EFFECT OF A DEFICIENCY OF CERTAIN VITAMINS OF THE B GROUP ON THE EXOCRINE ACTIVITY OF THE PANCREAS

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Antagonists such as antivitamins are nowadays used extensively in various branches of biology and medicine, especially for the study of nutritional problems [1-4, 6, 7]. In the present paper the state of the secretory function of the pancreas is compared in dogs with hypovitaminosis caused by administration of aminopterin and β-acetylpyridine.

EXPERIMENTAL METHOD

Experiments were conducted on six adult dogs with a permanent pancreatic fistula formed by Pavlov's method. To prevent the development of a pathological state as a result of the chronic loss of pancreatic juice, after the operation a course of subcutaneous injections of pancreatic juice was given by the method of Jackson and Milusckewicz, with a few unimportant modifications. For this purpose, pancreatic juice was collected from the fistula in aseptic conditions directly into a syringe and injected subcutaneously in a volume of 4-5 ml. For a period of 4-5 weeks 4-5 injections were given at weekly intervals. During the first 3-4 days after each injection of juice, 500,000 units of penicillin was injected. By means of this number of injections the dog could be maintained in a satisfactory general condition for long periods of time (as much as 2 years). After only 3 injections the skin changes resulting from the escape of pancreatic juice disappeared, and no subsequent signs of injury to the abdominal skin were observed. At the end of the fifth-sixth week the animals regained their original weight and they recovered completely from the operation. After they had done so, the dogs required no further special nursing and their condition was good. The animals were given small, frequent meals, and were kept on the normal diet of the Institute, supplemented by 1 liter of milk each per day.

TABLE 1. State of Secretory Function of Pancreas in Dog No. 1 With a Chronic Pancreatic Fistula, 6 Months after Operation

Food stimulus	Volume of juice in 5 h (in ml)	Amylase (in units/ml)	Lipase (in units/ml)	Trypsin (in ml 0.2 N KOH)	pH	Alkalinity (in ml 0.1 N HCl)
Meat	193.9	3,370	4,500	1.0	8.6	1.50
Butter	33.6	11,000	11,200	1.7	7.8	1.12

As food stimuli, 100 g of meat and 50 g of butter were given. The volume of juice was measured at hourly intervals and its total volume determined during an experiment lasting 5 h. The content of amylase, lipase, and trypsin in the juice was determined by the methods generally used in the laboratory. Determinations were also made of the titratable alkalinity, expressed in milliliters of 0.1 N HCl/ml of juice, and also of the pH of the juice (by means of a potentiometer with a glass electrode).

The initial level of secretion in dogs was established by control experiments.

When the animals were prepared in this way, the ability of their pancreas to react to the quality of applied food stimuli persisted for a considerable time. The chronic loss of juice from these animals did not affect the work-

ing of the organ adversely. The course of secretion of juice hour by hour, in response to administration of meat or butter, corresponded to the classical descriptions in the literature. The enzyme content of the juice was high, and in most cases bore an obvious relationship to the quality of the food used to stimulate secretion. Typical indices of the process of secretion in these dogs are shown in Tables 1 and 2.

TABLE 2. State of Secretory Function of the Pancreas of Dog No. 2 With a Chronic Pancreatic Fistula 1 Year after Operation

Food stimulus	Volume of juice hour by hour					Total volume of juice (in ml)	Amylase (in units/ml)	Lipase (in units/ml)	Trypsin (in ml 0.2 N KOH)
	1	2	3	4	5				
Meat	50	23.6	17.6	8.8	5.6	105.6	2,250	4,500	0.8
Butter	6.6	9.3	28.4	4.5	6.4	55.2	10,000	10,000	1.3

After the results of the control investigations had been obtained (not less than 4 initial experiments with each food stimulus) administration of the antivitamins began. Depending on the object of the investigation—the study of pancreatic activity in the acute or chronic form of insufficiency—different doses of antimetabolites were given. Aminopterin, dissolved in physiological saline, was injected parenterally once daily in doses of 0.5-2.0 mg, and β-acetylpyridine in doses of 0.5-2.0 g per animal daily or on alternate days. In lower doses, β-acetylpyridine is known to be able to act as a vitamin [5].

EXPERIMENTAL RESULTS

During administration of 0.5-1.0 mg aminopterin or 0.5-0.8 g β-acetylpyridine to the dogs, changes in the animals' general condition appeared after several injections had been given, and in both cases these took the form of a very slight loss of appetite, lethargy, and a fall in body weight (by 5-8%). After further administration of antagonists in these dogs, or administration of larger doses (2 g β-acetylpyridine and 2 mg aminopterin for 2-3 days) the ensuring symptoms differed in the case of the two substances.

