COMPARISON OF THE ANTIGENIC PROPERTIES OF SOME TISSUES OF THE GARFISH (BELONE BELONE EUXINI GÜN)

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Research in noninfections immunology has undergone extensive development. Fishes are a very convenient object for many such investigations, yet information concerning the antigenic properties of fish tissues is largely confined to a few studies of blood groups [1, 3-7]. No systematic investigation of the organ-specific properties of individual fish tissues has yet been undertaken.

Before investigating the changes in the antigenic properties of the developing ova of the garfish, we considered that a useful purpose would be served by making an immunological comparison of the muscles, heart, ovary, and crystalline lens of the adult fishes. In the present communication we describe the results of cross ring-precipitation reactions between immune sera produced against the above-mentioned tissues and saline extracts of these tissues.

EXPERIMENTAL METHOD

Approximately equal pieces of the muscles and ovary, and the entire heart and lenses were taken from 5 female garfish, cleaned, and washed in physiological saline. Weighed samples of the homologous tissues were diluted with 9 parts of physiological saline containing merthiolate, and blended in a homogenizer for about 2 min. The final merthiolate concentration was 1:10,000.

The supernatant fluid after centrifugation (30 min at 2000 rpm) was used as antigen. The protein concentration of the extracts was estimated by the micro-Kjeldahl method.

Rabbits were immunized with the use of a stimulator as described by Freund [7], by the scheme described previously [2]. As a result of immunization 2 active sera each were obtained against the extracts of the muscles, heart, ovary, and lens. From the original solution of antigen with a protein concentration of 1 mg/ml, serial 1:2 dilutions were made. The antisera for the experiments were diluted 5 times with physiological saline. The ring precipitation reaction was carried out in the usual manner at 18-20°. Observations lasted for 2 h. During the first hour readings were taken every 5 min, and thereafter at intervals of 90 and 120 min. As neutral serum control the concentrated antigen (1 mg/ml) was added, and as immune serum control—physiological saline. The experiments were repeated 2-3 times and the mean time taken for the ring to appear at a given antigen dilution (titer of the reaction) was calculated.

EXPERIMENTAL RESULTS

Sera against muscles in homologous reactions formed clear rings after 5 min in a titer of 1:16 and after 2 h in a titer of 1:128 (Table 1). In repeated experiments the precipitate in the first 3 tubes consisted of double rings. The upper ring was much weaker than the lower; as the dilution of the antigen increased, it was situated further from the level of the serum and its intensity diminished. Whereas in the 4th tube the intensity of the lower ring could still be read as ++++, that of the upper ring was reduced to a trace (an amorphous clouding of the antigen). With extracts of the lens rings were observed 5-10 min after the beginning of the experiments only in the first 2 2 tubes, and after 2 h as far as a dilution of 1:8. With extracts of the heart the reaction was weaker still than with antigens from the lens: an obvious precipitate was found only after 20-25 min and in dilutions up to 1:4-1:8.

TABLE 1. Reaction Between Antimuscle Sera and Tissue Extracts (Time Taken for Rings to Appear, in min)

Anti-muscle serum	Antigens (tissue extracts)	Dilution of antigen 1 mg/ml											Control (neutral serum)
		1	2	4	8	16	32	64	128	256	512	1024	Dilution of antigen
No. 2	Muscles	5	5	5	5	5	23	65	120	–	–	–	–
	Heart	23	30	48	–	–	–	–	–	–	–	–	–
	Ovary	40	25	25	15	18	33	–	–	–	–	–	–
	Lens	10	10	20	40	–	–	–	–	–	–	–	–
	Control (physiological saline)	Reaction negative											
No. 9	Muscles	5	5	5	5	5	25	82	120	–	–	–	–
	Heart	25	35	120	–	–	–	–	–	–	–	–	–
	Ovary	–	120	90	50	38	43	45	–	–	–	–	–
	Lens	5	5	10	35	–	–	–	–	–	–	–	–
	Control (physiological saline)	Reaction negative											

TABLE 2. Reaction Between Antiheart Sera and Tissue Extracts (Time Taken for Rings to Appear, in min)

Anti-heart serum	Antigens (tissue extracts)	Dilution of antigen 1 mg/ml											Control (neutral serum)
		1	2	4	8	16	32	64	128	256	512	1024	Dilution of antigen
No. 4	Muscles	5	5	5	5	12	48	75	–	–	–	–	–
	Heart	5	5	5	5	5	5	15	35	–	–	–	–
	Ovary	60	60	–	–	–	–	–	–	–	–	–	–
	Lens	5	5	8	55	–	–	–	–	–	–	–	–
	Control (physiological saline)	Reaction negative											
No. 11	Muscles	5	5	5	10	20	68	120	30	–	–	–	–
	Heart	5	5	5	5	5	5	15	–	–	–	–	–
	Ovary	43	38	60	–	–	–	–	–	–	–	–	–
	Lens	5	8	13	45	–	–	–	–	–	–	–	–
	Control (physiological saline)	Reaction negative											

TABLE 3. Reaction Between Anti-ovary Sera and Tissue Extracts (Time Taken for Rings to Appear, in min)

