Dtsch. Z. ges. gerichtl. Med. 65, 32—43 (1969)

The Time Dependence of the Concentration of 5-Hydroxytryptamine and Staining Characteristics of Enterochromaffin Cells in the Human Duodenum Post-Mortem

ANTTI PENTTILÄ* und KARI OJALA**

Department of Anatomy (Head: Prof. O. ERÄNKÖ, M.D., Ph. D.), and Department of Forensic Medicine (Head: Prof. U. UOTILA, M.D.), University of Helsinki

Eingegangen am 14. September 1968

Introduction

In several mammalian species most of the total body 5-hydroxytryptamine (5-HT) is located in the gastrointestinal mucous membrane (cf. [8]). The main site of the 5-HT occurrence in the alimentary tract of vertebrates is the duodenum [8]; this also includes man [21, 24]. 5-HT, determined histochemically by formaldehyde-induced fluorescence, locates selectively in the enterochromaffin cells (EC) of the duodenum [21]. The physiological function of the intestinal 5-HT is scarcely known, but it seems to participitate in the regulation of intestinal motility [9]. In addition, 5-HT is released into the venous circulation by increased intestinal motility and intralumenal pressure [2, 14] and is obviously excreted by EC into the intestinal lumen [19].

Norepinephrine is bound in isolated adrenomedullary granules by forces not dependent on metabolic processes [13, 26]. Morphologic [29] and chemical studies on isolated enterochromaffin granules [15, 22] suggest a similar kind of metabolically inert storage for 5-HT by enterochromaffin granules. In the isolated but intact duodenum of the cow, 5-HT and dopamine concentrations decreased gradually during post-mortem incubation at body and room temperature in experimental conditions [20]. The purpose of the present study was to estimate if there is a correlation between the post-mortem intestinal 5-HT level and the time of presence of human bodies at room temperature after death and their preservation at cold (4° C) temperature before autopsy.

Material and Methods

The material of human bodies was obtained from the Department of Forensic Medicine. For this study bodies were taken for which the time of death, the time of presence of bodies at room temperature and the time of their preservation at

This work was supported by a grant from the Yrjö Jahnsson Foundation, Helsinki (*), and by an institutional grant from the Sigrid Jusélius Foundation, Helsinki (**).

4° C before autopsy were exactly known. The material consisted of human adults of both sexes, the average age being about 55.8 years. Before autopsy, the bodies were at room temperature 2 to 77 hours and at 4 °C to 215 hours, the respective mean values being 11.0 and 54.7 hours.

At autopsy following sudden death the causes found were:

"Natural" death, 71 cases, of which 57 died in arteriosclerotic and degenerative heart diseases, 4 in cerebral hemorrhage, 2 in pulmonary embolism, 2 in aortic rupture, 3 in bronchopneumonia, 1 in malignant bronchial neoplasm with concomitant pneumonia, 1 in hepatic cirrhosis and 1 in general sepsis.

Group of violent deaths, 30 cases, of which 15 died in traumatic lesions of various organs, 2 by hanging, 3 by drowning, 6 by poisoning with barbiturates, 1 by carbon monoxide and 3 by ethyl alcohol.

For 5-HT determinations pieces were taken from the duodenum immediately adjacent to the pyloric ring, washed under tap water, dissected into suitable specimens (200 to 1000 mg of weight and including all intestinal layers), frozen, and stored in liquid nitrogen until homogenization. After thawing the specimens were weighed and homogenized in a Ultra-Turrax homogenizer for 10 sec in 0.1 N aqueous HCl solution. Bottles including homogenate together with bottles containing a standard amount of 5-HT (Serotonin-creatinin-sulfat Monohydrat, Fluka AG) were stored in an ice box at —20° C for 1—4 days. 5-HT was determined by the method of WEISSBACH [28] using an Aminco-Bowman spectrophotofluorometer. The amine content was expressed in μg/g wet weight of tissue.

