DISTRIBUTION OF GLUTAMINASE ACTIVITY IN THE STRUCTURE OF THE FUNDAL GLANDS OF THE STOMACH

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The study of the processes of formation and fixation of ammonia in the gastric mucosa in connection with its secretory function has shown [3] that the glutamine content of the mucosa increases as a result of the combination of ammonia with glutamic acid. It has also been shown [4] that the glutamine formed from ammonia acts as the source of amino nitrogen during the biosynthesis of hexosamines.

On the other hand it has been suggested that glutamine in the gastric mucosa acts at the same time as one source of ammonia formation. This has been confirmed by the discovery of a relatively high activity of the enzyme glutaminase in homogenates of the gastric mucosa, in individual cases close to the level of glutaminase activity of the brain and kidneys, in which the highest levels of activity of this enzyme are found [1].

The object of the present investigation was to determine the glutaminase activity of various cells of the fundal glands of the gastric mucosa in order to identify the role of this enzyme in the different secretory functions of the stomach (the formation of mucin, hydrochloric acid, and pepsinogen).

EXPERIMENTAL METHOD

To determine the glutaminase activity in the various cells of the gastric mucosa, the method of standard sections was used, as described by Linderström-Lang and Holter [10]. The mucous membrane of the stomach was isolated in decapitated cats and dogs in the region of the fundal glands. After freezing, by means of a special drill, cylindrical pieces of the mucosa 5 mm in diameter were cut out. Serial sections, 25 μ in thickness, were cut from these pieces, parallel to the surface of the mucosa, on a freezing microtome.

Incubation of 4 or 8 sections (total thickness 0.1 or 0.2 mm respectively) was carried out in flasks under penicillin in a volume of 10 ml in the presence of 0.2 ml of a 0.02 M solution of glutamine or 0.2 ml of 0.2 M phosphate buffer, pH 9.0 (the optimal pH for the gastric glutaminase [2]). Incubation continued for 1 h at 37°. The activity of the enzyme was expressed in μg ammonia nitrogen liberated during hydrolysis of glutamine. For this purpose the ammonia was made to diffuse from the incubation mixture by the Seligsons' method [16] and determined with phenol-hypochloride reagent by Russell's method [9] on a type FÉK-M photoelectric colorimeter.

In control experiments the amount of performed ammonia in the tissues and the amount of ammonia liberated during the spontaneous breakdown of glutamine in phosphate buffer were determined and compared with the results of the experimental analyses.

To study the distribution of glutaminase activity in the cells of the gastric mucosa, a piece of tissue was taken from an area next to the part of the gastric mucosa which had been removed, fixed in Carnoy's fluid, and embedded in paraffin wax. Sections were cut perpendicularly to the surface of the mucous membrane, treated by the PAS method, and stained with methylene blue at pH 5.6 [13]. As a result of these procedures the mucoid cells appeared reddish-violet, the oxyntic cells were pink, and the chief cells blue in color. The thickness of the glandular layer was measured by means of an ocular micrometer.

EXPERIMENTAL RESULTS

Maximal glutaminase activity was found in the accessory portion of the fundal glands, where the oxyntic cells, producing hydrochloric acid, are mainly concentrated (Table 1, Fig. 1). That the high glutaminase activity was in fact due to the oxyntic cells, and not to the mucoid cells situated in the same portion of the gland (stained darkly

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TABLE 1. Glutaminase Activity of Fundal Glands of the Stomach in Dogs (in μg ammonia nitrogen/8 sections)

