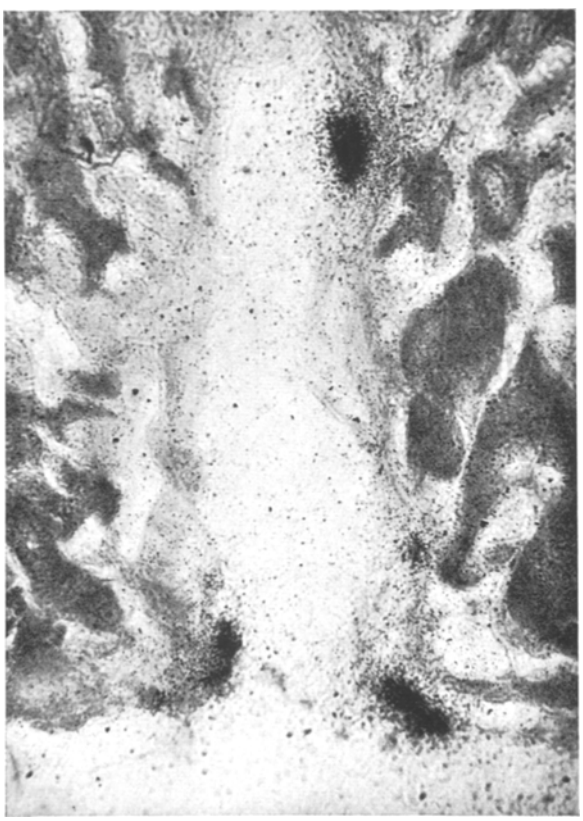
Characteristic High Label of Stomach Mast Cells after Subcutaneous Injection of 5-Hydroxytryptamine in Mice

By previous investigations, mast cells in the peritoneal fluid have been shown to be capable of taking up administered labelled 5-hydroxytryptamine (5-HT) (FURANO et al.[1]) or 5-hydroxytryptophan (5-HTP) (RITZÉN et al.[2]). We have recently observed that characteristically strong radioactivity appeared in mast cells in the stomach wall following subcutaneous or intraperitoneal injection of C^{14}-5-HT in mice by means of radioautography.

Adult male mice were injected subcutaneously with C^{14}-5-HT (5-HT-3'-C^{14} creatine sulphate) (sp. a. 32.0 mc/m*M*), 0.4 μc/g body weight, or intraperitoneally with C^{14}-5-HT (5-HT-2'-C^{14} hydrogen oxalate) (sp. a. 2.5 mc/m*M*), 0.5 μc/g body weight. The animals were sacrificed under ether narcosis 5, 15 and 30 min and 1, 3, 6 and 24 h after injection. The excised specimens from the abdominal skin, tongue, stomach (pyloric portion), duodenum, small and large intestine, heart, and kidney were fixed in 10% neutral formalin, embedded in paraffin, and cut into 10 μ thick sections. Following deparaffinization, unstained sections were coated with Kodak AR 10 by the stripping method. After exposure for 1, 2 and 3 months, the slides were developed, fixed and stained with Giemsa solution. A grain count was made on 20 mast cells each in various tissues, and the number of grains was recorded as the average number per 25 μ^2. The number of mast cells was also counted in 10 visual fields using a light microscope with a 40× objective and a 10× eyepiece.



Autoradiographic picture of the stomach mast cells of the mouse injected subcutaneously with C^{14}-5-hydroxytryptamine (0.4 μc/g body weight) 6 h before sacrifice. Three mast cells present in the submucosa are heavily labelled. × 370.