The decrease in the 5-HT concentration of the duodenum was also studied experimentally in standard conditions at 4° C for 96 hours. For these experiments bodies were used that were stored about 4 hours (mean) at room temperature and about 20 hours (mean) at 4° C before autopsy. Large pieces of the duodenum were dissected and placed in a closed glass vessel. Specimens were taken close to the pylorus at intervals of 24 hours, always from the same edge of the duodenum. 5-HT was determined as mentioned above but the 5-HT decrease was expressed in per cents using the specimen taken at autopsy as reference (= 100%).

Small specimens adjacent to pieces for the 5-HT determination were taken for routine histology and for freeze-drying procedure. For histology pieces were fixed in a 3.5% (v/v) aqueous formalin solution for 24 hours and thereafter dehydrated in graded alcohol and butanol before embedding in paraffin wax. The procedure for demonstrating the biogenic amines in histological sections, in this case presumably 5-HT, followed the principles of ERÄNKÖ [6, 7] and FALCK [10]. Small tissue pieces were placed on a piece of paper, frozen in liquid nitrogen and dried *in vacuo* at —40° C for two days. After treatment in formaldehyde vapour derived from paraformaldehyde at 80° C for one hour the specimens were rinsed in distilled xylene mounted in paraffin, and sections were cut from paraffin blocks at 7 μ. The paraffin wax was removed by xylene before ultraviolet microscopy. To demonstrate autofluorescence pieces were treated identically but not exposed to formaldehyde vapour.

The following staining methods were used: Haematoxylin-eosin [25], the argyrophil reaction [1], the argentaffin [12], the diazo coupling (see [17]), the ferric ferricyanide [16] and the indophenol (see [17]) reaction.

In statistical analysis Student's *t*-test and analysis of variance were used.

Results

1. Quantitative Studies

In Fig. 1 the duodenal 5-HT level is plotted against the total time for which the human bodies were left at room and cold (4° C) tem-

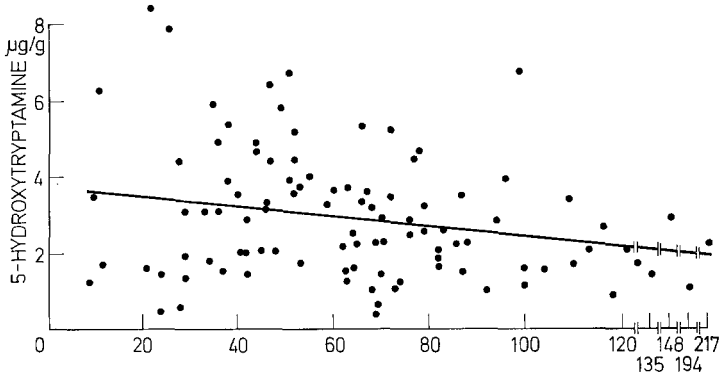


Fig. 1. 5-HT content of the duodenum in human autopsy material. *Abscissa:* the total preservation time of human bodies at room temperature and at 4° C before autopsy. *Ordinate:* 5-HT content in µg per g of wet weight of tissue. Each spot represent one human body. Note the great variance of 5-HT results and a low correlation ($P<0.01$) between the amine level and the total preservation time before autopsy. $y = 3{,}72 - 0.0128$ X, $n = 101$, $r = -0.279$

perature before autopsy. Clearly measurable amounts of 5-HT were detected in the duodenum of the human autopsy material and only in one of 101 specimens the 5-HT level was zero. Individual variations in the present material were large and statistically only a low significance ($P < 0.01$) was found between the amine level and the storage time of bodies before autopsy.

When analysis of variance was performed using the 5-HT level as ordinate and the storage time of human bodies at room temperature or at 4° C before autopsy as abscissa no significant dependence was found between the amine level and the storage time at these temperatures, respectively.

In Fig. 2 the bodies were stored at room temperature for 2—4 hours before their storage at 4° C. In this selected material ($n = 53$ bodies) a highly significant ($P < 0.001$) correlation was found between the amine level and the storage time of bodies at 4° C before autopsy.

Fig. 3 presents the effect of storage on the duodenal 5-HT level at room temperature. The storage time at 4° C was 20—39 hours ($n = 23$) and 60—79 hours ($n = 29$). No significant correlation was found between the amine level and the storage at room temperature if storage at 4° C was approximately constant. In Figs. 2 and 3 the groups were selected so that they comprised more than 50% of the total material. For the total material a diffuse distribution of storage time at 4° C and at room temperature was characteristic.

