PHOSPHOLIPIDS IN THE INTESTINAL JUICE OF DOGS

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In recent years the attention of research workers has been increasingly drawn to the role of phosphorus compounds, excreted into the gastro-intestinal tract, in the composition of digestive secretions. When the content of these compounds in saliva, gastric juice, pancreatic juice, and intestinal juice of dogs was studied, it was found that intestinal juice is the richest in phosphorus compounds, and in particular, the quantity of phospholipids in intestinal juice was 100 times higher than in the other secretions studied by L. M. Babushkins [1]. Frazer [9] attaches considerable significance to the presence of phospholipids in the intestinal lumen in connection with their role in the formation of a stable emulsion during absorption of neutral fat.

D. É. Grodzenskii and K. S. Zamychkina [2] established that a considerable quantity of phosphorus-containing compounds, largely phospholipids related to lecithin, are excreted in the bile.

A study of phosphorus compounds in particular, phospholipids, in digestive juices [1] also revealed the presence in intestinal juice of an enzyme which could breakdown the phospholipids of the same juice. The fact that intestinal juice contains both these compounds and an enzyme breaking them down indicates that intestinal juice can be a source of a certain quantity of metabolically active choline. As is well known, choline is an important lipotropic factor [4].

The endogenous formation of choline in the digestive system has received very little study.

In accordance with the facts set forth above, we set ourselves the problem in the present study of clarifying the nature of the phospholipids secreted into the digestive tract by way of the intestinal juice.

EXPERIMENTAL METHODS AND RESULTS

The work was carried out on dogs [3], each of which had a duodenal loop isolated by Thiry's method. Juice was collected during periodic secretion 16-18 hours after the animals had been fed. Soon after the juice had been secreted, it was centrifuged, and the fluid fraction was discarded, while the dense fraction of the intestinal juice, otherwise known as the "mucus" bodies, was put on ice.

Phospholipids were determined only in the dense fraction, since earlier work in the Laboratory of Digestion had established that organic phosphorus in intestinal juice is restricted to the dense fraction. The collected aliquots of this fraction of intestinal juice were pooled from all three dogs and retained for analysis.

Phospholipids were extracted from the dense fraction of intestinal juice by a mixture of chloroform and methyl alcohol (2:1) with frequent grinding in a mortar, reckoning 20 ml of extracting mixture per gram of tissue.

TABLE 1

Content of Phospholipids in the Dense Fraction of Intestinal Juice

Sample number	Quantity of phospholipids (mg) per g juice
1	3.5
2	3.4
3	3.5
4	4.0
5	3.9
6	5.0
7	3.2
8	4.6
9	3.2
10	4.0
Average	3.9 ± 0.6

TABLE 2

Content of Choline-Containing and Cephalin Fractions in Phospholipids of Intestinal Juice (as percentages of total quantity of phospholipids)

Sample number	Choline- containg	Cephalin
	fractions	
1	57.2	37.3
2	57.1	31.1
3	60.3	32.4
4	57.2	39.5
5	63.1	26.7
6	58.4	29.6
7	59.6	28.3
8	56.7	31.4
9	55.8	40.1
10	54.2	39.2
Average	57.8 ± 0.8	33.6 ± 0.7

TABLE 3

Chromatographic Separation of Phospholipids (qualitative)

Sample number	Composition of phospholipid hydrolyzates
11	Choline, serine, ethanolamine, inositol
12	Choline, ethanolamine, inositol, glutamic acid, aspartic acid, lysine, unidentified spot
13	Choline, ethanolamine, serine, inositol
14	Choline, ethanolamine, inositol, aspartic acid, lysine, unidentified spot
15	Choline, inositol, ethanolamine

After each grinding, the homogenate was centrifuged and filtered. The tissue residue was covered with chloroform and methyl alcohol (2:1) and left in a cold room overnight. On the next day the extracts were pooled and washed free of inorganic phosphorus with 0.1 N or 0.01 N HCl.

After the phospholipid extract had been washed free of inorganic phosphorus, it was evaporated in a vacuum under a nitrogen atmosphere at 40-50°. The dry residue was dissolved in methyl alcohol.

Separation of phospholipids into fractions was effected by adsorption on magnesium oxide by the method of Dawson, as modified by A. A. Smirnov and E. V. Chirkovskaya [3]. The result was that two fractions were obtained: the fraction of choline-containing phospholipids, consisting of lecithin and sphingomyelin, and the cephalin fraction, consisting of ethanolamine serine, and acetal phospholipids. Aliquots were taken from the extract of each fraction to determine inorganic phosphorus. The aliquots were converted to inorganic phosphorus by concentrated sulfuric and nitric acid. Phosphorus was determined by the method of Fiske and Subbarow. To convert the phospholipid, the quantity of phosphorus (in milligrams) multiplied by 23.5.

