EVALUATION OF THE AMOUNT OF PEPSIN AND HYDROCHLORIC ACID SECRETED BY A PAVLOV STOMACH POUCH IN DOGS*

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Curves representing rate of secretion of gastric juice, its acidity, and its digestive power, after feeding dogs with bread, meat, or milk were first derived by I. P. Pavlov, and are familiar to all physiologists. It appeared to us that the secretory function of the stomach might be more accurately assessed from a consideration of the absolute amounts of hydrochloric acid and of pepsin units secreted by the gastric glands, rather than from the values of the acidity of the juice and of its enzymic activity, although the importance of such data cannot be denied. The volume of the juice is in the first place an indication of the amount of water secreted. The volume of juice does not always vary parallel with the amount of hydrochloric acid and of pepsin. The results of our experiments confirm the opinions of those authors who believe that secretion of hydrochloric acid takes place independently of secretion of pepsin.

Although it is desirable to make use of three tests for an appraisal of the secretory function of the stomach (amount of juice, number of milligrams of hydrochloric acid, and number of pepsin units), papers continue to appear in which the authors make use of acidity and peptic activity data. They all come to the conclusion that these two indices do not reveal any regularities whatsoever.

One of the obstacles to the application of the method proposed by us is the lack of any simple and accurate procedure for determining the number of pepsin units. As is known, Mett's method is not suitable for this purpose. N. P. Pyatnitsky (2) suggested in 1937 that peptic activity could be evaluated from the milk-curdling power of the gastric juice. The same proposal was made earlier by V. V. Savich and F. M. Migal [6], on the basis of I. P. Pavlov's view that pepsin and chymosin were identical. At that time, however, the majority of workers ascribed the milk-curdling activity of gastric juice to a special enzyme, chymosin, and, moreover, the milk curdling technique was not sufficiently simple and accurate. It is now believed that chymosin is absent from the gastric juice of adult animals and humans.

S. Bichs [8], who studied the digestion of edestin by hydrochloric acid extracts of the gastric mucosa of animals, in buffer solutions (glycine – hydrochloric acid), found two pH optima, at pH 2 and 4.5. He hence concluded that gastric mucosa contain two enzymes, pepsin and cathepsin. Tolekmitt [11] was unable to confirm the presence of two pH optima for digestion of protein by gastric juice. For this reason he supported the view that the existence of two proteolytic enzymes has not been established, in gastric juice. We believe, on the basis of the results obtained by M. N. Selyukova [7] in our laboratory, that cathepsin is certainly present in hydrochloric acid extracts of gastric mucosa. As for gastric juice, it either does not contain any cathepsin, or there are not more than traces of it.

Northrop's crystalline pepsin exerts a marked milk-curdling action. The milk-curdling activity is proportional to the amount of pepsin.

Instead of whole milk we now use milk diluted with buffer solution, for the evaluation of milk-curdling

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TABLE 1

Curdling time (minutes)	Compared with normal	Approximate number of units per 1 ml	Recording of results
Less than 3	Very strong	More than 2500	++++
3—4	Moderately strong	2001—2500	+++
4—10	Normal	1001—2000	++
10—15	Weak	501—1000	+
More than 15	From zero to traces	0—500	0 or traces

activity. In a study of the rate of conversion of pepsinogen into pepsin, made in 1933, Ege and Menk-Thygesen [9] used acetate buffer at pH 5 (42 g of sodium hydroxide and 115 ml of 80% acetic acid per 1000 ml of water). This buffer was added in equal volume to fresh cow's milk. Five ml of milk-acetate mixture was added to 0.1 ml of gastric juice in a test tube, and the time required, at 25°, for the appearance of floccules of casein on the walls of the test tube was recorded.

Kleiner [10] considers that rennin is absent from the gastric juice of human adults, and, similarly to us, considers that milk-curdling activity can be taken as a measure of pepsin activity. As he considered that the method of pepsin assay previously devised by Borowski, Tauber and Kleiner was too complicated, Kleiner worked out a simpler modification of this method. Gastric juice is diluted 1:50 with water, and 1 ml of the solution is added to 10 ml of milk-acetate mixture at 20°, the mixture is placed in a water bath at 20°, and the number of minutes required to curdle the milk is recorded. The results are read according to Table 1.

