CHANGES IN THE ANTIGENIC PROPERTIES OF Belone belone euxini Gün. EGGS DURING THE PROCESS OF EMBRYONIC DEVELOPMENT

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Immunological methods of research, with their high degree of sensitivity and accuracy, have been widely used in elucidating many embryological problems. Recent progress in this field has been reviewed in the book by O. E. Vyazov. Although the range of material investigated by such methods is not large it includes most objects of study in traditional zoological and medical laboratories. Nevertheless little work has been carried out on representatives of such an enormous class as the fish. Research into fish embryology is not merely of theoretical interest but is of practical importance also, for it throws light on to problems of systematics, ontogeny, hybridization, etc.

We have attempted to elucidate the nature of those changes which occur in certain antigenic substances associated with the developing eggs of garfish, Belone belone euxini Gun. using data from ring-precipitation reactions.

EXPERIMENTAL METHODS

Extracts were prepared from the tissues of five, sexually mature, female garfish and these were used as research material. The extracts were prepared as follows: five fish were taken immediately after they had deposited their spawn and it had been fertilized and from the fish we removed approximately equal sized pieces of muscle and ovarian tissue* (in addition the whole heart and crystalline lens were removed). Weighed portions of homologous tissues were mixed with 9 parts of physiological saline solution (0.85% NaCl) and merthiolate was added. The resultant mixture was homogenized for 2 min when the final concentration of merthiolate amounted to 1:10,000. The homogenates were kept in a refrigerator for a month and then centrifuged for 30 min at a speed of 2,000 revs/min. The supernatant liquid was passed through a sterile Seitz filter and used as antigen. Extracts of muscle, ovary, heart and crystalline lens from adult fish were obtained in this way.

The fertilized ova were incubated in an aquarium†. Extracts of the eggs were prepared in the same way as those from the tissues of adult fish. Five extracts were prepared from a limited amount of embryonic material at the following times of development: 6 h – segmentation period; 58 h – gastrulation period, yolk sack four fifths overgrown; 80 h – period when the body of the embryo is formed, development of tail bud; 7 days – period of development of erythrocytes; 14 days – pigmentation of optic cup.

In order to obtain immune sera we used male albino rabbits, 8 months old and approximately 3 kg in weight. Immunization was carried out using Freund's adjuvant [5]. The animals were injected according to the scheme described by M. G. Zaks and M. M. Sokotova [2]. As a result of immunization two antisera were obtained with activity towards muscle, ovary, heart and crystalline lens antigens and eggs of 80 h development.

* In the garfish, spawning takes place in such a way that batches of eggs are emitted over a considerable period [3]; after each emission of ripe ova a certain number of immature oocytes still remain in the ovary.
† At a temperature of 20-21.5° embryonic development in Belone takes 21 days.

TABLE 1. Interaction between Serum Immunized Against Ovarian Tissue (No. 3) and Embryos (No. 12) with Extracts of Garfish Spawn at Various Stages of Development. Time of appearance of ring (in minutes)

Antigen (spawn extracts)	No. of serum, diluted with physiological saline in ratio 1:4	Dilution of antigen										Control – neutral serum; diluted antigen
		1	2	4	8	16	32	64	128	256	512	
Ovarian extract	3	5	5	5	5	5	5	5	53	80	–	–
	12	5	5	5	5	5	5	5	70	–	–	
6 h development – segmentation stage	3	5	5	5	5	5	5	10	55	90	–	–
	12	5	5	5	5	5	5	10	53	90	–	
58 h – gastrulation stage, yolk sack four fifths overgrown	3	5	5	5	5	5	5	7	15	43	–	–
	12	5	5	5	5	5	5	5	15	60	–	
80 h – body of embryo formed, development of tail bud	3	5	5	5	5	5	5	13	90	–	–	–
	12	5	5	5	5	5	5	5	65	–	–	
7 days – development of erythrocytes	3	5	5	5	5	5	5	18	–	–	–	
	12	5	5	5	5	5	5	5	120	–	–	
14 days – pigmentation of optic cup	3	5	5	5	5	5	5	8	90	–	–	–
	12	5	5	5	5	5	5	5	10	–	–	–
Control – physiological saline	3	Reaction negative										
	12	" "										

The tissue and egg antigens were compared by means of ring precipitation reactions, which were carried out at 18-20° by the usual method. The proteins in the extracts were determined by a micro-Kjeldahl technique before carrying out the reactions.

The results of the protein content analyses indicated a concentration of up to 1 mg/ml in the original material. From this material a series of diluted antigens was prepared, each member being half as concentrated as the last.

