island cells of certain animals (duck, guinea-pig, cat, dog and horse, but *not* of mouse or rat) contain a monoamine. In the case of guinea-pig, the monoamine probably belongs to the tryptamine group, and is localized in the B-cells.[40]

The present findings indicate that 5-HT might be normally present in the pancreatic islands not only in the species mentioned, but also in others. The concentrations might, however, be too low to be detectable by histochemical techniques. With Falck's and Hellman's investigations in mind, it seems probable, that the irregular distribution of radioactivity in the different cells within the islands corresponds to the distribution of B-cells.

*Adrenal medulla*

Bertler *et al.*[41] have shown that one hour after the administration of a large dose of 5-HTP (100 mg per kilogram body weight) to a rabbit, 5-HT will appear in a granular fraction of the adrenal medulla, the same fraction as the one where noradrenaline and adrenaline accumulate after administration of dihydroxyphenylalanine (DOPA). These authors considered this accumulation of 5-HT to be due to the well-known unspecificity of 5-HTP-ase, which probably is identical with DOPA-decarboxylase.[42] The accumulation of 5-HT after 5-HTP-administration was, however, much weaker than the increase of noradrenaline and adrenaline after DOPA-administration. On the contrary, Conners and Rosenkrantz[43] found less uptake *in vitro* of 5-HT from the medium in whole adrenals of rat and rabbit than in separated adrenal cortex.

The autoradiographic findings are in good agreement with those of Bertler *et al.*[41] The adrenal medullary cells are, however, also capable of taking up not only the amino acid precursor, when administered in a low dose, but also the ready-made amine (5-HT), and the unspecificity seems to prevail both in the decarboxylating and the storage mechanism. The uptake of 5-HT (maximal within 2 min) was more rapid than the uptake of the amino acid, but once taken up both of them persisted for a long time at the storage sites.

The general pattern of the autoradiograms obtained after administration of radioactively labelled 5-HT or 5-HTP has a striking resemblance to that obtained by Rosell, Sedvall and Ullberg[44] with DOPA-$^{14}$C. In the present investigation radioactivity has accumulated partly in organs that are known to contain 5-HT but also in tissues that generally are considered to be catecholamine containing, e.g. adrenal medulla, myocardium and salivary glands. This indicates that there is an overall lack

of specificity of the amine storing sites, when it comes to differentiating between catecholamines and indolalkyl amines, and their precursors. It is remarkable that 5-HT is so firmly bound in the adrenal medulla that it remains in high concentrations even 4 days after administration of comparatively small doses.

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