A METHOD OF RECORDING GASTRIC SECRETORY FUNCTION IN MICE

I. L. Kalantar, L. V. Nozdracheva,
and K. P. Gutiontova

UDC 612.323-084.087

KEY WORDS: gastric mucosa, muscular and glandular layers of mucosa, fundus, body, cardia, and antrum of stomach, gastric contents, secretion, mitochondria, proteolytic and succinate dehydrogenase activity, RNA, DNA.

The combined study of the stomach in one experiment by biochemical, morphological, and histochemical methods provides additional opportunities of obtaining objective information about the secretory activity of the stomach both under normal conditions and during testing of biologically active substances. The study of function of the gastric glands in laboratory mice presents familiar technical difficulties, for the weight of the stomach in this case is only 250-350 mg. The writers have therefore developed a method whereby, in the stomach of a single mouse, it is possible to study its contents and tissue by biochemical, morphological, and histochemical methods simultaneously.

Experiments were carried out on 25 mature female CC57W/MvS mice from the collection of the Research Institute of Biological Models, Academy of Medical Sciences of the USSR. The mice were killed by decapitation after deprivation of food for 18 h and the stomach was immediately removed. The stomach was washed 5 times through the cardial orifice, into which a tube 0.1 cm in diameter was introduced, with distilled water which flowed from the antral orifice. The washings (gastric contents, total volume 5 ml) were used to determine pH, proteolytic activity [4], and total protein [5]. The stomach was again filled with water, quickly frozen in a cryostat, and serial sections 6-8 μ thick were obtained from it, starting from the lesser curvature and parallel to the long axis of the stomach. By means of this method preparations of all parts of the stomach (fundus, body, antrum, cardia) could be obtained separately and together in the same preparation, taken from a definite level of the stomach. By means of the histochemical reaction succinate dehydrogenase (SDH) activity was determined in the sections quantitatively, and by its localization in the gastric tissue cells [6]. Identical sections through the gastric mucosa were stained with hematoxylin and eosin. A series of sections through the fundus and body of the stomach from 15 mice with a total weight of 50-60 mg was used to determine protein [15] and nucleic acids [7].* The gastric contents for biochemical study gave a weak acid reaction in most mice. Only in four of 15 mice was an acid reaction of the contents (pH 3.6 ± 0.9) combined with a higher protein content (4.0 ± 1.0) and proteolytic activity (653.5 ± 151).

Quantitative assay of SDH showed that it is not uniformly distributed in the various parts of the stomach. Highest activity was found in the body of the stomach (46.5 ± 3.2). SDH activity in the fundus was somewhat lower (27.9 ± 1.1) and about half as high in the antrum (10.6 ± 1.2) and cardia (10.2 ± 0.6).

Morphohistochemical analysis of the localization of SDH in the various parts and layers of the gastric mucosa shed some light on the quantitative experimental data. For instance, the glandular layer of the gastric mucosa is the primary location of SDH. Virtually none of the enzyme was present in the muscular layer of the mucosa, and the surface epithelium occupied an intermediate position for the presence of SDH. The uneven distribution of SDH activity in the various parts of the stomach is due to its uneven distribution within each gland. For instance, the chief cells have highest activity, as regards both size of the granules and density of their packing. The accessory cells, characterized by very small granules, have

*The experimental data were analyzed by computer and presented in the form M ± m.

Research Institute for Biological Testing of Chemical Compounds, Kupavna. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 4, pp. 501-503, April, 1987. Original article submitted June 14, 1986.

 0007-4888/87/0004-0572$12.50

TABLE 1. Characteristics of Gastric Contents of Mice (n = 15)

Statistical parameters	pH	Protein, mg	Proteolytic activity, μg pepsin	
			in contents	in 100 mg protein
M	5,6	3,6	18,4	511,1
m	0,3	0,8	3,5	135,7
σ	1,1	2,7	14,7	334,4

TABLE 2. Proteins and Nucleic Acid Levels and SDH Activity in Mouse Stomach

Statistical parameters	Protein, mg	RNA, μg	DNA, μg	RNA, μg	DNA, μg	SDH activity	
						n = 1	*n* = 4
	per 100 mg tissue			(n = 15) per mg protein			
M	14,5	57,0	20,8	3,9	1,4	50,9	60,6
m	1,3	5,7	2,1	0,7	0,3	3,7	9,2
σ	4,3	18,3	6,7	—	—	16,6	20,4

the least activity. Granules of different sizes with an uneven distribution of them within the cells are characteristic of the parietal cells.

Comparison of the results of the histochemical and biochemical study of the gastric tissues and contents showed that SDH activity in the stomach depends on the secretory activity of the organ and its content of protein and nucleic acids (Tables 1 and 2).

According to the data in Table 1 and mentioned previously, four mice with higher secretory activity of the chief and parietal epithelium (proteolytic activity and pH of the contents, respectively) had higher SDH activity in the body and fundus of the stomach.

The results of this investigation and those given in previous publications [1] are evidence that the secretory function of the stomach is linked with succinate dehydrogenase activity. The RNA level per unit of protein and per unit of DNA (Table 2) indicates that the tissue of the gastric mucosa is a highly differentiated epithelium with a well developed protein-synthesizing apparatus. In turn, the DNA content in the gastric tissue and per unit of tissue protein may be evidence of the existence of a pool of renewed cells in the gastric glands, a conclusion supported by the morphological data.

The use of the techniques mentioned above makes it possible to study gastric secretory activity in mice simultaneously by biochemical, histochemical, and morphological methods, so that the objectivity and productivity of the investigations are enhanced. These methods also enable the biological activity of preparations to be tested under screening conditions.

LITERATURE CITED

1. I. L. Kalantar, Yu. M. Toropov, and T. D. Kononova, The Use of Models of Pathological States in the Search for Biologically Active Preparations [in Russian], Part 1, Moscow (1983), pp. 70-71.
2. A. A. Pokrovskii, M. M. Gapparov, and L. G. Levin, Fiziol. Zh. SSSR, 59, No. 10, 1567 (1973).
3. A. M. Ugolev, Zh. Evol. Biokhim. Fiziol., 19, No. 4, 390 (1983).
4. V. P. Chernikov, Technical Instructions for the Use of Laboratory Methods of Investigation [in Russian], Moscow (1977), pp. 316-320.
5. J. Bailey, Methods in Protein Chemistry, Elsevier (1967).
6. M. M. Nachlas, W. H. Walker, and A. M. Seligman, J. Biophys. Biochem. Cytol., 4, 29 (1958).
7. F. Wannemacher et al., Anal. Biochem., 12, 320 (1965).