On grounds of economy and increased specificity, the serum used in our experiments was diluted with 5 times its volume of physiological saline solution. The course of the reaction was observed over a period of 2 h. During the first hour readings were taken every 5 min; after that readings were taken at 90 and 120 mins. As controls we used the following: in place of the series of diluted antigen – proantigen with a protein content of 1 mg/ml; for the antisera – tubes in which the immune sera had been covered with a layer of physiological saline. The experiments were carried out twice or three times with each serum.

As the specificity was expressed not only by the titer but also by the speed at which precipitation occurred, we were able to express the results in the form of graphs.

In determining specific absorption we followed the technique recommended by Boyd [4] and after finding the optimum proportions of reagents we took four parts of whole serum for one part of ovarian extract having a protein content of 8 mg/ml.

TABLE 2. Changes in the Antigenic Properties of Garfish Eggs during the Process of Their Embryonic Development as Revealed by Absorbed Sera Immunized Against Ovarian Extract (No. 12)

Antigen extracts from eggs	Protein content of extract (in mg/ml)						Control – neutral serum (protein – in extract 2 mg/ml)
	2	1	0.5	0.375	0.25	0.125	
Extracts from ovary	–	–	–	–	–	–	–
6 h development – segmentation	–	–	–	–	–	–	–
58 h – gastrulation yolk sack four fifths overgrown	±	–	–	–	–	–	–
80 h – body of embryo formed, developed of caudal bud	+	+	–	–	–	–	–
7 days – development of erythrocytes	+	+	+	+	+	±	–
Control – physiological saline	–	–	–	–	–	–	–

Symbols: + definite ring; ± traces of ring; – reaction negative.

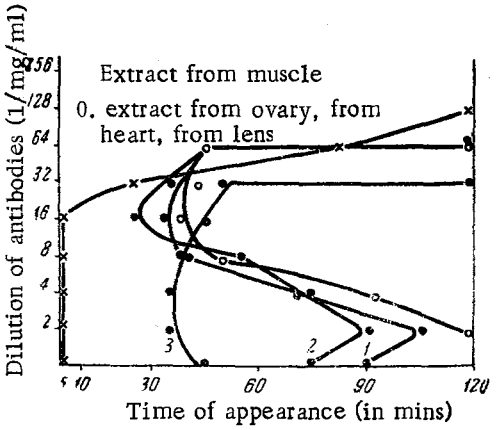

Fig. 1. Reaction of serum immunized against muscle (No. 9) with extracts from garfish eggs at various stages of development. 1) Extract from eggs after 80 h development; 2) 7 days development; 3) 14 days development.

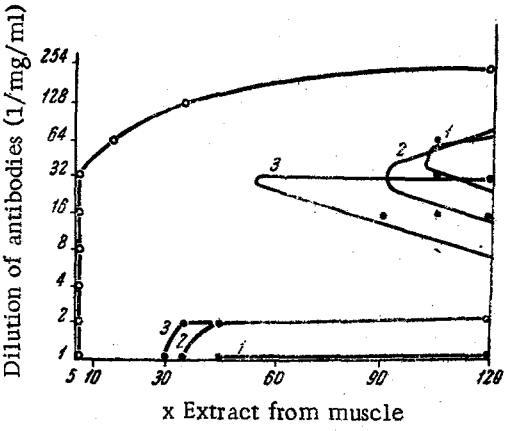

Fig. 2. Reaction of immune serum against heart tissue (No. 4) with egg extracts at various stages of development. Symbols as in Fig. 1.

EXPERIMENTAL RESULTS

In the first series of experiments we compared the reaction of serum immunized against the tissue of embryos at the caudal bud stage with extracts of spawn at various stages of development and the reaction of serum immunized against ovarian tissue with the same extracts.

It is evident from Table 1 that there is no essential difference in the reaction of serum immunized against embryo tissue and that immunized against ovarian tissue. However in every experimental series there is a definite weakening of the reaction between serum and egg extracts as embryonic development proceeds. Thus, if extract is taken from eggs at the segmentation stage a ring forms at a dilution of 1:256, whereas with extract from embryos at the stage when the optic cup is pigmented – the ring is only formed at a dilution of 1:64. Evidently such a weakening of the reaction is related to the reduction which takes place in the amount of yolk present during embryogenesis. It is characteristic of these results that sera immunized against embryo tissue react with homologous antigen more freely than they do with antigen from eggs at the segmentation or gastrulation stages. It may be supposed that the yolk, as antigen, is present in a considerably greater amount than the other egg components and by promoting the production within itself of most of the antibody it completely masks the interaction of the other reagents.