Anti-ovary serum	Anitgens (tissue extracts)	Dilution of antigen 1 mg/ml											Control (neutral serum)
		1	2	4	8	16	32	64	128	256	512	1024	Dilution of antigen
No. 3	Muscles	17	35	90	–	–	–	–	–	–	–	–	–
	Heart	20	43	87	–	–	–	–	–	–	–	–	–
	Ovary	5	5	5	5	5	5	5	53	80	–	–	–
	Lens	73	180	–	–	–	–	–	–	–	–	–	–
	Control (physiological saline)	Reaction negative											
No. 10	Muscles	5	10	120	–	–	–	–	–	–	–	–	–
	Heart	Experiments not performed											
	Ovary	5	5	5	5	5	5	13	63	–	–	–	–
	Lens	27	55	–	–	–	–	–	–	–	–	–	–
	Control (physiological saline)	Reaction negative											

TABLE 4. Reaction Between Antilens Sera and Tissue Extracts (time Taken for Rings to Appear, in min)

Anti-lens serum	Antigens (tissue extracts)	Dilution of antigen 1 mg/ml											Control (neutral serum)
		1	2	4	8	16	32	64	128	256	512	1024	Dilution of antigen
No. 1	Muscles	5	15	33	63	–	–	–	–	–	–	–	–
	Heart	33	53	–	–	–	–	–	–	–	–	–	–
	Ovary	40	45	–	–	–	–	–	–	–	–	–	–
	Lens	5	5	5	5	5	5	5	5	8	33	90	–
	Control (physiological saline)	Reaction negative											
No. 8	Muscles	10	15	35	65	–	–	–	–	–	–	–	–
	Heart	30	35	120	–	–	–	–	–	–	–	–	–
	Ovary	25	90	–	–	–	–	–	–	–	–	–	–
	Lens	5	5	5	5	5	5	5	10	28	52	105	–
	Control (physiological saline)	Reaction negative											

As regards its antigenic properties revealed by experiments with muscle antisera, the ovary occupied a special place among the tissues being compared. As will be clear from Table 1, the reaction with extracts of the ovary was visible soonest of all in the dilution zone 1:8-1:64. In the first tubes with a higher protein concentration in the extract, on the other hand, rings developed later or were not formed at all (with serum No. 9), presumably indicating delay in precipitation as a result of excess of antigen. The precipitates were distinct, but the intensity of the reaction was low and could be designated as + or ++. The final dilution was 1:64 and was almost indistinguishable from the titers of the homologous rections.

The antisera against the heart and muscles had rougly identical titers—1:128. However, the homologous reactions of these two sera differed considerably in character. Whereas the antimuscle sera obviously fixed their corresponding antigen at a dilution of 1:32 after 30 min, 1:64 after 90 min, and 1:128 after 120 min, the homologous reactions of the antiheart sera were positive within the first 30 min to a dilution of 1:128, which remained as the limiting value. It will be clear from Table 2 that the muscle tissues were closer to the heart tissues, which was not found in the experiments with the antimuscle sera. This suggests that antigens common to both the heart and the muscles were present in large amounts in the extracts of the heart. The antiheart serum gave a weak reaction with the lens proteins, as also did the antimuscle serum. In all repetitions of the experiment, double rings were formed after addition of the layer of antigen. The reaction with extracts of the ovary was weakest of all: the precipitate appeared only after 30 min and in dilutions of up to 1:2-1:4. It should be noted that the antiheart and antilens sera gave no reaction with extracts of the developing ova at the cleavage stage. Immune sera against the ovary and lens were distinguished by their high reactivity and specificity (Tables 3 and 4), probably associated with the greater antigenic homogeneity of the extracts of these tissues than of the muscle and heart. From the differences observed in the cross experiments conclusions could be drawn regarding the presence of common or closely related antigens in the investigated tissues. For instance, sera against the ovary reacted weakly with extracts of muscles or heart. In experiments with muscle extracts rings were formed above the serum—antigen boundary, indicating an excess of the corresponding antibodies. The optimum of the reaction between antimuscle sera and ovary fell at a dilution of 1:8-1:64 of the extract (see Table 1), indicating excess of antigen. Hence it may be assumed that antigens common to ovary and muscles were present in a significantly larger amount in the ovarian extract, where they were presumably associated with the properties of the yolk. The amounts of these components in heart extracts were less than in muscle extracts.

Besides organ-specific antibodies, other antibodies were found in the lens which were also present in extracts of the muscles, heart, and ovary. The muscles and heart, judging by the reactions of the sera against these tissues with extracts of the lens, contained roughly equal amounts of antigens also characteristic of the lens, but more than in the lens itself. Still small amounts of components common to the extracts of the lens and ovary were detected. In cross reactions using these extracts and the corresponding antisera, weak rings were observed only after 30 min and in antigen dilutions of up to 1:2.

SUMMARY

Hence, the results demonstrate both the similarity and the essential immunological differences between the saline extracts of the investigated tissues. The cross ring precipitation reactions revealed that each extract contained both common antigens and antigens characteristic of the corresponding tissue. It may be assumed that the presence of common antigenic properties in different organs is to some extent due to the presence of species-specific antigens in the tissues.

The complexity of the antigenic composition of the extracts was brought out by the ring precipitation extracts, where it led to the formation of double precipitation rings.

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