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Fluorescence Microscopy of the 5-HTP Turnover in the Exocrine Pancreas of Mice and Rats*

P. Alm

Institute of Anatomy and Histology, Department of Histology, University of Lund, Lund, Sweden

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Summary. The turnover of L-5-HTP, D-5-HTP and 5-HT in the exocrine pancreas have been studied by means of the fluorescence method of Falck and Hillarp. L- and D-5-HTP are easily taken up by the acinar cells, whereas 5-HT seems to pass into the cells only to a minor extent. After the administration of L-5-HTP (and in some cases after 5-HT administration), specific fluorescence is seen in the form of apically located granules (probably identical with the zymogen granules) for a short period, which is prolonged, if the animals are pretreated with a MAO inhibitor. Decarboxylase inhibition prevents the appearance of these fluorescent granules. Administration of D-5-HTP does not give rise to this granular fluorescence but to a diffuse fluorescence throughout the cells. Thus, there are reasons to assume that the granular fluorescence derives from 5-HT. The results obtained in this work correspond well with those from a similar study with L-DOPA and some of its analogues.

The exocrine pancreas has a very great capacity to take up amino acids and analogues of amino acids from the blood, presumably because of its high protein synthetizing capacity. By applying a combination of chemical and histochemical methods for studying the monoamines and their immediate precursors, it has been established, that the amino acid L-DOPA is taken up and handled by the acinar cells in a special way (Alm, Ehinger and Falck, 1967, 1969). Thus, rapidly after the injection of L-DOPA, a specific diffuse fluorescence appears in the cytoplasm and the nuclei of the cells. Somewhat later, specific fluorescence is seen only in coarse apical granules (probably identical with zymogen granules). It was also shown, that the substance taken up into the granules is by all probability not L-DOPA, but its decarboxylated derivative, DA. As 5-HTP is accumulated to a very high extent in the pancreas (Ritzén, Hammarström and Ullberg, 1965; Gershon and Ross, 1966a and b) and also can be detected with the procedure of Falck and Hillarp, it was considered of interest to perform a fluorescence microscopical study of the metabolism of 5-HTP in the exocrine pancreas and to compare it with that of DOPA.

Materials and Methods

Experimental Procedure. Albino mice and albino rats of both sexes (weighing 20—25 g and 180—250 g; Anticimex, Sweden) were used. All the animals were given standard pellets (Teknosan, Sweden) and water *ad libitum*. Injections were performed intravenously as

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The following abbreviations have been used in this article: DOPA = 3,4-dihydroxyphenylalanine, DA = dopamine, NA = noradrenaline, A = adrenaline, 5-HTP = 5-hydroxytryptophan, 5-HT = 5-hydroxytryptamine, MAO = monoamine oxidase.

previously described (ALM *et al.*, 1969). At various times after the injections, the animals were sacrificed by a blow in the neck whereupon the pancreas was dissected out, frozen in liquid-nitrogen-cooled propane, freeze-dried, treated with formaldehyde of standardized humidity (FALCK and OWMAN, 1965; HAMBERGER, 1967), embedded in paraffin wax *in vacuo* sectioned, mounted on glass slides and studied in a fluorescence microscope (for details see e. g. FALCK and OWMAN, 1965).

Drugs. The following substances, dissolved in 0.9% saline, were injected: L-5-HTP (Fluka AG), D-5-HTP (Calbiochem), and 5-HT creatinine sulphate (Regis Chem. Corp.).

Nialamide solution (Niamid, Pfizer Ltd.) was prepared as described by BERTLER, FALCK and OWMAN (1964) and injected (100 mg/kg) 3 hours before the injection of L-5-HTP.

NSD 1015 (m-hydroxybenzylhydrazin; Smith and Nephew Ltd. through Ferrosan AB, Sweden), dissolved in 0.9% saline, was given (100 mg/kg) one hour before the injection of L-5-HTP.

Microscopy. Microscopy was performed according to FALCK and OWMAN (1965). After the injection of 5-HTP or 5-HT, there appeared a yellowish fluorescence in the exocrine pancreatic cells (see Results). This fluorescence fulfilled the criteria formulated for the identification of the fluorophore formed from some 5-hydroxylated indole amine derivatives and is referred to as "specific fluorescence" in this paper (cf. FALCK and OWMAN, 1965; CORRODI and JONSSON, 1967). The fluorescence picture was judged by two criteria: the intensity of the fluorescence and its cellular localization. If the fluorescence appeared more or less uniformly over the cell and could not be localized to any cellular structure, it was considered diffuse. The intensity of the fluorescence was judged subjectively: 0 = absence of fluorescence, 1 = weak, 2 = moderate, 3 = strong, 4 = very strong. This was to make clear very simply the changes in intensity of the subjectively estimated observations.

