PRESENCE OF A SPECIFIC ANTIGENIC FACTOR IN THE DUODENAL CONTENTS OF DOGS WITH EXPERIMENTAL PANCREATIC NECROSIS

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The duodenal contents of dogs with experimental pancreatic necrosis cause an allergic reaction when injected intradermally into dogs with pancreatic necrosis. The presence of a factor X, antigenically connected with the duodenum but not with the tissue of the normal or pathologically changed pancreas, can be demonstrated serologically in the duodenal contents of the affected animals.

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Attempts to isolate specific antibodies against tissues of the normal and diseased pancreas have been described by both Soviet and Western authors [1, 6, 7].

However, we found no data in the literature relating to the use of duodenal contents during pancreatic necrosis in allergologic and immunologic investigations.

The results of our previous investigations into the etiology and pathogenesis of acute pancreatic necrosis in dogs suggested that the duodenal contents (the name conventionally given to the pathological duodenal juice) of these animals contains a certain factor concerned with the allergic and immunologic mechanisms of this disease [2, 4, 5].

To test this hypothesis we decided to carry out allergy tests, accompanied by histopathological controls, on dogs with pancreatic necrosis and also to detect a specific antigenic factor in the duodenal contents of these animals by means of the complement fixation reaction (CFR).

EXPERIMENTAL METHOD

Experiments were carried out on 34 dogs and 37 rabbits.

An operation was performed on the dogs to form a duodenal fistula, so that normal duodenal contents and, after reproduction of experimental pancreatic necrosis, pathological duodenal juice could be obtained.

Allergic skin tests were performed on the same dogs by injecting 0.1 ml of their own pathological duodenal juice intradermally 26-28 h after production of experimental pancreatic necrosis. Injection of the same dose of juice into healthy animals served as control. The results were read 6 and 24 h after injection.

Serologic investigations were carried out as the CFR with three types of antisera. These sera were obtained by immunizing rabbits with material obtained from the dogs: pathological duodenal contents, normal duodenal contents, and pathological duodenal contents preliminarily "blocked" by rabbit serum against normal duodenal contents (factor X).

The rabbits were immunized in accordance with the following scheme: 4 intravenous injections of antigens in increasing doses at intervals of 2 days. Blood was taken 8 days after the last injection.

To detect the hypothetical specific antigenic factor present in pathological duodenal contents, we used the method of blocking and purifying tissue antigens suggested in 1965 by G. P. Tribulev and A. K. Saankov. To 10 ml rabbit serum against normal duodenal contents poured into centrifuged tubes, 2-3 drops

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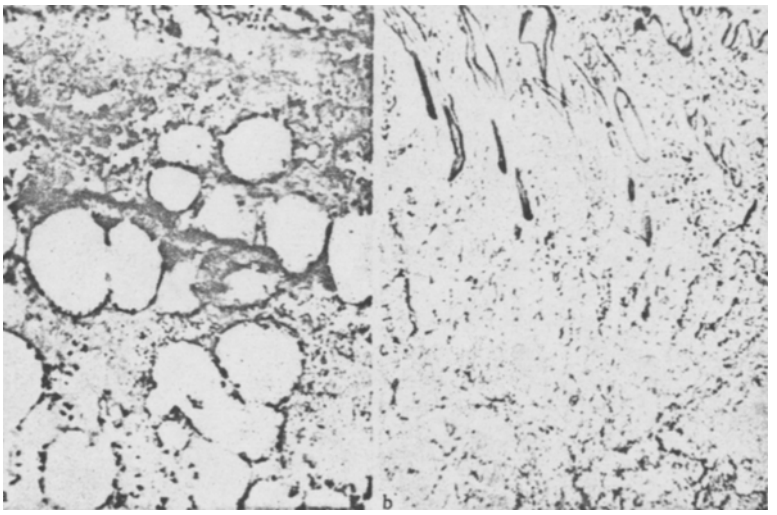

Fig. 1. Site of injection of pathological duodenal juice into dog with pancreatic necrosis (a) and healthy dog (b). Hemorrhages, fibrinoid necrosis, and degeneration of the vessel wall, with accumulation of lymphoid cells can be seen in the experiment (a). Only slight edema of the subcutaneous cellular tissue is present in the control (b).

of undiluted pathological duodenal contents were added in order to block common antigens present in both normal and pathological contents. The precipitate thus formed was sedimented after 20 min by centrifugation for 15 min at 3000 rpm. The procedure was repeated until addition of pathological duodenal juice, drop by drop, produced no further precipitate. The supernatant, when separated from the precipitate, according to our hypothesis contains active factor X, not precipitated by antibodies against normal duodenal contents. This supernatant, which we called antigen X, was used for immunizing rabbits and for producing X antiserum. The CFR was carried out in a volume of 2.5 ml at 37°. Besides normal and pathological duodenal contents, three control antigens were used, namely tissue extracts from normal and necrotic pancreases, and the duodenal wall of dogs with pancreatic necrosis; each experiment was repeated two or three times. The results were read twice: at the end of incubation and after the samples had been kept at room temperature for 20 h.

The results of the reaction after incubation for 20 h are given in Tables 1-3, because they were more precise in character.

