AN ELECTROPHORETIC AND IMMUNOELECTROPHORETIC STUDY OF THE PROTEIN COMPOSITION OF THE INTESTINAL JUICE OF DOGS AFTER PARTIAL GASTRECTOMY

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The notion that the blood proteins enter the digestive tract as an important stage in the intermediate metabolism of proteins was first suggested by I. P. Razenkov in 1946, and since then it has been developed extensively by his collaborators in his laboratory [2-6].

Besides the isotope method, however, immunochemical methods may also be used to verify this hypothesis experimentally.

Facts accumulated during recent years confirm to some extent that the serum proteins may pass into the lumen of the stomach [8, 9], and considerable study has been made of the passage of the serum proteins into the intestine. Evidence has been obtained [7, 10, 11] indicating that labeled serum albumin is secreted along with the intestinal juice; however, no details are available in relation to the remaining serum proteins.

In the present investigation the protein composition of the intestinal juice of dogs before and after gastrectomy was studied by means of electrophoretic and immunoelectrophoretic methods.

EXPERIMENTAL METHOD

Experiments were conducted on nine dogs in which the composition of the proteins of the intestinal juice was studied before and after resection of $\frac{2}{3}$ of the stomach. To obtain the intestinal juice an intestinal loop was isolated in all the animals by the Thiry-Vella method. The intestinal juice was collected in a fasting state for a period of 1 h, always at the same time of day. The juice obtained was centrifuged for 15 min at 3000 rpm. The total protein content was determined refractometrically, and the protein fractions by the method of electrophoresis on paper.

Electrophoresis was carried out in the EFA-1 chamber, using a boroborate buffer, pH 9.0. The conditions of electrophoresis were: voltage 400 V, current 0.3 mA/cm width of the strip, duration 8 h. The relative proportions of the protein fractions in per cent were determined photocolorimetrically.

The serum proteins were detected and identified among the proteins of the intestinal juice by the method of immunoelectrophoresis in agar, using rabbit antiserum against the serum proteins of an adult dog. The conditions of immunoelectrophoresis and the technique of obtaining the antiserum were described previously [1]. The precipitation arcs were identified from the scheme given in the literature [12].

EXPERIMENTAL RESULTS

It is clear from Fig. 1 that four protein fractions were detected in the composition of the intestinal juice by the method of electrophoresis on paper, three of them with the mobility of globulins and one with the mobility of the albumins of the blood serum.

TABLE 1. Changes in Protein Composition of Intestinal Juice of Dogs After Gastrectomy

	- -						Glob	Globulins (in %)		
Time of investigation	Number of dogs	Number of expts.	Statistica1 index	Volume of juice(in m1)	Volume of Totalpro- juice(inml) tein(ing%)	Albumins (in %)	α	В	۰.	A/G ratio
Before operation	2	24	M	3,8	76,0	44,73	17,09	26,26	11,92	0,89
1	· • • • •		ш	±0,54	±0,17	$\pm 2,36$	$\pm 1,51$	±1,18	±1,33	⁺⁰ ,1
			ь	±1,08	∓0,38	上5,29	±3,38	±2,64	±2,98	+0,22
After operation	ĸo	ro	W	3,9	1,73	29,47	22,95	26,99	20,59	0,43
3rd-5th day			ш	∓0,91	±0,62	$\pm 2,67$	$\pm 2,46$	±2,94	$\pm 3,33$	40,0€
(m			ь	±2,04	±1,39	$\pm 5,98$	$\pm 5,51$	±6,58	土7,46	±0,13
			ď	>0,5	>0,2	<0,01	>0,05	>0,5	<0,05	<0,001
8th-10th day	r.	ro	M	2,8	1,42	30,01	22,60	28,26	19,13	0,43
			ш	±0,49	±0,3	土1,45	$\pm 2,35$	土1,45	$\pm 2,59$	±0,03
			ъ	H1,1	∓0,67	士3,25	$\pm 5,26$	±3,25	 	±0.07
			Ь	>0,2	>0,2	<0,001	>0,05	>0,5	<0,05	<0,001
13th-15th day	4	4	M	2,5	1,38	33,26	33,76	17,85	15,13	0,51
	•		ш	±0,61	±0,18	十3,76	$\pm 3,61$	±3,67	$\pm 3,27$	+0,0 1
		-10-weight	ď	±1,22	∓0,36	±7,52	±7,22	±7,34	十6,54	±0,18
			Ъ	>0,1	>0,1	<0,05	<0,01	>0,05	>0,5	<0,001
18th-20th day	4	4	M	2,1	2,22	28,7	25,61	25,58	20,11	0,41
		***********	ш	±0,51	±0,46	$\pm 2,48$	±0,5	$\pm 2,16$	∓0,93	90,0∓
			ם	±1,02	+0,92	+ 4,96	1	±4,32	1 1,86	±0,12
			Ъ	>0,05	<0,05	<0,01	<0,001	>0,5	<0,02	<0,001
1 year after	4	91	M	5,4	1,05	31,46	25,16	26,61	16,77	0,46
		<u></u> -	ш	±0,13	±0,18	±1,18	±1,11	±0,92	±0,85	±0,05
			b	∓0,26	±0.36	$\pm 2,36$	$\pm 2,22$	+1,84	$\pm 1,7$	±0,1
			ď	<0,05	>0,5	<0,01	<0,01	>0,5	<0,02	<0,001

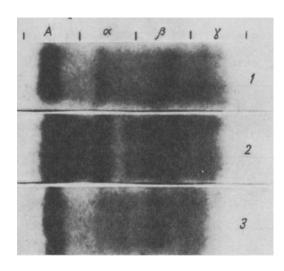


Fig. 1. Electrophoresis of intestinal juice and serum protein of a dog. 1,3) Intestinal juice; 2) serum.