Results

Only small differences were noted between the two species used. After the injection of L- or D-5-HTP, there appeared a yellowish fluorescence in the exocrine pancreatic cells. In a pilot experiment, L-5-HTP was given in different doses to mice (10, 20, 40, 60 and 100 mg/kg) and the animals were killed after 5, 15, 30 and 60 minutes. Up to 30 minutes after the injection, the specific fluorescence was intense and diffusely distributed throughout the acinar cells (but little or none occurred in the connective tissue). 60 minutes after the injection, the specific fluorescence occurred only in the apical parts of the acinar cells and in the form of densely aggregated coarse granules (compare Fig. 1). 60 mg/kg produced the appearance of discrete granules, displaying a sufficiently strong fluorescence intensity. In a preliminary experiment, when L-5-HTP was given to rats in a dose of 60 mg/kg, nearly the same morphological picture and fluorescence intensity as that of mice was obtained at the different times after the injections. Although granular fluorescence was also seen at lower dose levels, this dose was chosen for the further study of the effect of various drugs on the metabolism of L-5-HTP in the two species.

In the following experiments, L-5-HTP (60 mg/kg) was injected and the animals were killed after different times (see Table 1 and 2). In mice, a strong specific diffuse fluorescence was found already 10 minutes after the injection (compare Fig. 2). 40 and 60 minutes after the injection, the specific fluorescence was mainly confined to the apical parts of the cells (see Table 1) and appeared as granules, which correspond to zymogen granules, as seen in the phase contrast picture (compare Fig. 3). This type of fluorescence is in the following referred to as "granular". Though there occurred granular fluorescence in all mice at these

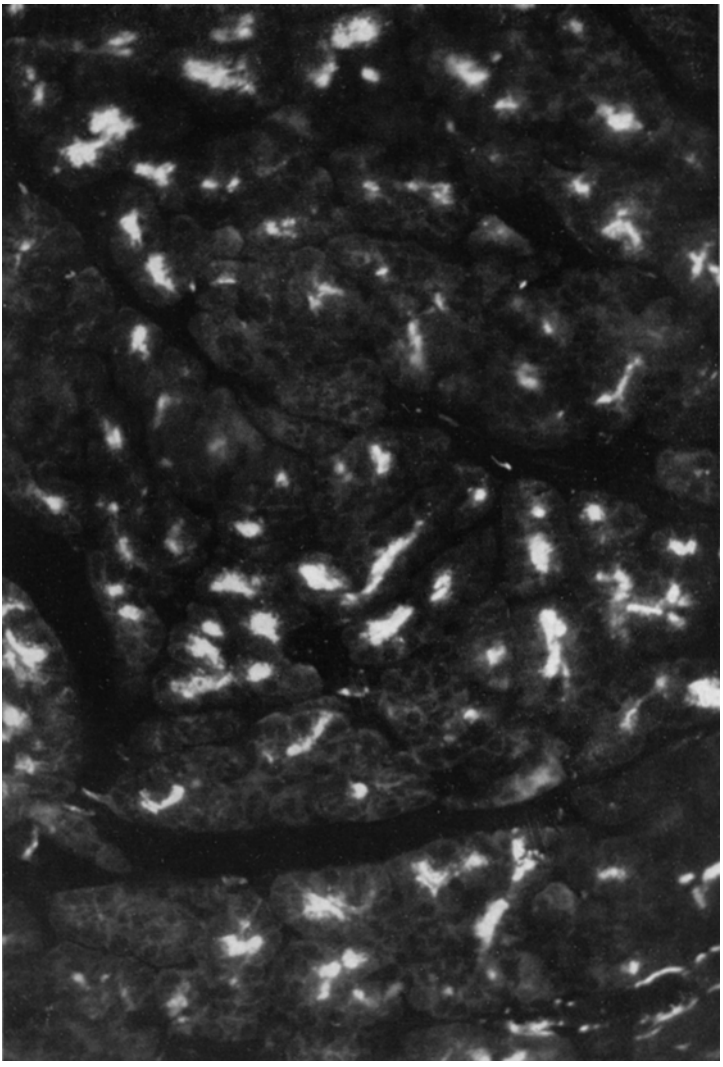

Fig. 1. Rat pancreas, 90 min after the injection of L-5-HTP (60 mg/kg). Specific fluorescence is localized only to the apical parts of the exocrine cells. Fluorescence micrograph, ×304