As is evident from Table 1, the determination of activity of gastric juice in Kleiner units is very approximative and inconvenient.

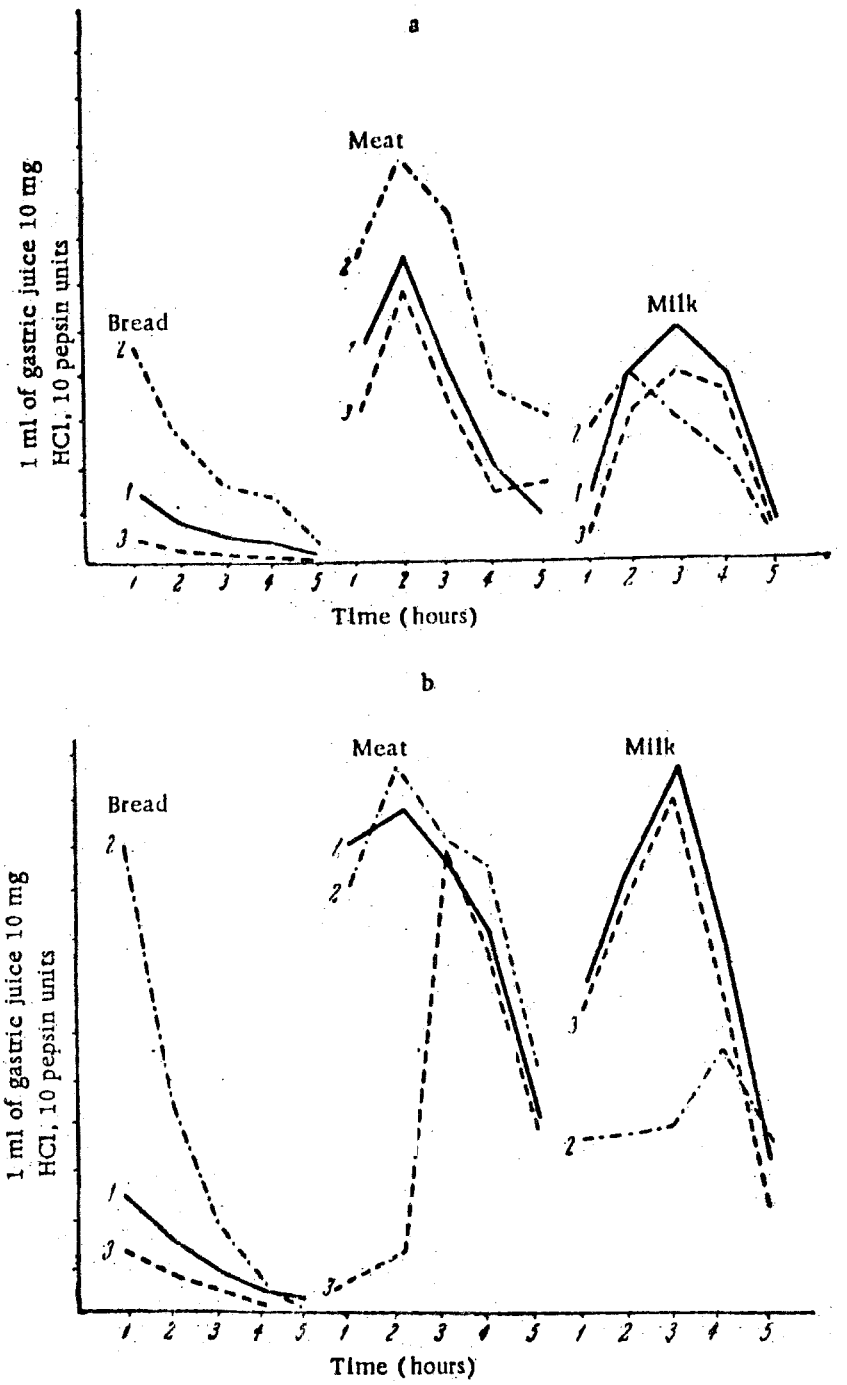
N. P. Pyatnitsky suggested in 1954 that a unit of pepsin should be taken as that amount which causes curdling of 5 ml of milk-acetate mixture in 60 seconds at 25°. Dividing 60 by the number of seconds required with a measured aliquot of gastric juice gives the number of pepsin units in the aliquot. For example, if 0.1 ml of undiluted juice curdles 5 ml of milk-acetate mixture in 20 seconds, 1 ml of juice will contain 30 pepsin units ($10 \times 60/20 = 30$).