The dogs with folic acid deficiency developed diarrhea, becoming profuse. The stools lost their fecal appearance and consisted of a blood-stained fluid. Dehydration and a rapid fall of body weight thus developed rapidly: the sclerae became dry and the eyes were sunken. The dogs developed a marked leukopenia and aniso- and poikilocytosis.

The animals with nicotinic acid deficiency developed salivation and their oral mucous membrane became friable and lost its shiny appearance. Ulcers developed on the anterior surface of the tongue and a purulent odor escaped from the mouth. Lacrimation, photophobia, a divergent stabismus, and purulent conjuctivities appeared. Some animals developed paresis, and later paralysis, of the hind limbs. In nicotinic acid deficiency, as opposed to folic acid deficiency, disturbances of intestinal function took the form of loosening of the stools, which remained fecal in character, were mixed with mucus, and had a foul odor.

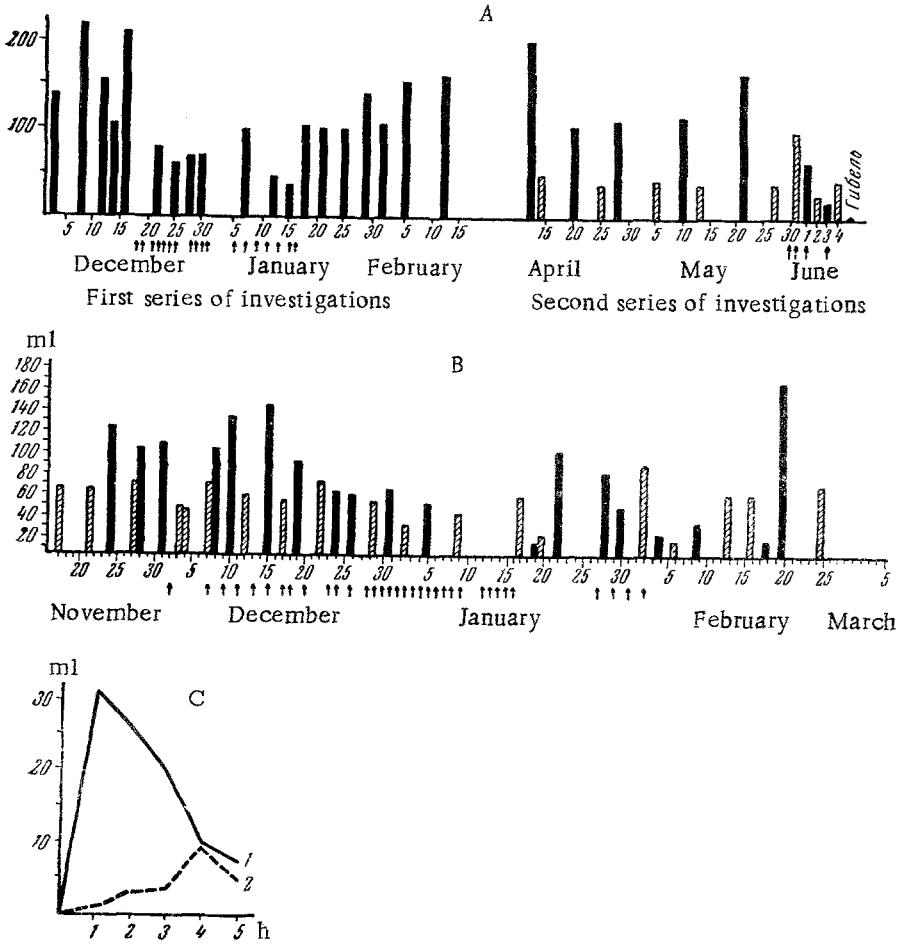
The changes in the general condition described above were not observed if the corresponding vitamin was injected at the same time as the antimetabolite.

The investigation showed that administration of folic and nicotinic acid antagonists was accompanied by a marked change in the volume of pancreatic juice secreted. In deficiency of both folic and nicotinic acid, the secretion of juice fell in the initial period of administration of the antimetabolites, before the development of signs of a change in the general condition of the animals. When changes in the general condition were apparent, the inhibition of pancreatic secretion was quite marked. This state was observed in the experiments with both meat and butter.

Besides a change in the total volume of pancreatic juice secreted during the experiment, the hourly course of secretion was also modified. In the experiments with meat the secretion of juice during the first hours as a rule fell sharply, in contrast to the controls, and the period of maximal secretion was postponed to a later hourly period. This demonstrates a change in the nervous regulation of the secretion of juice in vitamin deficiency (see the figure).