two groups of time, there was an evident variation between the animals as to the number of acini containing granular fluorescence. In some animals, granular fluorescence occurred in nearly all acini, while in others there were found acini, which were totally lacking any fluorescence. 90 minutes after the injection, the granular fluorescence still existed in nearly all mice. Variations in the number of acini containing granular fluorescence were still seen between the different animals. 180 minutes after the injection, granular fluorescence was seen only in a few of the animals. 240 minutes after the injection, granular fluorescence of a very low intensity could be seen in some few acini.

Table 1. *Mouse*

Time (min) Drugs		10	40	60	90	180	240
L-5-HTP 60 mg/kg	D	$2^1/_2$	0 (I)	0 (I)	0 (II)	0 (III)	0 (IV)
	G	0	2	2	$1^1/_2$, in $^5/_6$	2, in $^2/_6$	1, in $^5/_6$
	N	6	5	5	6	6	6
D-5-HTP 60 mg/kg	D	2	2	2	1	0	
	G	0	0	0	0	0	
	N	3	3	3	3	3	
5-HT 180 mg/kg	D	0 (V)		0 (VI)			
	G	2, in $^2/_6$		2, in $^6/_7$			
	N	6		7			

D = diffuse fluorescence, G = granular fluorescence; N = number of animals used, h = hour, a = acini, $^1/_5$ = one out of five animals, $^3/_7$ = three out of seven animals, etc.

(I) in $^1/_5$ G. in 50% of a., in $^4/_5$ G. in all a.
(II) in $^2/_6$ G. in a few a., in $^2/_6$ G. in 50% of a., in $^1/_6$ G. in all a.
(III) in $^1/_6$ G. in all a., in $^1/_6$ G. in a few a.
(IV) in $^4/_6$ G. in a few a., in $^1/_6$ G. in 40% of a.
(V) G. in a few a.
(VI) in $^3/_7$ G. in a few a., in $^1/_7$ G. in all a., in $^1/_7$ G. in 70% of a., in $^1/_7$ G. in 20% of a.

In rats there also occurred a diffuse fluorescence 10 minutes after the L-5-HTP injection. In contrast to mice, however, the apical parts of most exocrine cells contained specific fluorescence of a very low intensity. In phase-contrast microscopy, these weak fluorescent cellular parts correspond quite well to those parts, which contain the zymogen granules (see Fig. 2). 40, 60 and 90 minutes after the injection, there also appeared granular fluorescence (see Fig. 1 and 3) although to a lesser extent when compared to mice. In contrast to mice, a rather strong diffuse fluorescence persisted simultaneously with the granular fluorescence in the last-mentioned three groups. This diffuse fluorescence decrease only slightly between the 40 and the 90 minutes groups (see Table 2).

Shortly after the injection of D-5-HTP (60 mg/kg), a diffuse fluorescence appeared in both species all over the exocrine pancreatic cells in the same way as after the L-5-HTP administration (see Table 1 and 2). However, granular fluorescence never appeared, and the fluorescence remained diffuse. In mice, it slowly diminished and remained longer than after the L-5-HTP administration. In rats, the fluorescence also remained diffuse. No great changes in intensity occurred at the times tested.

5-HT (180 mg/kg) was taken up by the exocrine pancreas of both mice and rats but to a much lesser extent than both D- and L-5-HTP (see Table 1 and 2). In none of the groups did the acinar cells show any specific fluorescence comparable to that after the L-5-HTP injection. Ten minutes after the injection, the acinar cells were totally lacking fluorescence in rats, whereas in mice granular