Expt. No.	Lesser curvature				Greater curvature			
	epithelium of surface+crypt	accessory portion	chief portion	tissue of subgland. layer.	epith. of surf. and crypts	accessory portion	chief portion	tissue beneath glan. lay.
In a resting state								
1	— 0,47	1,15 0,75 0,79	1,02 0,75 0,73	1,23 — —	— —	0,78 0,97 —	0,78 0,76 —	— — —
2	0,56 —	0,80 0,95 0,75	0,59 0,83 0,65	0,91 1,13 0,99				
3	0,56 — —	0,61 — 0,78	0,46 0,20 0,22	0,91 — —				
4	0,85	0,87 1,08	0,70 0,66	0,92 —	0,58	0,57 0,70	0,53 0,89	0,97 —
Mean	0,60	0,86	0,63	1,02	0,58	0,76	0,74	0,94
1-1.5 h after feeding								
5	0,63 0,43	0,96 0,77	0,72 0,53	0,94 —				
6	0,86	1,82 1,30	1,46 1,07	0,97 —	0,68	1,19 1,18	0,80 0,82	0,92 —
7	0,33	0,56 0,79	0,53 0,52	— 0,99	0,33	0,66 0,79	0,58 0,56	— —
Mean	0,57	1,03	0,80	0,97	0,50	0,96	0,69	0,92

in Fig. 1), was confirmed by the fact that the glutaminase activity of the mucoid cells of the surface epithelium was very low. Since a certain amount of connective tissue, whose glutaminase activity is relatively high (as shown by determinations of the activity of sections of connective tissue from beneath the mucous membrane), is present beneath the surface epithelium, it may be concluded that the glutaminase activity of the mucoid elements of the gastric glands was in fact lower than is shown in Table 1 and Fig. 1.

Hence, the high glutaminase activity of the accessory portion indicates that the ammonia formed from glutamine by the action of glutaminase may be important in the formation of hydrochloric acid. This was in agreement with previous findings [3]. On the other hand, the low glutaminase activity of the mucoid cells of the gastric glands was evidently attributable to the fact that the glutamine in these elements was a source of amino nitrogen for the biosynthesis of components of the mucoids – hexosamines and sialic acids [11, 12], and its hydrolysis is therefore undesirable.

The glutaminase activity diminished toward the bottom of the fundal glands, obviously in connection with the decrease in the number of oxyntic cells. It is clear from Fig. 1 that the glutaminase activity of the lowermost portion of the fundal glands, 100 μ in thickness, containing mainly chief cells producing pepsinogen, was approximately half that in the accessory portion. This was rather unexpected, for it might be assumed that it was here that intensive hydrolysis of glutamine into ammonia and glutamic acid took place, with incorporation of the

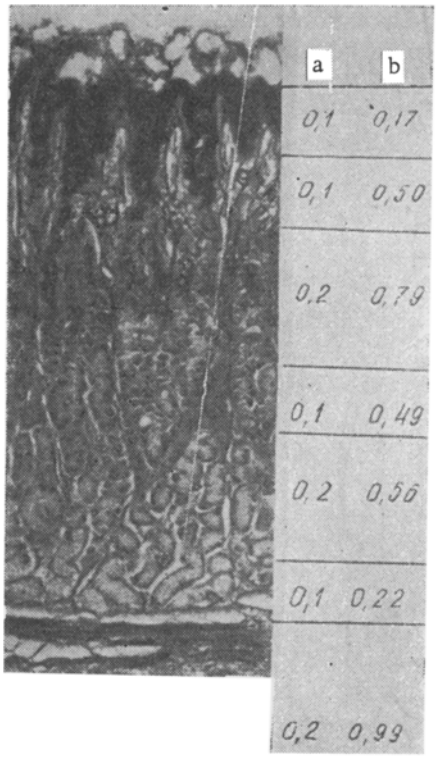

Fig. 1. Distribution of glutaminase activity (in μg ammonia nitrogen) in the mucous membrane of the fundal part of a dog's stomach. a) Thickness of investigated part (in mm); b) glutaminase activity.