Observations on the secretory function revealed some difference between the actions of aminopterin and β-acetylpyridine. This concerned the period of normalization of the changes noted above. In folic acid deficiency, changes in the work of the pancreas developed, as a rule, after an improvement in the general condition of the animals, and this period was characterized by a series of abrupt rises and falls. A lowered or normal secretion alter-

nated with a higher level (2-3 times greater than the initial level) of secretion of juice on certain days. This period lasted 3-4 weeks after the cessation of aminopterin administration. After administration of β-acetylpyridine had ceased, however, the secretion of pancreatic juice rapidly returned to its initial level and the period of rises and falls was not observed.



Secretion of pancreatic juice in dogs in experiments with meat (black columns) and with butter (shaded columns) after administration of β-acetylpyridine (A) and aminopterin (B). The arrows indicate injection of antimetabolites; C) course of secretion of juice hour by hour before (1) and during (2) administration of antimetabolites.

In contrast to the antagonists, administration of the vitamins themselves, in doses 2-5 times greater than the therapeutic doses of nicotinic and folic acids, for 21-28 days had no effect on the secretion of pancreatic juice.

An example illustrating the various aspects of the secretory process in animals during administration of the two antagonists is given in the figure. In one of the dogs the initial secretion before giving meat was between 120 and 250 ml of juice. After the second injection of 0.5 g of β-acetylpyridine the volume of juice fell to 78 ml, and a further injection led to a continued fall (33-65 ml). Besides causing changes in the general condition of the animal, administration of 1-2 g of the preparation led to a sharper fall in the secretion of juice. After administration of aminopterin a similar picture was observed (see the figure). Injections of 1 mg of aminopterin lowered the secretion of juice to 51-64 ml; continued injections gave rise to the development of clinical manifestations together with a distinct inhibition of secretion—on certain days down to 24 ml. In the initial period, in the experiments with meat, the volume of juice secreted was 115-145 ml. The same effect of depression of the secretion of juice was observed when the dog was fed with butter. Whereas in the initial period the volume of juice in response to butter was 67-74 ml, during administration of aminopterin it fell to 35-55 ml. It is also clear from the figure that during administration of the antagonists the secretion of juice fell sharply during the first hour of the secretory period.

The results of the investigation of the enzyme-secreting function of the pancreas showed that during administration of the antagonists no significant variation of the enzyme content from the control values was observed. The concentration of enzymes, and also the relative proportions of the various enzymes remained as before during administration of aminopterin and β-acetylpyridine, and were unchanged when the volume of juice was reduced.

Comparison of indices such as the pH and titratable alkalinity of the juice revealed no marked differences in their values during the period of administration of the antimetabolites (Tables 3).

TABLE 3. pH and Titratable Alkalinity of Pancreatic Juice During Administration of Antimetabolites

Food stimulus	pH		Alkalinity (in ml 0.1 N HCl)	
	Before injection	During administration	Before injection	During administration
β-Acetylpyridine (Dog No. 3)				
Meat	8.0 -8.6	7.9-8.3	1.0 -1.55	1.2 -1.65
Butter	7.45-8.2	7.6-8.5	0.9 -1.25	1.15-1.3
Aminopterin (Dog No. 6)				
Meat	7.5 -8.1	7.5-8.2	0.9 -1.0	0.8 -1.1
Butter	7.0 -7.5	7.1-7.8	0.65-0.8	0.6 -0.9

Hence, in folic and nicotinic acid deficiency following administration of the corresponding antimetabolites, a marked change is observed in the activity of the pancreas.

SUMMARY

A study was made of the effect produced by the folic and nicotinic acids deficiency caused by the administration of aminopterin and β-acetylpyridine on the external secretory activity of the pancreas. Experiments were carried out on 6 dogs with a chronic fistula of the pancreatic duct after I. P. Pavlov. Aminopterine was administered parenterally in daily doses of 0.5-2.0 mg. The number of injections in dogs differed from 9 to 34 for 33-63 days. During antimetabolite administration there was a reduction of secretion in response to food stimuli for a period of 5 h; the course of secretion according to hourly intervals, characteristic of different food stimuli, has also changed. There were no changes in the concentration of amylase, lipase, and trypsin in the juice.

LITERATURE CITED

1. S. Ya. Mikhlin, N. P. Bochkov, and Z. N. Pavlova, Arkh. pat., 1, 44 (1960).
2. S. Ya. Mikhlin and M. F. Nesterin, Vrach. delo, 8, 3 (1961).
3. S. Ya. Mikhlin, Arkh. pat., 9, 24 (1962).
4. S. Ya. Mikhlin and M. F. Nesterin, Vrach. delo, 5, 57 (1963).
5. W. T. Beher and W. L. Anthony, J. biol. Chem., 203, p. 895 (1953).
6. R. S. Harris, In Book: The Vitamins, New York, 2, p. 452 (1954).
7. D. W. Woolley, Antimetabolites [Russian translation], Moscow (1954).

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