Fig. 4 demonstrates the reduction of the 5-HT level during storage *in vitro* of the duodenal wall at 4° C in standard experimental conditions.

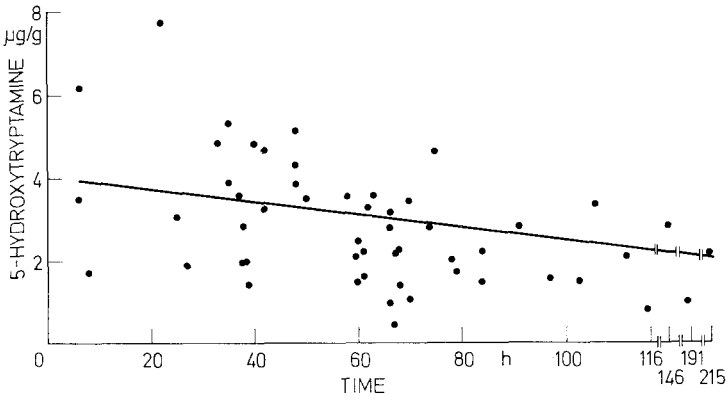


Fig. 2. 5-HT content of the duodenum in the selected human autopsy material. The human bodies were left for 2—4 hours (constant time) at room temperature before autopsy. *Abscissa:* the preservation time of human bodies at 4° C before autopsy. *Ordinate:* as in Fig. 1. Each spot represents one human body. A highly significant ($P < 0.001$) correlation was found between the amine level and the storage time at 4° C. $y = 4.00 - 0.0173\ X$, $n = 53$, $r = -0.467$

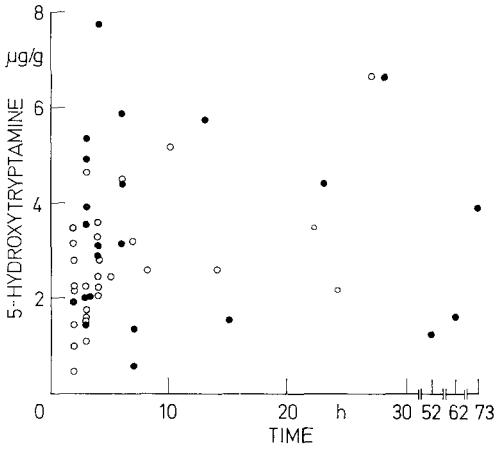


Fig. 3. 5-HT content of the duodenum in the selected human autopsy material. The human bodies were stored for 20—39 hours (constant time) (•) and 60 to 79 hours (constant time) (o) at cold temperature (4° C) before autopsy. *Abscissa:* the preservation time of human bodies at room temperature before autopsy. *Ordinate:* as in Fig. 1. Each spot represents one human body. No significant correlation was found between the amine level and the storage time at 4° C in these two groups, respectively. $y = 3.61 - 0.010\ X$, $n = 23$, $r = -0.105$ for the first group (•) and $y = 3.32 - 0.0940\ X$, $n = 29$, $r = 0.487$ for the second group (o)

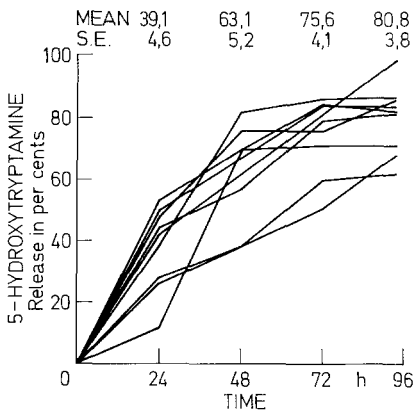


Fig. 4. Decrease of the human duodenal 5-HT content in the autopsy material. Large pieces of the duodenum were dissected and placed in a glass vessel at 4° C. Pieces for the 5-HT determination were taken always from the same edge of the specimens at interval of 24 hours. *Abscissa:* the preservation time of bodies at 4° C in hours. Means and standard errors. *Ordinate:* the 5-HT content determined in per cents using the specimen taken immediately after autopsy as reference (= 100%)

In 96 hours the 5-HT amount of the duodenum decreased significantly with the incubation time.