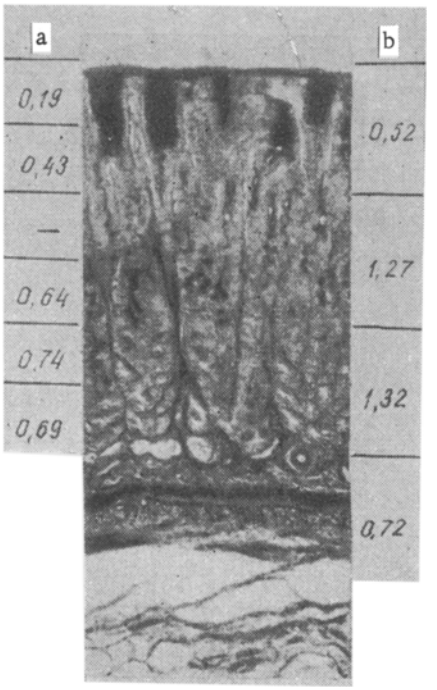

Fig. 2. Distribution of glutaminase activity (in μg ammonia nitrogen) in the mucous membrane of the fundal part of a cat's stomach. a) analysis of 4 sections (0.1 mm); b) analysis of 8 sections (0.2 mm).

latter into pepsinogen, which contains considerable amounts of glutamic acid [8]. Evidently the incorporation of this dicarboxylic amino acid into pepsinogen takes place while still in the form of glutamine and not of glutamic acid, a conclusion supported by the fact that glutamine passes more readily than glutamic acid through cell membranes [14].

It is clear from Table 1 that in all the dogs studied the ratio between the glutaminase activity of the different portions of the fundal glands was the same. Although the results of some experiments (Nos. 1 and 4) showed that fairly high glutaminase activity was present in the chief portion of the fundal glands, not lower than the activity of the accessory portion, nevertheless these differences in the experiments cited were evidently attributable to peculiarities of the structure of the gastric mucosa in these animals: the oxyntic cells were distributed uniformly throughout the gland, and the chief cells, arranged in the direction of the lumen of the gland, reached to the bottom of the crypt, so that both types of cells were distributed uniformly both in the chief and in the accessory portion of the gland.

In cats (Fig. 2 and Table 2) the distribution of glutaminase activity in the fundal glands of the stomach differed from its distribution in dogs: the glutaminase activity of the chief portion was higher than in the accessory portion. However, the glutaminase activity of the surface epithelium, as in dogs, was very low. This species difference was evidently not connected with differences in the structure of the gastric mucosa. According to the available evidence [7], the ability of the gastric glands to secrete hydrochloric acid is weaker in cats than in dogs. It is possible, therefore, that the lower glutaminase activity discovered in the accessory portion of the fundal glands in cats was associated with the less intensive production of hydrochloric acid.

The glutaminase activity of different organs, according to data in the literature, depends on their functional state. In muscle tissue during work the glutaminase activity rises [6] and, conversely, during inhibition (in brain tissue after administration of sedatives) if falls [5]. In the present experiments on both dogs and cats (see Tables 1 and 2) the glutaminase activity of the gastric mucosa varied within wide limits in individual animals.

Taking these facts into account, the author investigated the glutaminase activity of the fundal glands during stimulation of secretion by food and by histamine.

TABLE 2. Glutaminase Activity of the Fundal Glands of the Lesser Curvature of the Stomach in Cats (in μg ammonia nitrogen/8 sections)

Expt. No.	Stimulus of secretion	Epithelium of surface and crypts	Accessory portion	Chief portion	Tissue of subglandular layer
1	—	1,0 0,75 —	1,70 1,35 1,65	1,90 1,90 2,30	— — —
2	—	— —	2,60 2,50	2,20 3,00	— —
3	—	0,70 —	1,38 1,07	1,84 1,28	— —
4	—	0,10	0,65 0,62	1,06 0.62	— 1,18
5	—	0,38	1,14 0,93	0,98 0,93	0,79 —
6	—	0,59	1,13 1,18	1,31 1,43	0,99 —
Mean		0,50	1,34	1,57	1,05
7	Histamine	— —	1,05 0,75	1,50 0,75	— —
8	Histamine	— 0,60 —	1,95 1,95 2,25	2,05 2,40 2,40	— — —
9	Carbachol	0,90 —	1,35 1,40	1,40 1,55	— —
10	Food	0,12 0,52	1,22 0,78	1,82 1,22	— 0,62
11	Food	0,48 0,52	1,35 1,27	1,42 1,32	0,72 —
12	Histamine	0,90	1,22 1,19	1,19 1,34	1,21 —
13	Food	0,74	1,00 0,92	1,30 1,18	1,32 —
14	Food	0,59	0,75 1,14	0,92 1,17	0,92 —
15	Food	0,33	0,76 0,53	0,91 1,01	— —
Mean		0,61	1,15	1,37	1,00