The immunologic investigations were carried out jointly with Dr. V. Vylchanov in the Laboratory of Cytoimmunology of the Bulgarian Academy of Sciences.

EXPERIMENTAL RESULTS

In the allergy tests, we found erythema and swelling at the site of injection of the pathological duodenal juice in the affected dogs 6 h after injection, and large areas of hemorrhagic infiltration with central necrosis 24 h after injection. No skin reaction was found in the control dogs.

Histopathological investigation (Fig. 1, a and b) showed only slight edema of the subcutaneous cellular tissue in the control dogs, whereas edema in the deep layers of the skin and massive hemorrhages were found in the affected dogs. Areas of fibrinoid necrosis with an abundant nuclear debris (leukocytoclassia), fibrinoid degeneration of the vessel wall, accumulation of lymphoid cells, and other changes were observed indicating that components of allergic inflammation were present against the background of a general inflammatory reaction.

The results of the complement fixation test are given in Tables 1-3.

The results of cross investigations on antiserum against normal and pathological duodenal contents are shown in Table 1. It is clear that antiserum against pathological duodenal contents reacted with antigen

TABLE 1. CFR with Antisera against Pathological and Normal Duodenal Contents

Serum	Antigens					
	dilution of serum	pathological duodenal contents	normal duodenal contents	extract of duodenal tissue from dogs with pancreatic necrosis	extract of tissue of necrotic pancreas	extract of tissue of normal pancreas
Antiserum against pathological duodenal contents (No. 4a)	1:4	++++	+++	++++	+	–
	1:8	++++	++(+)	+++	±	–
	1:16	++++	–	+	–	–
	1:32	++++	–	±	–	–
	1:64	+++	–	–	–	–
	1:128	+	+	–	–	–
	1:256	–	–	–	–	–
Antiserum against normal duodenal contents (No. 27)	1:4	+++	++++	+(+)	–	–
	1:8	+(+)	++++	+	–	–
	1:16	–	++++	–	–	–
	1:32	–	+++	–	–	–
	1:64	–	+(+)	–	–	–
	1:128	–	–	–	–	–
	1:256	–	–	–	–	–

TABLE 2. CFR with Antisera against Factor X Obtained by "Blocking" Method

Serum	dilution of serum	pathological duodenal contents	Antigens		extract of tissue of necrotic pancreas	extract of tissue of normal pancreas
			normal duodenal contents	extract of duodenal tissue		
X antiserum (No. 29)	1:4	++++	+++	++(+)	–	–
	1:8	++++	++	++	–	–
	1:16	++++	–	±	–	–
	1:32	++++	–		–	–
	1:64	–	–		–	–
	1:128	–	–		–	–
	1:256	–	–		–	–
X antiserum (No. 29; after keeping for 2 months at 4°)	1:4	++++	–	–	–	–
	1:8	++++	–	–	–	–
	1:16	++++	–	–	–	–
	1:32	+++	–	–	–	–
	1:64	+++	–	–	–	–
	1:128	+	–	–	–	–
	1:256	–	–	–	–	–

from pathological duodenal contents in much higher titer than with antigen from normal duodenal contents (1:128 compared with 1:8). Meanwhile, although this serum reacted in a titer of 1:16 with extract from duodenal tissue of a dog with acute pancreatitis, it hardly reacted at all with antigen from tissue of the necrotic pancreas and completely failed to react with tissue from the normal pancreas.

TABLE 3. CFR with Antiserum against Factor X Obtained by the "Blocking" Method

Time of reading	Antigen	Dilution of antiserum against factor X					
		1:4	1:8	1:16	1:32	1:64	1:128
After incubation	Factor X	++++	++++	++++	++++	+++	–
	Pathological duodenal contents	++++	++++	++++	–	–	–
20 h later	Factor X	++++	++++	+++	+ –	–	–
	Pathological duodenal contents	++++	+	–	– –	–	–

The most interesting results were obtained with X antiserum obtained by "blocking" antigens of normal duodenal contents.

It follows from Table 2 that antiserum against factor X likewise reacted preferentially with antigen from pathological duodenal contents (1:32) and in lower titers with antigen from normal duodenal contents (1:8) and from duodenal tissue (1:5). After storage of this X antiserum at 4° for 2 months we found that it reacted only with pathological duodenal contents (titer 1:128) did not react with either of the other antigens. Later, when factor X had been isolated in sufficient quantity, we carried out the reaction with X antisera and antigens from factor X and from pathological duodenal contents. The preferential reaction between the X factors and X antiserum is clear from Table 3.

It can be concluded from the results of the allergy and serology tests that an antigenic factor, which we have named factor X, really exists or, more precisely, is formed. This factor is associated with and probably influences the course of acute experimental pancreatic necrosis in dogs. This specific antigen is found in the duodenal contents of dogs with pancreatic necrosis.

Since the X antiserum did not react in the CFR with antigens from tissues of the necrotic and normal pancreas, but reacted only with pathological and normal duodenal contents and also with duodenal tissue from dogs with pancreatic necrosis, it can be postulated that the specific factor X is in fact connected with the duodenal wall.

Later investigations will be carried out to study the possibility of isolating this factor and to investigate its nature.

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