2. Morphological Study

The duodenum of most bodies showed EC with a positive Bodian's silver reaction. A positive argentaffin reaction was found only in 10 bodies of the total material and as seen in the table these bodies were stored in general for a short time at 4° C before autopsy.

In the Bodian's reaction, individual cytoplasmic granules were easily identifiable (Figs. 6 and 7). In the autolyzed tissue EC with disintegrated but still strongly positive cytoplasmic granules were seen only

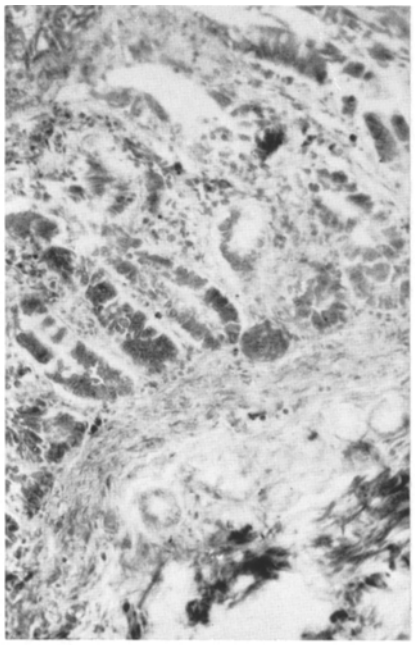
Fig. 5. Argentaffin reaction in the human duodenum. The body was stored 4 hours at room temperature and 6 hours at 4° C before autopsy. With certainty no argentaffin EC were discerned. × 200

Figs. 6—8. The human duodenum preserved 2 hours at room temperature and 42 hours at 4° C before autopsy

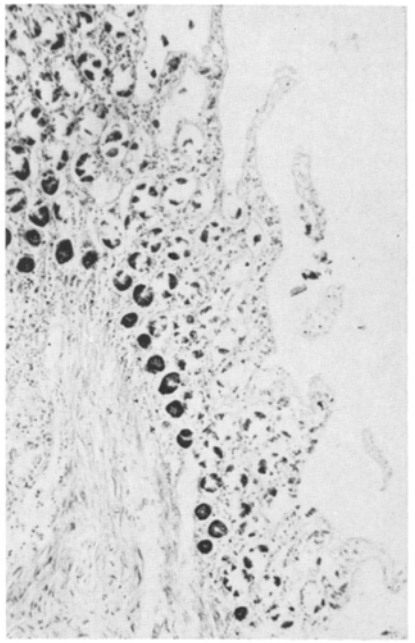
Fig. 6. Hemalaun-eosin reaction. Note some tissue separation from the surface of the mucous membrane. The superficial parts of the mucosa are only slightly stainable but intact glandules are well preserved at the bottom of Lieberkühn crypts with their pyknic nuclei. × 80

Fig. 7. Argyrophil reaction. The mucous membrane is unevenly stained but some strongly stained EC are discerned in the mucous membrane. × 80

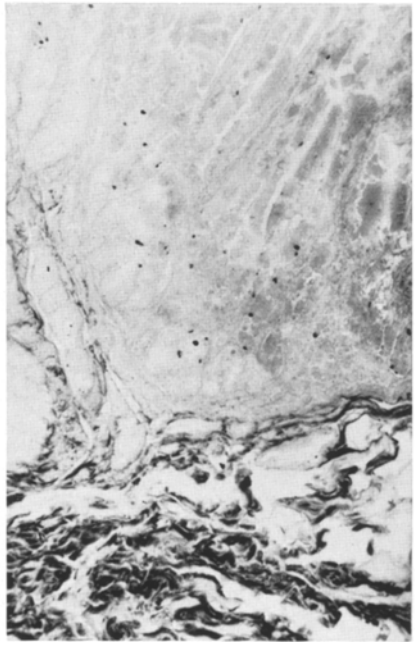
Fig. 8. Argentaffin reaction. No positive EC are to be found. × 80