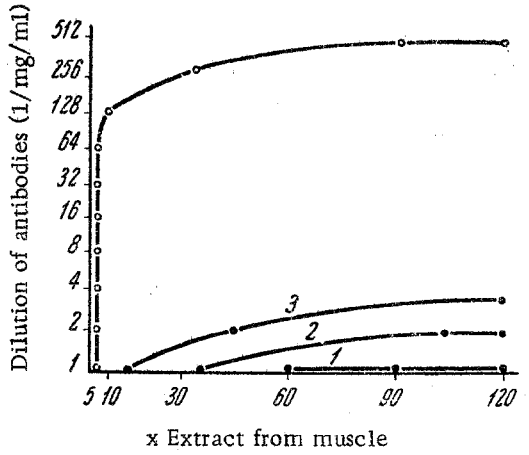

Fig. 3. Reaction of immune serum against crystalline lens (No. 1) with extracts of garfish eggs at different stages of development. Symbols as in Fig. 1.

In order to eliminate the masking effect of the anti-yolk antibodies we carried out a special absorption of the serum against ovarian extract. The absorbed serun did not react with either ovarian antigen or antigen from eggs after 6 h development; there was, however, a noticeable reaction with antigen from eggs at the gastrulation stage. In all these replicate experiments the reaction was expressed by a definite turbidity where the antigen came into contact with the serum (Table 2). When extracts derived from eggs after 80 h of development were used a distinct positive reaction was noticed 30 min (mean time) after setting up the experiment. With extracts of eggs which had undergone 7 days development, precipitation occurred as early as 10 min after setting up the experiment and at the maximum dilution of the antigen. These results were confirmed by precipitation reactions in agar using a previously described technique [1].

Then, by using absorbed sera we were able to follow changes in the antigenic properties of developing eggs from the time of gastrulation onwards. As development proceeded the antigenic properties were intensified.

Experiments involving sera immunized against specific tissues were carried out in order to elucidate the manner in which organ-specific antigens arise and increase in amount in the developing spawn.

Immune serum against muscle reacted with egg extracts from early stages (segmentation and gastrulation) in almost the same way as did ovarian extract. The optimum reaction took place with 1 : 16 and 1 : 32 dilutions of antigen. When the dilution was slight 1 : 1 and 1 : 2, i.e., where the amount of protein was high, ring formation occurred considerably later or usually did not occur at all (Fig. 1). This phenomenon is apparently connected with the retarding effect on precipitation which excess antigen has. However, from the caudal bud stages onwards the nature of the reaction changed somewhat, as is shown in the first two tests. As development of the eggs proceeded (7 days) the ring began to appear earlier in zones of little dilution (1 : 1 – 1 : 2) and when 14 day embryo extracts were used the ring did not develop even when we used proportions found to be optimum for ring development with ovarian antigen. At dilutions ranging from 1 : 1 to 1 : 32 the ring first appeared at almost the same time. The titer of the reaction fell. In its general form the reaction approximated to a precipitation of muscle antigen by the sera.

Heart antigens were observed in the spawn after 80 h development. Sera immunized against heart tissues from adult fish gave two reaction zones with the extracts. One zone lay within dilution limits of 1 : 1 – 1 : 2, the other within limits of 1 : 8 – 1 : 64. All the replicate experiments gave similar results and in both cases the reaction was intensified as the development of the embryos proceeded. With extracts from later embryos the rings not only formed earlier in the dilution zone 1 : 8 – 1 : 64 but optimum formation shifted to higher concentrations of protein in the extracts (Fig. 2). It may be supposed that in this case we were dealing with stage-specific antigens, possibly related to changes in the properties of the yolk.

Crystalline lens antigens appeared in the spawn at the gastrulation stage but at this stage only traces of ring formation were detectable and then only in the first tube of the dilution series. A definite ring was noticed in experiments involving extracts of 80 h embryos. As development of the embryo proceeded the reaction intensified; the rings appeared earlier and at greater dilutions (Fig. 3). Such intensification of the reaction testifies to an increased amount of crystalline lens antigen in the extracts.

These results suggest that the antigenic properties of eggs undergo qualitative and quantitative changes during embryonic development.

SUMMARY

To ascertain the character of changes in antigenic properties of developing Belone belone euxini Gün the author obtained immune sera against tissues of the muscle, heart, ovary and lens of adult fish, as well as sera against the embryo at the stage of the caudal bud development. Experiments involved the use of the ring precipitation reaction between the sera and the extracts from the eggs at their various developmental periods.

In experiments with antiembryonic sera and absorbed extracts from the ovary it was established that a change of the antigenic properties of the roe occurred beginning from gastrulation. These changes enhance with its further development. In experiments with sera against individual tissues antigens of the muscles, heart and lens were revealed, starting from the stage of the caudal bud development. During embryogenesis the amount of antigens in individual tissues was seen to increase.

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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.