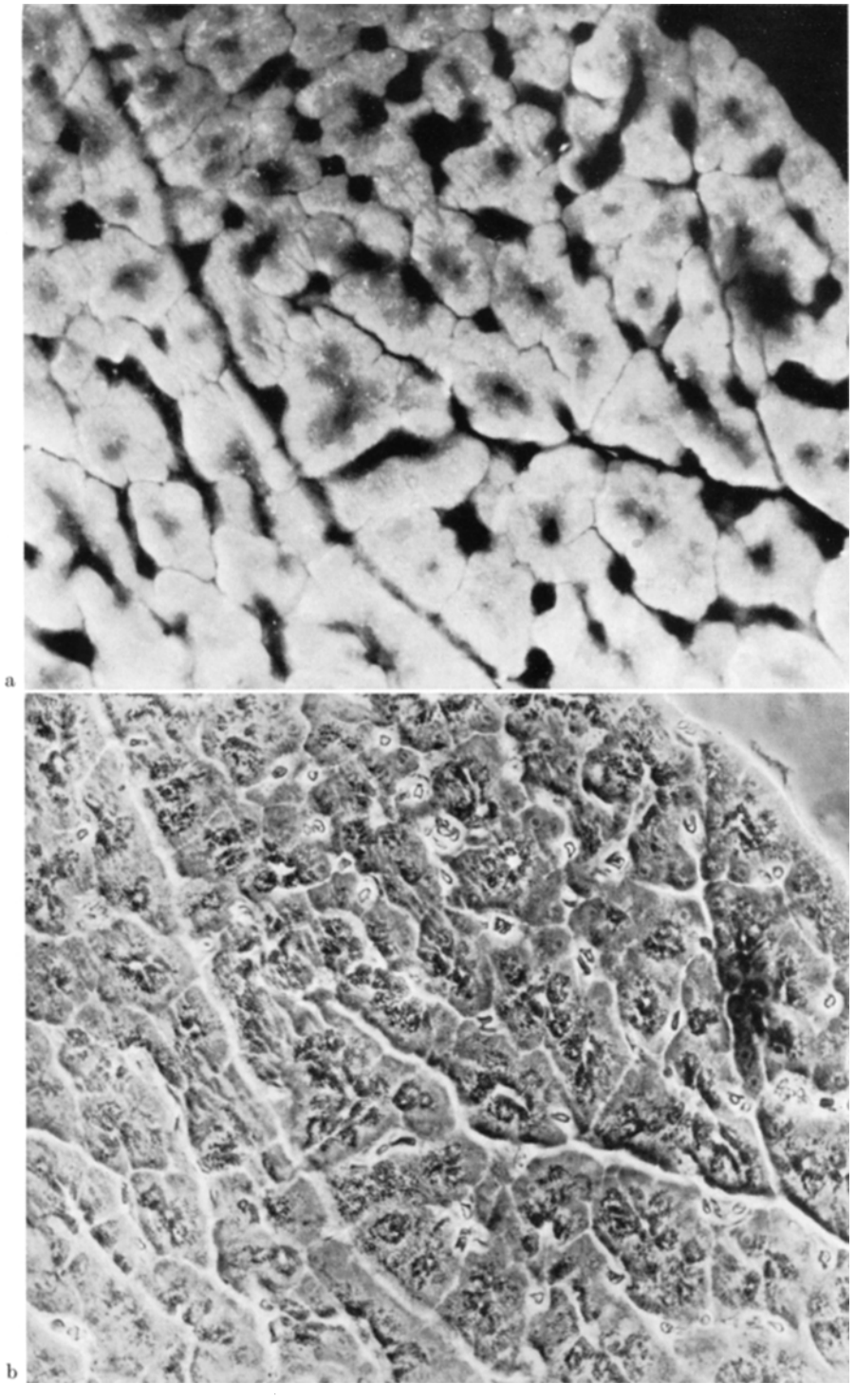

Fig. 2a and b

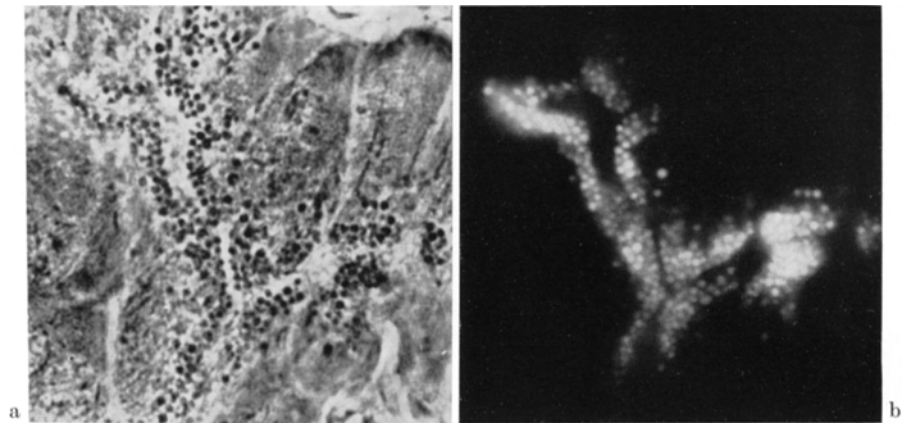

Fig. 3. a) Rat pancreas, 40 min after the injection of L-5-HTP (60 mg/kg). The coarse black spots are zymogen granules. There is close correspondence between a zymogen granulum and a coarse fluorescent granulum (compare with Fig. b). Phase-contrast micrograph, × 1,500. b) Fluorescence micrograph, same area and magnification as Fig. a

Table 2. *Rat*

Time (min) Drugs		10	40	60	90
L-5-HTP 60 mg/kg	D	$2^1/_2$	2 (I)	$1^1/_2$ (II)	1 (III)
	G	0	3	3	2
	N	3	3	3	3
D-5-HTP 60 mg/kg	D	2	$1^1/_2$	2	
	G	0	0	0	
	N	3	3	3	
5-HT 180 mg/kg	D	0		0 (I)	
	G	0		2, in $^2/_3$	
	N	3		3	

(I) G. in a few a.
(II) in $^2/_3$ G. in 25% of a., in $^1/_3$ G. in 50% of a.
(III) in $^1/_3$ G. in 30% of a., in $^1/_3$ G. in 50% of a., in $^1/_3$ G. in a few a.

fluorescence was seen in a few animals. 60 minutes after the injection, both mice and rats showed granular fluorescence. However, there were great variations between the different animals as to the proportions of acini containing granular

Fig. 2. a) Rat pancreas, 10 min after the injection of L-5-HTP (60 mg/kg). There is a strong specific fluorescence in all exocrine cells. In the apical parts of the cells, there are areas, where the fluorescence is very weak, if present at all. Fluorescence micrograph, × 500. b) Phase-contrast micrograph, same area and magnification as Fig. a. Around the acinar lumen, zymogen granules are stored and seen as accumulations of black spots. There is close correspondence between these and the weak fluorescent areas in Fig. a

fluorescence and acini lacking granular fluorescence (see Table 1 and 2). Great differences could be noted even between two adjacent lobes.

After inhibition of MAO prior to administration of L-5-HTP, a granular fluorescence persisted in all acini up to 4 hours in all mice tested (see Table 3). It should be noted in this connection, that no specific fluorescence has been observed in the pancreatic ducts.