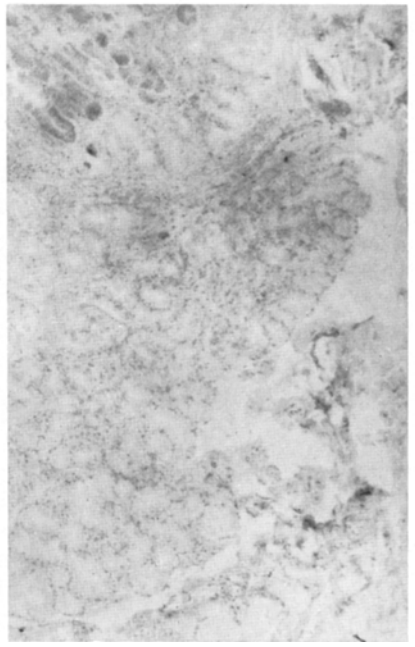
5



6



7



8

Figs. 5–8

Table. *The number of human bodies exhibiting positive EC in the argyrophil and argentaffin reactions in specimens taken various times after death from the autopsy material. The material is arranged according to the time of storage of bodies at 4° C before autopsy and the arbitrary time intervals are seen in the first column*

The time of storage of human bodies at 4° C before autopsy (hours)	Number of specimens	The time of human bodies at room temperature before their storage at 4° C (hours)		Number of specimens exhibiting positive cells in the argyrophil reaction	Number of specimens exhibiting positive cells in the argentaffin reaction
		Mean	S.D.		
0—9	8	11.4	8.0	8	1
10—19	3	25.0	—[a]	2	1
20—29	14	21.6	23.7	12	2
30—39	10	4.4	3.2	7	4
40—49	19	17.6	21.2	11	1
50—59	3	4.3	—[a]	2	0
60—69	18	6.2	6.9	11	1
70—79	11	6.4	7.1	8	0
80—99	6	6.3	6.3	1	0
100—149	7	4.3	3.2	2	0
150—215	2	2.5	—[a]	0	0

[a] S. D. was not determined.

occasionally. In contrast to the argyrophil reaction, the argentaffin reaction weakened gradually and in general only weakly positive EC were found or no positive EC (Figs. 5 and 8). Positive diazo reactions only were found in three bodies, no positive ferric ferricyanide and indophenol reactions in the EC. EC exhibiting formaldehyde-induced fluorescence were found only occasionally in the duodenum. Along with autolytic changes a strong yellow-greenish autofluorescence of the background was found and this obviously faded the specific monoamine fluorescence emitted by EC after treatment in formaldehyde vapour.

Discussion

The present quantitative results indicated that there were analyzable amounts of 5-HT in the duodenal mucosa of the human autopsy material. The slow degradation of 5-HT (mean preservation time of human bodies was 11.0 hours at room temperature and 54.7 hours at 4° C) indicates that 5-HT is either bound in enterochromaffin granules by forces not dependent on metabolic processes as suggested earlier for granule types carrying catecholamines [13] or that 5-HT after liberation from enterochromaffin granules remains unchangeable in the autolyzing tissue.

Several factors have an influence on the amine binding post mortem. In human bodies certainly a passive diffusion of 5-HT from entero-

chromaffin granules occurs at room temperature and obviously also at 4° C. The autolyzing process in the duodenum has an influence on the architecture of duodenal cells which affects the amine binding. In the present study enterochromaffin granules especially were resistant to the effect of autolysis and the seemingly intact argyrophil granules in EC suggest their ability to retain 5-HT long times after death.

The metabolic degradation of 5-HT occurs subsequently obviously by monoamine oxidase, the activity of which is known to remain unchanged in the human intestinum for 24 hours after death [5]. Possibly the inactivation of the liberated 5-HT occurs in the cytoplasm of EC, since at least in some other mammalian species the cytoplasm of this cell type exhibits monoamine oxidase activity determined histochemically [20].