Autoradiograms showed scattered presence of heavy concentrations of silver grains in the interstitial connective tissues of some organs after subcutaneous injection of C^{14}-5-HT (Figure). The cells, on which large amounts of silver grains were concentrated, showed strong metachromasia following Giemsa stain and were therefore considered as mast cells. Besides silver grains on the mast cells, a moderate number of grains was disseminated around the mast cells, especially after short intervals which were decreased after 6 h. Mast cells in the stomach walls showed marked label 15 min after subcutaneous injection, still high radioactivity even after 6 h, but no label after 24 h (Table I). Mast cells present in three lyers (submucosa, muscularis and subserosa) revealed a similar number of grains each time. As seen from Table I there were some differences of uptake of administered C^{14}-5-HT in mast cells of different organs. Skin mast cells, except in muscularis, showed little if any label compared to the stomach mast cells. Tongue mast cells showed a small number of grains, but a considerable number after 24 h. After intraperitoneal injection (Table II), the stomach mast cell exhibited a small amount of label at

Table I. Grain counts on the mast cells of the stomach (submucosa, muscularis, subserosa), tongue, and skin (dermis, subcutaneous, muscularis) after subcutaneous injection of C^{14}-5-hydroxytryptamine (0.4 μc/g body weight). The number of grains represents the mean number per 25 μ^2 in 20 mast cells. Exposure time 1 month

	Time after injection			
	15 min	1 h	6 h	24 h
Stomach				
submucosa	21.5 (8)	22.0 (15)	22.4 (18)	0 (20)
muscularis	20.0 (35)	22.2 (9)	19.7 (4)	0 (2)
subserosa	25.9 (27)	22.8 (15)	10.4 (9)	0 (1)
Tongue				
muscularis	2.3 (65)	7.1 (58)	0.1 (76)	12.6 (60)
Skin				
dermis	0.7 (83)	1.7 (43)	1.5 (86)	0 (100)
subcutaneous	1.0 (127)	3.4 (37)	1.5 (69)	1.3 (55)
muscularis	12.7 (24)	7.2 (4)	13.4 (12)	7.0 (2)

The number of mast cells in 10 visual fields using a light microscope with a 40 × objective and a 10 × eyepiece.

Table II. Grain counts on the mast cells of the stomach (submucosa, muscularis, subserosa) after intraperitoneal injection of C^{14}-5-hydroxytryptamine (0.5 μc/g body weight). Exposure time 2 months

	Time after injection				
	15 min	1 h	3 h	6 h	24 h
Stomach					
submucosa	5.7 (3)	6.0 (10)	15.1 (28)	23.2 (28)	13.5 (46)
muscularis	0 (1)	4.8 (10)	14.7 (11)	21.7 (9)	17.3 (7)
subserosa	0 (1)	5.6 (6)	12.8 (11)	28.0 (1)	0 (0)

[1] A. V. FURANO and J. P. GREEN, J. Physiol. *170*, 263 (1964).
[2] M. RITZÉN, L. HAMMARSTRÖM, and S. ULLBERG, Biochem. Pharmacol. *14*, 313 (1965).

15 min, followed by a slight increase at 1 h, maximal uptake after 6 h, and a considerable amount of label at 24 h, which is markedly different from the case of subcutaneous injection. Skin and tongue mast cells also showed maximal deposition similar to stomach mast cells after 6 h, but their grain numbers were less than those of the stomach. The grain counts in other organs showed that mast cells of the duodenum, small intestine and myocardium had relatively high radioactivity, but the number of mast cells in these organs was too small to be compared with the stomach, tongue and skin, which contained a considerable number (Tables I, II).

From our radioautographic study mentioned above, the uptake of C^{14}-5-HT was characteristically high in the stomach mast cells compared with other tissue mast cells in the case of subcutaneous injection. In this respect it is very interesting that gastric ulcerations were reported to be caused by 5-HT administration (Wilhelmi[3], Hedinger et al.[4], and Nikodijević et al.[5]). Accordingly, we suppose that characteristic high uptake of C^{14}-5-HT in stomach mast cells might be related to the induction of gastric ulcerations.

Our observations also showed that there were some differences of uptake of C^{14}-5-HT in mast cells according to the method of administration. Gershon et al.[6] found that radioactive serotonin was synthesized and bound in the myenteric plexus of the mouse intestine after intravenous injection of its radioactive precursor 5-HTP, a result which is entirely different from our present result. Therefore, it seems necessary to make a further research on what connections or relations exist between mast cells and the myenteric plexus (or nervous system in general) in the uptake and transport of serotonin.