Inhibition of the 5-HTP decarboxylase with NSD 1015 resulted in mice in a very strong diffuse fluorescence after the L-5-HTP injection, which remained up to 180 min after the L-5-HTP injection (Table 3). However, granular fluorescence was constantly seen 180 min after the injection (see Table 3). In rats only one time was studied. 180 min after the injection, a diffuse fluorescence was seen but no fluorescent apical granules could be detected (3 animals).

Table 3. *Mouse*

Time (min) after the last injection. Drugs		30	60	120	180	240
Nialamide 3h + L-5-HTP 60 mg/kg	D	2	1 1/2	1	0	0
	G	0	3	1 1/2	1 1/2	1 1/2
	N	5	6	5	5	5
NSD 1015 1 h + L-5-HTP 60 mg/kg	D	2 1/2	2	1	1 (I)	0 (II)
	G	0	0	1 1/2, in 1/6	1 1/2	1 1/2
	N	6	6	6	5	6

(I) in 3/5 G. in 50% of a., in 1/5 G. in all a., in 1/5 G. in a few a.
(II) in 2/6 G. in 50% of a., in 3/6 G. in all a., in 1/6 G. in a few a.

Discussion

The fluorescence method of FALCK and HILLARP for the histochemical demonstration of certain monoamines and their immediate precursors is both chemically and histochemically well defined (see CORRODI and JONSSON, 1967) and has been used in the study of a large number of monoamine-containing structures (see review by NORBERG, 1967; FALCK and OWMAN, 1968). The results of the present experiments are in several respects similar to those previously obtained with L-DOPA and DA (ALM *et al.*, 1969). Shortly after the injection of L-5-HTP, there was a much more intense fluorescence in the acinar cells than in the surrounding connective tissue, suggesting an active uptake mechanism of L-5-HTP just as of L-DOPA. The two stereoisomers, D-5-HTP and L-5-HTP, also seem to be taken up to roughly the same extent just as D- and L-DOPA. However, only after L-5-HTP administration (and not after D-5-HTP administration), specific fluorescence could be demonstrated in the islets of Langerhans in mice and rats and in the parafollicular cells of the rat thyroid (CEGRELL, 1968; SUNDLER, unpublished observations). Further, D-5-HTP does not seem to pass the blood-brain barrier in rats (OWMAN, personal communications).