The protein composition of the investigated samples and the volume of intestinal juice secreted are given in Table 1, which shows that the secretion of intestinal juice in the first days after gastrectomy remained essentially unchanged, although 1 year after the operation it had risen by approximately 50%, probably on account of the adaptation of the digestive system to the conditions created by gastrectomy.

The total protein concentration in the intestinal juice during the first days after resection remained unchanged, and not until the 18th-20th day could a slight increase be detected. After 1 year the protein concentration had returned almost to its preoperative level.

The fractional composition of the intestinal juice changed during the first days after resection: the albumin content fell significantly, whereas the concentration of α -, and β -globulins rose. The A/G ratio fell as a result of the lowering of the albumin level and the elevation of the globulin content. These changes in

the fractional composition of the intestinal juice presisted with some variation not merely for the first 18-20 days after operation, but for a year.

It was found by the method of immunoelectrophoresis that the intestinal juice included certain proteins immunologically identical with the blood serum proteins (Table 2). When the complete antigenic set of proteins of the intestinal juice and the serum was being determined, the stains on the paper after immunoelectrophoresis were developed with antiserum to the serum proteins of an adult dog, preliminarily exhausted with intestinal juice. It was found that the set of antigenic components of the intestinal juice corresponded fully to the antigenic composition of the blood serum, for after exhaustion with intestinal juice the antiserum ceased to react with the blood serum (Fig. 2). Gastrectomy caused no significant changes in the amount of serum proteins secreted with the intestinal juice, also probably in connection with the high degree of adaptation of the organism to the disturbance of the integrity of the alimentary tract.

The results of these investigations show that the intestinal juice contains proteins with the electrophoretic mobility of the serum albumins and globulin, with a higher relative proportion of the latter. In the early (until the 20th day) and late (1 year after) periods after gastrectomy the total protein content in the intestinal juice was essentially unchanged. However, the albumen content was diminished while the content of α - and γ -globulins was increased.

TABLE 2. Comparative Immunoelectrophoretic Analysis of the Blood Serum and Intestinal Juice of Dogs

Materialstudied	la i	er ts	lin	Globulins							
	mber ani- ils mber expts		A1bumin	αι	α_2	α_3	α,	βı	β₂	γ	
	Z to E	N b	A11	number of antigenic components							
Before Operation											
Blood serum Intestinal juice	5 5	10 10	1—3 2—3	2—3 2—3	2—3 1—3	2-4 2-4	3—4 1—3	2-3 2-3	1-2 1-2	1	
After Operation Intestinal juice 3rd-5th day 8th-10th day 13th-15th day 18th-20th day 1 year after	5 5 4 4 4	5 5 4 4 8	2—3 3 2—3 2—3 1—2	2—3 2—3 2—3 2—3 1—2	2—3 1—2 1—3 2—3 1—2	2-4 2-4 2-3 2-3 2	2-4 1-3 1-3 2-3 1	2-3 2-3 2 2 1-2	$\begin{bmatrix} 2 \\ 2 \\ 2 \\ 1-2 \\ 1 \end{bmatrix}$	1 1 1 1	

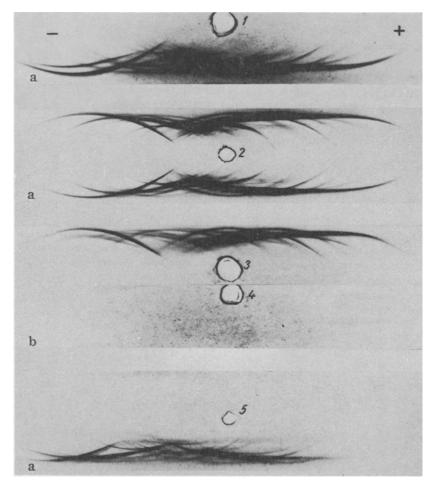


Fig. 2. Immunoelectrophoretic characteristics of the proteins of the intestinal juice of a dog. 1, 3, 4) Intestinal juice; 2, 5) blood serum of a dog; antiserum against serum of a dog before (a) and after (b) exhaustion with excess of proteins of the intestinal juice.

The fractional composition of the intestinal juice was changed after the first postoperative days and remained unchanged 1 year after resection.

It is interesting that among the proteins of the intestinal juice there should be a complete set of the antigenic components belonging to the normal blood serum of the dog. The similarity between the proteins of the intestinal juice and the blood serum as regards their antigenic structure confirms I. P. Razenkov's hypothesis that the serum proteins may pass out into the lumen of the digestive tract.

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