Zusammenfassung. Nach s.c. und i.p. Injektion von C^{14}-5-Hydroxytryptamin an Mäusen wurde die Radioaktivität verschiedener Organe autoradiographisch untersucht. Eine charakteristische starke Radioaktivität, welche bis zu 6 h anhielt und nach 24 h verschwand, wurde bei s.c. Injektion schon nach 15 min in den Gewebsmastzellen der Magenwand festgestellt. Mastzellen anderer Organe zeigten nur schwache Radioaktivität. Bei i.p. Injektion tritt eine verzögerte Speicherung der radioaktiven Substanz in den Magenmastzellen auf. Die charakteristische Affinität von Serotonin zu den Magenmastzellen dürfte die Ursache des experimentellen Magengeschwürs nach Serotoninbehandlung sein.

N. Shimizu, K. Kawabata, and M. Kaneda

Department of Neuroanatomy, Institute of Higher Nervous Activity, Osaka University Medical School, Osaka (Japan), September 21, 1965.

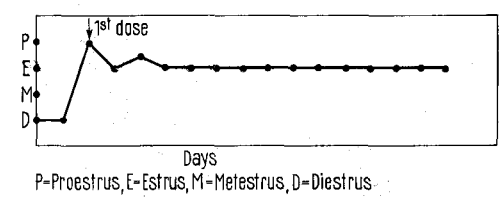
[3] G. Wilhelmi, Helv. physiol. Acta *15*, 83 C (1957).
[4] C. Hedinger and F. Veraguth, Schweiz. med. Wschr. *87*, 1175 (1957).
[5] B. Nikodijević and T. Trajkov, Arch. int. Pharmacodyn. *143*, 442 (1963).
[6] M. D. Gershon, A. B. Drakontides, and L. L. Ross, Science *149*, 197 (1965).

Role of 'Adreno-Ephedrine' in the Induction of Persistent Vaginal Cornification

Adrenalin (epinephrine) is believed to be involved in the normal release of gonadotrophin from the pituitary[1], to cause an increase in size of adrenals in hypophysectomized rats[2], and to decrease the corticotrophic content of both the pituitary and the blood in the adrenalectomized rats[3]. In the present paper, the effect of the *adreno-ephedrine* (a long-acting adrenalin preparation) in the female sex cycle is reported.

Twenty-four normal cyclic female rats were selected for the experiments and were provided with a balanced vitaminized diet and water to drink ad libitum. In the proestrus phase of the cycle, eighteen of the animals were subjected to a chronic treatment of adreno-ephedrine (1 ml = adrenalin 1:1000 and ephedrine 15 mg) subcutaneously at a dose level of 0.2 ml/day/animal for a 15-day period. Six of the identically saline-treated animals were taken as controls. Vaginal smears of both the experimental and control animals were routinely recorded. At the end of the experiments, all the animals of respective groups were sacrificed. At autopsy, the pituitary, uteri and the adrenals of the animals were compared gravimetrically and the liver and ovaries histologically.

Injections of adreno-ephedrine apparently and identically induced constant vaginal cornification in all the experimental animals (Figure). But the estrous cycles of the saline-treated control animals, on the other hand, remained unaltered. The adrenal weight of the experimental animals was significantly greater than that of the controls. But no such difference in the pituitary weight of the respective groups of animals was noted (Table). Histological examination of the liver showed fatty infiltration of the parenchymal cells of the centrilobular areas in the experimental animals. Ovaries of the experimental animals contained follicles of varying size but lacked mature corpora lutea. In addition to constant cornification of vaginal epithelium, a greater uterine weight of the experimental animals compared with that of the controls revealed an increased concentration of circulating estrogen (Table).



Graph of vaginal smears, showing apparently persistent cornification after adreno-ephedrine administration.

[1] J. E. Markee, C. H. Sawyer, and W. H. Hollinshead, Endocrinology *38*, 345 (1946).
[2] R. A. Miller and A. W. Dockrell, Anat. Rec. *115*, 404 (1953).
[3] C. A. Gemzell, Endocrinology *50*, 399 (1952).