When comparing the effect of the incubation time in experimental conditions at 4° C on the duodenal 5-HT content and the values of the duodenal 5-HT found in the autopsy material it seems that the metabolic degradation of 5-HT occurred more quickly in experiments obviously due to the presence of oxygen necessary for the inactivation of 5-HT by monoamine oxidase (cf. [23]). Again in experimental conditions, in which duodenal tissue of the cow was cleaned from the bacterial flora and incubated in the presence of Neomycin, the inactivation of 5-HT occurred more slowly than in the present study at 4° C [20], indicating the importance of the bacterial flora in the autolyzing process and subsequent degradation of 5-HT.

In different vertebrate species the intestinal 5-HT level varies greatly from species to species and there are great individual variations in the same species (cf. [8]). This was stated also for the duodenum of man in which the 5-HT concentration was 4.2-6.2 μg/g in fresh surgical specimens [21]. In the present material only such human bodies were taken for which the cause of death was known and which had received no drugs known to alter the amine level in the intestinum or in the central nervous system. The present results indicated that individual variations of the duodenal 5-HT were large, and no significant correlation was found between the amine level and preservation time of human bodies at room or cold (4° C) temperature. A correlation with a low significance ($P < 0.01$) was found between the amine level and the *total* preservation time of bodies before autopsy but a highly significant dependence ($P < 0.0001$) between the amine level and the storage time at 4° C when keeping the preservation time of bodies at room temperature short and constant. Many factors in the present material certainly vary greatly and they may have an influence on the intestinal amine content after death or even before it: various somatic diseases, the nutritional status, the kind of bacterial flora in the intestinum, the cause of death *e.g.* by

alcohol, by barbiturate poisoning, by acute traumatic shock *etc.* In brief, the intestinal 5-HT level after death is not without other tools a suitable method for the determination of an approximative time of death but in experimental conditions and with short preservation times of bodies at room temperature the 5-HT level decreased gradually with the preservation time of duodena at 4° C.

In the present study the argyrophil reaction was the superior one for the demonstration of EC in histological sections. Argyrophil-positive EC were found in about 63% but argentaffin ones only in about 10% of the total of 101 bodies. The number of positive cells in the argyrophil and argentaffin reactions decreased greatly with a longer preservation time of bodies at 4° C. This kind of fluctuation in various stainings as found in the present study has been observed in specimens taken immediately after death from various gastrointestinal sites of different species (cf. [27]). It has been suggested that all staining reactions, except the argyrophil one, are due to the same substance in EC, *i.e.* the condensation product of 5-HT with formaldehyde [17]. The present results lend further support to this theory and to the assumption that the staining of EC in various reactions is dependent on the concentration of 5-HT. In the present study the argyrophilia in EC was always strong and the intensity of this reaction did not decrease similarly to that in the argentaffin one. It is likely that argyrophilia is not provoked by diffusable substances in EC but that the granular matrix in EC is responsible for this reaction. It seems that argyrophilia is first lost in EC with the total autolyzing degradation of the duodenal tissue.

The formaldehyde-induced fluorescence is the most sensitive and specific reaction in the histochemical localization of catecholamines and 5-HT [3, 10, 11], and this was stated also for 5-HT in EC of the gastrointestinal tract [18]. In the autopsy material the formaldehyde-induced fluorescence was not a good method for the demonstration of EC since the autofluorescence of the duodenum increased greatly after death and obviously faded the yellow monoamine fluorescence emitted by EC.

Zusammenfassung

An einem Untersuchungsgut von 101 forensischen Leichen wurden der Gehalt von 5-Hydroxytryptamin (5-HT), die vom Formaldehyd verursachte Fluoreszenz, die Bodiansche Argyrophilie, die Masson-Fontanasche Argentaffinie im Duodenum untersucht; es wurden auch drei andere Färbungen auf enterochromaffine Zellen (EC) angewandt.

