ANTIGENIC CHARACTERISTICS OF THE HUMAN AND RAT PANCREAS

S. K. Vel'bri

UDC 616.37-008.9-097.5

Organ-specific antigens were found in extracts of the human and rat pancreas. Results of the gel diffusion reaction showed that human pancreas contains two such antigens, and rat pancreas three. By immunoelectrophoresis, three organ-specific antigens were found in human pancreas and eight in rat pancreas.

Data in the literature [6, 8] on the study of porcine, bovine, and rabbit pancreas show that this organ possesses organ-specific antigens.

In this investigation the presence of organ-specific antigens was determined in human and rat pancreas.

EXPERIMENTAL METHOD

Saline extracts of the pancreas and other organs were prepared by the method of Rose and Witebsky [5]. The organs were carefully washed with 0.15 M NaCl, minced, ground in a mortar, and treated with phosphate buffer, pH 7.2 (2 ml buffer/1 g organ). After exposure for 18 h at 4° the suspension was centrifuged (40 min at 8000 rpm) and the supernatant was used as antigen. Antisera were obtained by subcutaneous immunization of rabbits with tissue extracts mixed with Freund's adjuvant.

Tests were carried out with antisera against rat and human pancreas, and in the control experiments antisera against the heart, liver, and kidney of the rat were used. The complement fixation reaction, Boyden's passive hemagglutination reaction, precipitation in agar, and immunoelectrophoresis were used for the investigation. The complement fixation reaction was carried out by V. I. Ioffe's method of prolonged fixation at 4° [3], the passive hemagglutination reaction by Boyden's method [4] as modified by Weinbach [7] with formalinized sheep's erythrocytes, precipitation in agar by Ouchterlony's method as modified by A. A. Gusev and V. S. Tsvetkov [2], and immunoelectrophoresis in the modification of G. I. Abelev and V. S. Tsvetkov [1].

EXPERIMENTAL RESULTS

The characteristics of rabbit antiserum against rat pancreatic extracts, as obtained by the passive hemagglutination reaction by Boyden's method are given in Table 1.

It is clear from Table 1 that antiserum against pancreatic extract reacted both with homologous antigen and with extracts from other organs, and the reaction was particularly strong with serum proteins. To determine the reasons for these cross reactions, the antisera were exhausted.

Preliminary experiments were carried out to determine the optimal dose of antigen for exhaustion, which proved to be 0.5 ml of normal rat serum to 1 ml antiserum or 20 mg protein of tissue extracts. Exhaustion experiments showed that the cross reactions were due mainly to serum proteins contained in the pancreatic extracts. After exhaustion with normal rat serum and antigen from liver or heart, the reaction with antigens from the pancreas persisted in its previous titer. Similar results were obtained with the complement fixation reaction, except that it was from 5 to 40 times less sensitive than the passive hemagglutination reaction. Similar results were also given by the study of the remaining three antisera against rat pancreas.

Central Medical Laboratory, Tartu University; Department of Microbiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR A. A. Smorodintsev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 66, No. 11, pp. 80-83, November, 1968. Original article submitted June 26, 1967.

TABLE 1. Characteristics of Rabbit Antiserum Against Rat Pancreas (Boyden's Passive Hemagglutination Reaction)

Antigen for exhaustion	Antigen						
	Pancreatic extract	Normal rat	Extracts of				
			Liver	Kidney	Heart	Lung	Spleen
–	3200	6400	200	400	400	160	80
Serum	3200	–	10	–	10	–	–
Serum and liver extracts	3200	–	–	–	–	–	–
Serum and heart extracts	3200	–	–	–	–	–	–
Serum and pancreatic extracts	–	–	–	–	–	–	–

Note. Numbers in all tables denote degree of dilution of antiserum still giving a positive result.

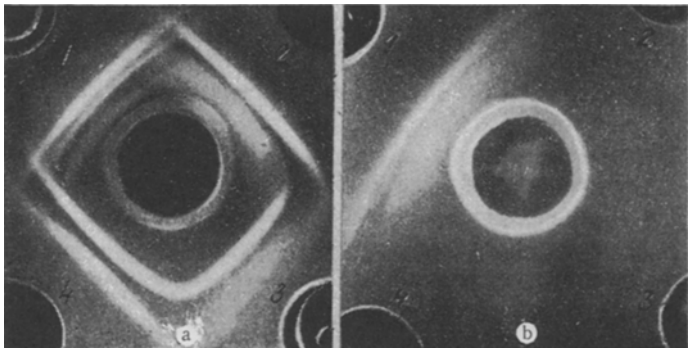

Fig. 1. Precipitation reaction in agar with antiserum against rat pancreas. In wells: 1) extract of rat pancreas (1 mg protein/ml); 2) rat serum, 1:1000; 3) extract of rat's heart; 4) extract of rat's liver (1 mg protein/ml); in center: a) antiserum against rat pancreas (undiluted); b) the same serum exhausted with normal rat serum and with rat liver extract.