These experiments showed (see Tables 1 and 2) that the distribution of glutaminase activity in the various structures of the fundal glands was unchanged by comparison with its distribution in a state of rest. Likewise no significant differences were found in the glutaminase activity of the gastric mucosa after stimulation of secretion.

Determination of the glutaminase activity of the fundal glands of the lesser and greater curvatures separately

in dogs (see Table 1) and cats revealed no appreciable difference either in the distribution of activity in the different structures of the glands or in the absolute activity.

These experiments showed that the activity of pieces of tissue situated close together (1-2 cm apart) often differed (see Tables 1 and 2). This "mosaic" pattern of activity of glutaminase was evidently associated with the irregular distribution of the oxyntic cells in different parts of the body of the stomach [15], a factor which complicates the comparative study of glutaminase activity after stimulation of secretion and also along the greater and lesser curvatures.

SUMMARY

Glutaminase activity in various cells of the fundal glands of the stomach was determined by means of the method of serial sections in cats and dogs.

In dogs the highest glutaminase activity was discovered in the accessory part of the fundal glands where oxyntic cells are mainly concentrated. In the basal part of the glands (zone of peptic cells) the glutaminase activity was somewhat less intense. Very low activity was shown by the mucoid elements of the superficial epithelium. This is evidence of the important role of glutaminase in the ability of oxyntic cells to secrete hydrochloric acid.

In cats the highest glutaminase activity was revealed in the principal part of the gland.

These specific differences are apparently connected with the smaller capacity of the cat's gastric glands for hydrochloric acid secretion.

LITERATURE CITED

1. Yu. Zalesskaya, É. Martinson, and L. Tyakhepyl'd, Biokhimiya, No. 2 (1961), p. 281.
2. S. Maramaa, Uchen. Zapiski Tartusk. Univ., No. 163, Trudy po Med., Vol. 9 (1964), p. 261.
3. É. Martinson and Kh. Lind. Biokhimiya, No. 5 (1955), p. 533.
4. É. É. Martinson and L. A. Villako, Biokhimiya, No. 3 (1962), p. 437.
5. L. I. Miloslavskaya, Biokhimiya, No. 3 (1958), p. 347.
6. D. L. Ferdman and A. I. Silakova, Biokhimiya, Nos. 1-2, (1957), p. 283.
7. E. J. Conway, The Biochemistry of Gastric Acid Secretion, Springfield (1953), p. 128.
8. M. Dixon and E. C. Webb. Enzymes [Russian translation], Moscow (1961), p. 456.
9. D. Glik, In: Methods of Histo- and Cytochemistry [in Russian], Moscow (1950), p. 228.
10. H. Holter and K. Linderström-Lang, Hoppe-Seylers Z. Physiol. Chem., Vol. 226 (1934), p. 149.
11. L. F. Leloir and C. E. Gardini, Biochim. Biophys. Acta, Vol. 12 (1953), p. 15.
12. Ibid., Vol. 20 (1957), p. 33.
13. R. D. Lillie, Histopathologic Technic and Practical Histochemistry, New York (1954), p. 156.
14. A. Meister, The Biochemistry of Amino Acids [Russian translation], Moscow (1961), p. 174.
15. A. M. Read and F. R. Johnstone, Anat. Rec., Vol. 139 (1961), p. 525.
16. D. Seligson and H. Seligson, J. Lab. Clin. Med., Vol. 38 (1951), p. 324.