Die Liegezeit der Leichen bei Zimmertemperatur (2—77 Std) und bei einer Temperatur von 4° C (0—215 Std) vor der Obduktion war bekannt. Bis auf 1 Fall wurden stets analysierbare Mengen von 5-HT im Duodenum vorgefunden. Eine auffallende Korrelation ($p < 0{,}01$) zwischen dem 5-HT-Gehalt des Duodenums und der Liegezeit der Lei-

chen vor der Obduktion war jedoch *nicht* festzustellen. Wenn die Körper jedoch eine konstante Zeit (2—4 Std) vor der Obduktion bei einer Temperatur von 23°C gelagert worden waren, war die Korrelation zwischen der 5-HT-Menge und der Liegezeit der Leichen bei 4°C auffallend hoch ($p < 0.001$).

Wegen der großen Uneinheitlichkeit des 5-HT-Gehaltes im Duodenum ist es jedoch nicht möglich, die Todeszeit ausschließlich mit Hilfe der Feststellung der 5-HT-Menge zu bestimmen.

In den meisten Leichen wurden argyrophile, aber nur in einigen argentaffine EC angetroffen. Die EC wurden gar nicht durch die Schmorlsche und Gibbsche Reaktion gefärbt und die Diazo-Reaktion sprach nur in einigen Leichen auf die EC an. Es kam zu Störung durch autolytische Veränderungen, die Autofluoreszenz nahm zu und vermischte sich mit der durch Formaldehyd verursachten Gelbfluoreszenz in den enterochromaffinen Zellen.

Summary

The post-mortem level of 5-hydroxytryptamin (5-HT), formaldehyde-induced fluorescence, argyrophilia, argentaffinity and three other staining reactions were studied in enterochromaffin cells (EC) of the human duodenum in 101 bodies for which the storage time at room and cold (4°C) temperature before autopsy were known.

The preservation time of human bodies was 2—77 hours at room temperature and 0—215 hours at 4°C. The duodenal 5-HT level was zero in only one body, in the others clearly analyzable amounts of 5-HT were found. The duodenal 5-HT level did not correlate with storage time of human bodies at room or at cold temperature, respectively, but a low significance ($p < 0.01$) was found between the amine level and the total storage time of bodies at these temperatures before autopsy. In selected material a highly significant ($p < 0.001$) correlation was found between the amine level and the storage time of human bodies at 4°C when the storage time at room temperature was constant (2—4 hours). In experimental conditions on the autopsy material a significant reduction of the duodenal 5-HT amount occurred with increasing the experiment time.

Argyrophil cells were found in the duodenum in most specimens but Masson-Fontana-positive argentaffin EC in only a few. The principal difference between argyrophil and argentaffin cells was discussed. Positive EC were found only occasionally in the diazo coupling reaction, but there were no positive EC in the ferric ferricyanide or indophenol reactions.

The post-mortem autolysis was followed by increased autofluorescence of the duodenal tissue that obviously faded the formaldehyde-induced fluorescence of EC and therfore formaldehyde-induced fluorescence was less suitable for the demonstration of EC than the above-mentioned silver reactions.