It is well known, that amino acids are mainly taken up into the pancreas in order to become incorporated into enzyme-proteins, which are then stored in the zymogen granules (see e.g. JAMIESON and PALADE, 1967). It has been noted, that the time necessary for this transport is 40—60 minutes in mice and rats kept under similar conditions as those of the present work (NADLER, 1963; WARSHAVSKY, LEBLOND and DROZ, 1963; VAN HEYNINGEN, 1964). The appearance of the granular fluorescence in both mice and rats after the administration of L-5-HTP at an approximately corresponding time (cf. GERSHON and ROSS, 1966b) is noteworthy. It has been proposed, that 5-HTP would be "taken up and distributed by the exocrine pancreas as a protein forming amino acid" (RITZÉN *et al.*, 1965). Still, however, 5-HTP has not been described to be a constituent of mammalian proteins, and nor has 5-HTP been demonstrated to be incorporated into mammalian proteins (GERSHON and ROSS, 1966a).

DOPA decarboxylase has been found in the pancreas from a number of different species (HOLZ, CREDNER and STRÜBING, 1942). As DOPA and 5-HTP are decarboxylated by the same enzyme (see e.g. HAGEN and COHEN, 1966), it was considered of obvious interest to see, if decarboxylase inhibitors would affect the turnover of L-5-HTP as they do in case of L-DOPA. It was found, that the appearance of the granular fluorescence was delayed by decarboxylase inhibition. Further, the administration of D-5-HTP [which is resistant to the decarboxylating enzyme (cf. e.g. LOVENBERG, WEISSBACH and UDENFRIEND, 1962)] did not give any granular fluorescence. This indicates, that the appearance of the granular fluorescence is dependent on the decarboxylation of L-5-HTP to 5-HT, which thus seems to be the substance taken up in the zymogen granules. The granular fluorescence after L-5-HTP administration had the same localization and appeared at about the same time as the granular fluorescence obtained after L-DOPA injection (ALM *et al.*, 1969).

The granular fluorescence after L-5-HTP administration remains somewhat longer than that after L-DOPA in both mice and rats. This seems to be in line with the observations on the difference in turnover between DOPA and 5-HTP (ROSELL, SEDVALL and ULLBERG, 1963; RITZÉN *et al.*, 1965). Shortly after L-5-HTP administration, the apical parts of the rat exocrine cells are almost devoid of fluorescence. Such a phenomenon was not observed in mice. Further, diffuse fluorescence in the basal $^2/_3$ rds of the mice exocrine cells could not be observed simultaneously with the apical granular fluorescence, which is the case in rats, and in addition, the diffuse fluorescence exists during a much longer time in rats than in mice. These facts seem to imply, that mice could have a more intense turnover of L-5-HTP than rats.

The way of disappearance of the 5-HT from the apical granules — which probably are the zymogen granules — is obscure. Inhibition of MAO prolongs the persistance of the granules, and it thus seems possible, that this enzyme is at least partially responsible for the elimination of the 5-HT.

The ability of the pancreas and other gastrointestinal organs to take up 5-HT is much lower than with its precursor 5-HTP (RITZÉN *et al.*, 1965; GERSHON and ROSS, 1966a; AIRAKSINEN and MATTILA, 1967). This readily explains the lack of fluorescence in the acinar cells shortly after the 5-HT injection. However, some 5-HT is obviously taken up since after longer times, granular fluorescence

is found. The results with DOPA and DA were similar (ALM *et al.*, 1969). The acinar cells resemble in this respect other cell systems such as the islets of Langerhans (CEGRELL, 1968), the enterochromaffin-like cells in the rat gastric mucosa (HÅKANSON, LILJA and OWMAN, 1967), and the brain capillaries, which easily take up L-5-HTP and L-DOPA but to a very small extent the decarboxylated analogues (OWMAN, personal communication; BERTLER, FALCK, OWMAN and ROSENGREN, 1966).

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P. ALM
Institute of Anatomy and Histology
Department of Histology
University of Lund
Lund/Sweden