The experimental results are set out in Tables 1 and 2.

Thus, the content of phospholipids in the dense fraction of intestinal juice is on the average 3.9 mg per g, corresponding to 16 mg% of phosphorus. By using special precautions during separation of the fluid fraction of intestinal juice from the dense fraction, one might obtain even larger amounts of phospholipids in the dense fraction of intestinal juice.

Table 2 shows that a large part of the phospholipids of intestinal juice consists of the choline-containing fraction (57.8%).

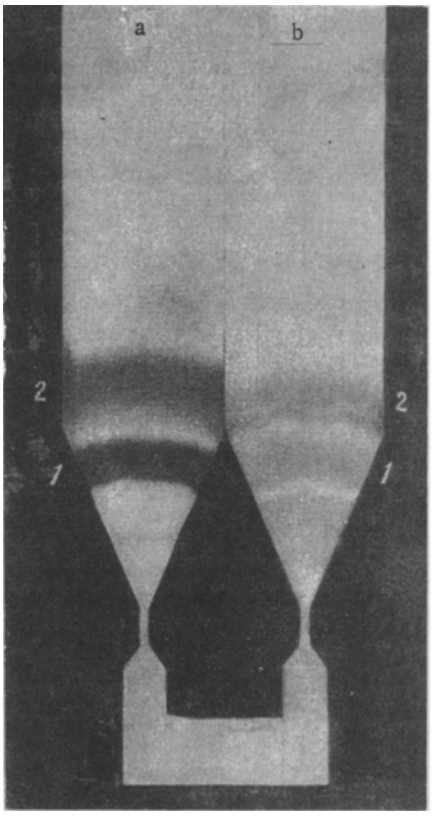

Fig. 1. Chromatographic estimation of ethanolamine and serine in phospholipids of intestinal juice. a) Control; b) experiment. 1) Serine; 2) ethanolamine.

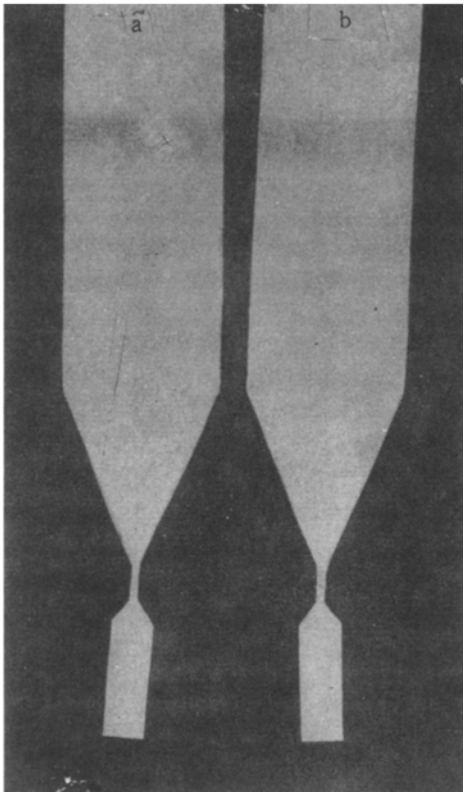

Fig. 2. Chromatographic estimation of choline in phospholipids of intestinal juice. a) Control; b) experiment.

TABLE 4

Composition of Phospholipid Fractions in the Dense Fraction of Intestinal Juice

Sample number	Quantity of phospholipids (mg) per g juice	Quantity of phospholipids undergoing hydrolysis (mg)	Quantity of the various phospholipid fractions (as % of the total)		
			choline-containing phospholipids	serine phospholipids	ethanolamine phospholipids
1	5.5	7.7	65.0	6.5	19.3
2	5.7	7.9	63.3	–	20.3
3	3.5	1.9	42.1	5.2	31.6
4	3.3	2.1	66.1	–	19.1
5	5.5	3.6	50.0	–	16.6

Since the content of phospholipids in intestinal juice is not large, and the method used allowed them to be separated into only two fractions, we turned to a chromatographic method for separating them.

We used the method suggested by Levine and Chargaff [10]. After removal of solvents in vacuum, the dry residue of phospholipids was subjected to acid hydrolysis with 5 N HCl at 125° for 12 hours. The hydrolyzate was freed of fatty acids by filtration and evaporated in a vacuum under a nitrogen atmosphere at 40-50°. The residue was dried in a desiccator over P_2O and dissolved in water (1 mg phospholipid in 0.05-0.1 ml H_2O).