These experiments showed that pancreatic extracts contain mainly organ-specific antigens and a small proportion of serum proteins and antigenic components common to other organs.

This was confirmed by experiments with rabbit antisera against antigens of the rat's heart, liver, and kidney. In Boyden's passive hemagglutination reaction these antisera reacted with pancreatic extract in low titer (1-20 to 1-160), but this reaction disappeared completely after exhaustion of the antisera with normal rat serum.

The cross reaction with pancreatic extract was thus due to serum proteins and not to common tissue antigens in the pancreas and other organs.

The presence of organ-specific components in the pancreas could also be demonstrated by the precipitation reaction in agar (Fig. 1, a). Antiserum against pancreas gave two precipitation lines with normal rat serum and extracts from rat's liver and heart, but pancreatic extracts gave these lines and also three other distinct precipitation lines. The latter appeared also after exhaustion of the antiserum with normal rat serum and with liver extract (Fig. 1, b). The results of the precipitation reaction in agar thus showed that rat pancreas contains three organ-specific components.

Immunoelectrophoresis with unexhausted antiserum revealed 8-10 antigenic components in rat pancreatic extract (Fig. 2). When the reaction was carried out with the same rabbit antiserum after exhaustion with normal rat serum and with heterologous rat organs, only one of the precipitation lines disappeared in the zone of albumins.

TABLE 2. Boyden's Passive Hemagglutination Reaction with Extract from Human Pancreas and Corresponding Rabbit Antiserum

Antigen for exhaustion	Antigen			
	Liver extract	Normal human serum	Heart extract	Liver extract
–	6400	100,000	10	10
Normal human serum	6400	–	–	–
Serum and liver extract	6400	–	–	–

TABLE 3. Boyden's Passive Hemagglutination Reaction with Pancreatic Extract of Different Species

Antiserum	Antigen for exhaustion	Extract of pancreas			
		Human	Rat	Rabbit	Guinea pig
Against human pancreas	–	6400	20	10	20
	Human serum	6400	–	–	–
Against rat pancreas	–	100	3200	100	200
	Rat serum	–	3200	–	–

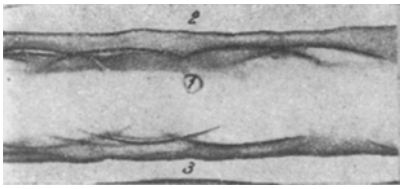

Fig. 2. Immunoelectrophoresis with extract of rat's pancreas. 1) Extract of rat's pancreas (whole); 2) antiserum against rat's pancreas; 3) the same antiserum exhausted with normal rat serum and with liver extract.

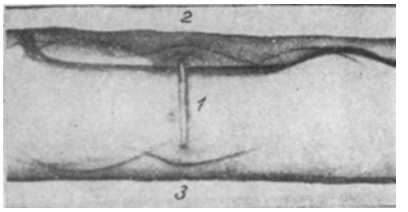

Fig. 3. Immunoelectrophoresis with extract of human pancreas. 1) Extract of human pancreas (whole); 2) antiserum against human pancreas; 3) the same antiserum exhausted with normal human serum and liver extract.

Further experiments showed that not only rat pancreas possesses organ-specific antigens. The same results were obtained with extract of human pancreas and the corresponding rabbit antiserum in Boyden's passive hemagglutination reaction (Table 2). Two lines corresponding to specific pancreatic antigens were found in the precipitation reaction in agar, while three organ-specific components were found by immunoelectrophoresis (Fig. 3).

In cross experiments with extract of human pancreas and the pancreas of other species of animals it was found that this organ possesses not only organ-specificity, but also species-specificity (Table 3). The cross reactions which were observed between extracts of the pancreas of different species, using unexhausted antisera, were due to common components in the serum proteins.

These experiments thus demonstrated the presence of several antigens characteristic of the pancreas of that species in extracts of the rat and human pancreas. They agree with analogous results obtained by investigation of antigens of the porcine, bovine, and rabbit pancreas [6, 8].

LITERATURE CITED

1. G. I. Abelev and V. S. Tsvetkov, Vopr. Onkol., No. 6, 62 (1960).
2. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
3. V. I. Ioffe, Arkh. Biol. Nauk, 58, No. 1, 3 (1940).
4. S. V. Boyden, J. Exp. Med., 93, 107 (1951).
5. N. R. Rose and E. Witebsky, J. Immunol., 83, 34 (1959).
6. N. R. Rose, R. S. Metzgar, and E. Witebsky, J. Immunol., 85, 575 (1960).
7. R. Weinbach, Schweiz, Z. Allg. Path., 22, 1 (1959).
8. E. Witebsky, N. R. Rose, and H. Nadel, J. Immunol., 85, 568 (1960).