References

1. BODIAN, D.: The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. Anat. Rec. **69**, 153—162 (1937).
2. BÜLBRING, E., and A. CREMA: The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J. Physiol. (Lond.) **146**, 18—28 (1959).
3. CORRODI, H., u. N.-Å. HILLARP: Fluoreszenzmethoden zur histochemischen Sichtbarmachung von Monoaminen. I. Identifizierung der fluoreszierenden Produkte aus Modellversuchen mit 6,7-dimethoxyisochinolinderivativen und Formaldehyd. Helv. chim. Acta **46**, 2425—2430 (1963).
4. — — Fluoreszenzmethoden zur histochemischen Sichtbarmachung von Monoaminen. II. Identifizierung des fluoreszierenden Produktes aus Dopamin und Formaldehyd. Helv. chim. Acta **47**, 911—918 (1964).
5. EPPS, H. M. R.: The development of amine oxidase activity by human tissues after birth. Biochem. J. **39**, 37—45 (1945).
6. ERÄNKÖ, O.: Distribution of fluorescing islets, adrenaline and noradrenaline in the adrenal medulla of the hamster. Acta endocr. (Kbh.) **18**, 174—179 (1955).
7. — Histochemical demonstration of catecholamines by fluorescence induced by formaldehyde vapour. J. Histochem. Cytochem. **12**, 487—488 (1964).
8. ERSPAMER, V.: Recent research in the field of 5-hydroxytryptamine and related indolealkylamines. Fortschr. Arzneimittel-Forsch. **3**, 151—367 (1961).
9. — Occurrence of indolealkylamines in nature. In: Handbook of experimental pharmacology (ed. O. EICHLER and A. FARAH). Berlin-Heidelberg-New York: Springer 1966.
10. FALCK, B.: Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta physiol. scand. **56**, Suppl. **197**, 1—25 (1962).
11. —, and CH. OWMAN: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Univ. (Lund) (II) **7**, 1—23 (1965).
12. HAMPERL, H.: Über die „gelben (chromaffinen)" Zellen im Epithel des Verdauungstraktes. Z. mikr.-anat. Forsch. **2**, 506—535 (1925).
13. HILLARP, N.-Å.: The release of catechol amines from the amine containing granules of the adrenal medulla. Acta physiol. scand. **43**, 292—302 (1958).
14. KÄRKI, N., M. K. PAASONEN, and P. PELTOLA: Factor influencing the release of 5-hydroxytryptamine from rabbit intestine into the venous blood. Ann. Med. exp. Fenn. **38**, 231—236 (1960).
15. LEMBECK, F., u. H. HELD: Serotoninfreisetzung aus isolierten Granula enterochromaffiner Zellen. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **253**, 409—420 (1966).
16. LILLIE, R. D.: Histopathologic technic and practical histochemistry. New York and Toronto: The Blakiston Comp. Inc. 1954.
17. PEARSE, A. G. E.: In: Histochemistry, theoretical and applied. London: J. & A. Churchill Ltd. 1960.
18. PENTTILÄ, A.: Histochemical reactions of the enterochromaffin cells and the 5-hydroxytryptamine content of the mammalian duodenum. Acta physiol. scand. **69**, Suppl. 281, 1—77 (1966).
19. — Embryological aspects of the relationship of 5-HT to EC. Ann. Med. exp. Fenn. (1968) (in press).

20. PENTTILÄ, A.: Effect of incubation in Krebs-Ringer solution or humid air on the amine content, fluorescence and staining characteristics of the duodenal enterochromaffin and dopamine cells. Virchows Arch. path. Anat. **1**, 269—282 (1968).
21. —, and M. LEMPINEN: Enterochromaffin cells and 5-hydroxytryptamine in the human intestinal tract. Gastroenterology **54**, 375—381 (1968).
22. —, and N.-E. SARIS: 5-Hydroxytryptamine release from enterochromaffin granules *in vitro*. Acta physiol. scand. (1968) (in press).
23. PLETSCHER, A., K. F. GEY, and W. P. BURKARD: Inhibitors of monoamine oxidase and decarboxylase of aromatic amino acids. In: Handbuch der experimentellen Pharmakologie, Bd. XIX, S. 593—735. Berlin-Heidelberg-New York: Springer 1966.
24. RESNICK, R. H., and S. J. GRAY: Distribution of serotonin in the human gastrointestinal tract. Gastroenterology **41**, 119—131 (1961).
25. ROMEIS, B.: In: R. OLDENBOURG, Mikroskopische Technik. München 1948.
26. SCHÜMANN, H. J.: Medullary particles. Pharmacol. Rev. **18**, 433—438 (1966).
27. VIALLI, M.: Histology of the enterochromaffin system. In: Handbuch der experimentellen Pharmakologie, Bd. XIX, S. 1—65. Berlin-Heidelberg-New York: Springer 1966.
28. WEISSBACH, H.: The estimation of serotonin in biological material. Stand. Meth. Clin. Chem. **4**, 197—203 (1961).
29. WETZSTEIN, R., W. DOERFLER u. A. SCHWINK: Die Feinstruktur der enterochromaffinen Zellen und ihrer spezifischen Granula. Elektronenmikroskopische Untersuchungen am Duodenum des Meerschweinchens. Protoplasma (Wien) **55**, 303—312 (1962).

Dr. ANTTI PENTTILÄ and Dr. KARI OJALA
The Department of Anatomy, and the Department of Forensic Medicine, University of Helsinki