The simplicity and accuracy of the chymosin method of assay of pepsin, and the convenient units for expression of its amount, encouraged us to repeat I. P. Pavlov's experiments on dogs fed with bread, meat, and milk.

We had four healthy dogs with Pavlov pouches at our disposal. We performed three experiments with each type of feeding. The secretory response varied individually for each dog, but was always the same for a given sort of food. For this reason, the results of only one experiment with a particular food are presented in Table 2.

The following regularities are evident from an inspection of the curves for total secretion, secretion of hydrochloric acid, and secretion of pepsin, plotted from our experimental results. After a bread meal (200 g) very little gastric juice and hydrochloric acid are secreted, but relatively large amounts of pepsin (see Figure). Much juice, hydrochloric acid, and pepsin are secreted in response to meat (200 g). Ingestion of 600 ml of milk leads to a smaller secretion of juice, hydrochloric acid, and pepsin than after meat. In all the experiments, the amount of acid secreted varies in the same direction as does the volume of juice and its pepsin content, although full parallelism is not observed. Secretion of acid and of pepsin also do not always vary parallel. As is known, the activity and the acidity of the juice vary reciprocally as a general rule: the higher the acidity of the gastric juice of a given dog, the lower is its peptic activity. The acidity of juice is lowest at the beginning and the end of the secretory period, and remains fairly constant at the height of secretion.

It follows from our observations that there is no strict parallelism between secretion of water, pepsin,



Secretion of (1) gastric juice; (2) pepsin; (3) hydrochloric acid, in response to bread, meat, and milk.
a) by the dog Palma, b) by the dog Laska.

TABLE 2

Indexes of the Work of a Pavlov Gastric Pouch Over a 5 Hour Secretory Period

Hour	ml of gastric juice	Activity in ml 0.1 N NaOH			Activity	HCl	Pepsin units	
		free	bound	total	1 ml	mg		
Dog Palma								
1	3	10	30	50	15	4.4	45	Fed 200 g of bread
2	2	18	28	58	13.3	3.4	26 6	
3	1.5	10	34	50	11	2.4	17.5	
4	1	6	37	50	13.5	1.5	13.3	
5	0.8	0	38	46	7.5	1.1	6	
Total amount of juice over 5 hours	8.3					12.8	108.4	
1	9	70	30	108	7.5	33	67	Fed 200 g of meat
2	13	100	25	132	6.6	59	85	
3	8	82	30	122	9.2	32	73	
4	4	60	32	104	9.4	14	36	
5	2.2	10	30	52	15	15	33	
Total amount of juice over 5 hours	36.2					153	294	
1	2.8	22	20	80	10	4.3	28	Fed 600 g of meat
2	8	80	27	120	5	31.4	40	
3	9.8	90	30	130	3.1	39.2	30 3	
4	8	93	28	129	2.7	35.2	21.6	
5	2	30	20	60	3.1	3.6	6.2	
Total amount of juice over 5 hours	30.6					113.7	126.1	
Dog Ryzhik								
1	1.2	10	50	80	8.5	2.6	10.2	Fed 200 g of bread
2	1	10	40	60	12	1.8	12	
3	0.9				20		18	
4	0.8				15		12	
5	0.5				13.3		6.6	
Total amount of juice over 5 hours	4.4					4.4	58.8	
1	4.5	40	30	90	12	10.5	54	Fed 200 g of meat
2	5	45	30	92	10	13.6	50	
3	3	20	40	72	8.3	6.6	24.9	
4	2.5	10	35	50	7	4.1	17.5	
5	2		30	40	8.5	2.2	17	
Total amount of juice over 5 hours	17					37	163.4	