For chromatograph we used Leningrad chromatographic paper, washed with "trilon." The paper was cut into the shape used by Matthias [12]. A solution (0.11-0.03 ml) was applied with a special micropipette.

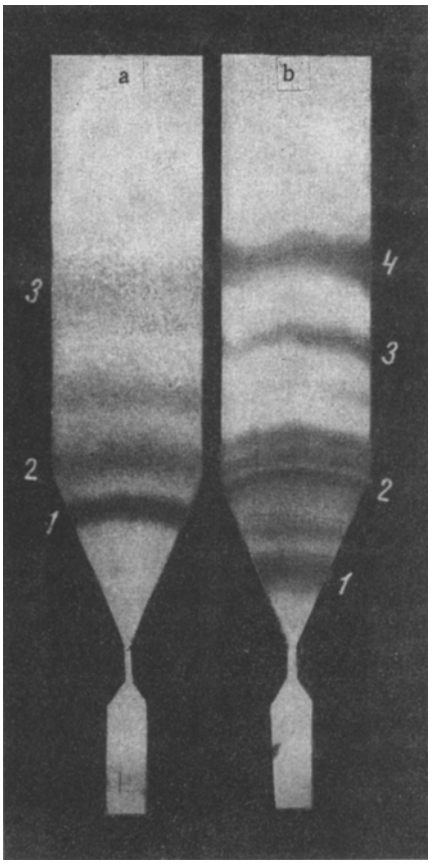

Fig. 3. Chromatographic estimation of amino acids in the phospholipid fraction of intestinal juice. a) Experiment; b) control. 1) Lysine; 2) aspartic acid; 3) glutamic acid; 4) unidentified band.

The solvent mixture used to separate inositol, serine, and ethanolamine consisted of n-butanol: acetic acid: water (4: 1: 5), while choline was separated by n-butanol: diethylene glycol: : water (4: 1: 1).

Chromatography was carried out at 23-24° for 18-20 hours.

To show up serine and ethanolamine chromatograms were sprayed with a 0.2% solution of ninhydrin in acetone and then warmed in a drying cabinet at 80° for 2-3 min. This caused blue-violet bands to appear on the paper (Fig. 1).

To show up choline, chromatograms were dried at 100°, dipped in 2% phosphomolybdic acid solution for one minute, washed with n-butanol and water, then treated with freshly-prepared 0.4% solution of $SnCl_2$ in 3 M HCl. This gave dark blue bands against a bluish-white background (Fig. 2).

To show up inositol, chromatograms were sprayed with 2.5% solution of $AgNO_3$ in methanol, dried at room temperature, and treated with ammonia vapor for one hour. Then the chromatograms were left in a drying cabinet at 100° for ten minutes. Inositol showed up as dark bands (Ag_2O, formed by the reaction of $AgNO_3$ with inositol in a medium of ammonia).

It was established by the chromatographic method that phospholipids of the dense fraction of intestinal juice contain choline, serine, ethanolamine, and inositol. In addition, glutamic acid, aspartic acid, and lysine were detected (Fig. 3). These results support the data of Westley and co-workers [13], who also found glutamic acid, aspartic acid, alanine, and cystine in phospholipids of rat brain and kidneys.

Table 3 shows the results of chromatographic study of five samples of the dense fraction of intestinal juice.

Quantitative estimation of serine, ethanolamine and choline allowed one to compute the content of serine-, ethanolamine, and choline-containing phospholipids.

Inositol could not be estimated quantitatively, since at present there are no chemical methods allowing the estimation of inositol in quantities under 0.04 mg.

After the chromatograms had been developed, the areas corresponding to the position of serine, ethanolamine, and choline (from an estimate of R_f values) were cut out of other, undeveloped chromatograms. Ethanolamine, serine, and choline were eluted with water at room temperature for 18-20 hours. Then choline was estimated by the method of Kushner [11], and serine and ethanolamine by the method of Cocking [5].

Table 4 shows the results of this experiment.

The chromatographic method of study,also showed that most of the phospholipid of the dense fraction of intestinal juice consists of choline-containing phospholipids.

SUMMARY

The author studied the phospholipid composition of the intestinal secretion in dogs. Intestinal juice contains considerable amount of phospholipids. The bulk of phospholipids in the intestinal juice is constituted by the choline-containing phospholipids, demonstrable by fractionation of phospholipids on magnesium oxide as well as with chromatographic method of examination. Along with lecithine, cholamine, serine and inosite-containing phospholipids are present too, although in smaller quantities.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