TABLE 2 (continued)

Hour	ml of gastric juice	Activity in ml 0.1 N NaOH			Activity	HCl	Pepsin units	
		free	bound	total	1 ml	mg		
1	3	30	50	98	11.5	8.7	34.5	Fed 600 g of meat
2	3.5	40	50	100	10	11.8	35	
3	4	50	30	89	6.6	11.7	26.4	
4	2	40	10	69	10.8	3.6	20.6	
5	1				12		12	
Total amount of juice over 5 hours	13.5					35.8	128.5	
Dog Dik								
1	3.5	8	16	52	14.3	3	50	Fed 200 g of bread
2	2.5	4	18	40	12	2	31	
3	1		12	22	8.5	0.4	8.5	
4	1				7.5		7.5	
5	0.5				6.6		3.3	
Total amount of juice over 5 hours	8.5					5.4	100.3	
1	9	98	10	112	6.6	34	59.4	Fed 200 g of meat
2	11	102	12	120	5.7	45.7	62.7	
3	8	90	18	116	9	30.7	72	
4	4	80	20	102	15	14.6	60	
5	1.5	40	30	80	20	3.4	30	
Total amount of juice over 5 hours	33.5					128.4	284.1	
1	3	30	30	102	8.5	6.6	25.5	Fed 600 g of meat
2	8	60	30	120	7.5	27	59	
3	11.5	80	30	130	6.7	26.1	77	
4	7	80	28	118	9	28.2	63	
5	2	30	20	60	12	3.6	24	
Total amount of juice over 5 hours	31.5					91.5	248.5	
Dog Laska								
1	5	53	22	80	20	13.6	100	Fed 200 g of bread
2	3	64	22	96	15	9.4	45	
3	2	40	16	50	10	4	20	
4	1	20	10	40	6	1.1	6	
5	0.3				5.4	—	1.6	
Total amount of juice over 5 hours	11.3					28.1	172.6	

TABLE 2 (continued)

Hour	ml of gastric juice	Activity in ml 0.1 N NaOH			Activity	HCl	Pepsin units	
		free	bound	total	1 ml	mg		
1	20	100	26	140	4 5	9.2	90	Fed 200 g of meat
2	22	130	15	150	5	11.6	115	
3	19	120	20	150	5.3	97	100	
4	16	110	23	140	6	78	96	
5	8	100	20	125	7.5	30.6	52.5	
Total amount of juice over 5 hours	85					413.6	453.5	
1	14	100	25	132	2.7	63.8	37.8	Fed 600 g of meat
2	19	110	18	136	2	85.7	38	
3	23	116	12	140	1.7	107.4	39	
4	16	112	12	130	3.5	72.3	56	
5	6	70	25	102	6	20.7	36	
Total amount of juice over 5 hours	78					349.9	206.8	

and acid by the gastric glands. The work of these glands cannot be appraised on the basis of the proteolytic activity of the juice, or of its acidity; it may be evaluated from the amount of pepsin and of hydrochloric acid produced during a given secretory period (5 hours). We found that not much juice or hydrochloric acid was secreted in response to feeding 200 g of bread, but that relatively large amounts of pepsin were secreted, the response to 200 g of meat was much juice, much pepsin, and much acid, and to 600 g of milk somewhat less juice, pepsin, and acid than to meat. The proteolytic power of the gastric juice of adult humans and animals, at pH 1.6 – 2, varies parallel with its milk-curdling power (at pH 5). For this reason, the chymosin method for the assay of pepsin merits wide application.

SUMMARY

The authors suggest that absolute quantities of stomach juice, hydrochloric acid and pepsin should be determined during the whole period of secretion for examination of the secretory function of stomach glands. Hemozinic method of pepsin determination, as well as new units of pepsin are proposed.

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