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Immunological analysis of serum proteins of redfish (genus Sebastes)

in connection with maturation

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## ABSTRACT

By the method of double immunodiffusion in agar gel the sexual differences in redfish serum proteins have been discovered. In blood of mature and first maturing females has been found the antigen complex (ovovitellin), absent in males and immature females. It is proposed to use this approach for estimating rate of sexual maturation in redfish female population.

By the methods of the paper electrophoresis (Vanstone & Ho, 1961) and the double immunodiffusion in agar gel (Ridgway et al., 1962) the sexual differences in salmon serum proteins (Oncorhynchus nerka and O.kusutch) have been discovered. This differentiation is associated with the process of sexual maturation and expressed in the appearance in the blood of the first maturing females of the lipoprotein complex, named by Vanstone and Ho (1961) as 'serum vitellin'. In the mature females the serum vitellin is always found contained, and, moreover, in high concentration in salmon eggs, as has been shown by Krauel and Ridgway (1963). In blood of males and immature females this complex is absent. *In recent papers (Utter and Ridgway, 1967a,b) the existence of 'maturity antigens' is shown in two more marine species.*

The present work is concerned with the immunological studies of maturation in the viviparous redfishes of the genus Sebastes. Investigations of such kind were undertaken by us because the sensitivity and expressivity of the immunological tests allow easily to identify the complex of the sexual antigens. In case they are present in blood of redfish it would be possible lay down a foundation for a very objective

experimental way of determining the relative number of the first maturing females in the stock of this commercially significant fish.

### Materials and Methods

The work took place in 1964-1967 on board the r/v of the Polar Institute, 'Sevastopol' and in the laboratory of Moscow University. There were studied 209 samples of blood serum<sup>a</sup> of S. marinus, S. mentella and the 'giants', to which we referred the specimens more than 60 cm in length. Samples of blood from S. marinus and S. mentella were collected in September-October 1964, and from the 'giants' - in August 1965. The places of trawling the fish are shown in the map (fig.1). Six samples<sup>i</sup> of serum are brought from the Barents<sup>s</sup> Sea. To this purpose four rabbits were immunized with a mixture of blood serum<sup>a</sup> from five to six specimens of 'marinus' and 'mentella' types of redfish. Friend's three-week cycle round of immunization with adjuvant was applied in a manner described in detail previously (Altukhov, Apekin & Limansky; 1964). As the result, three highly reactive serum<sup>a</sup> were obtained: two against the female serum proteins (anti-marinus N. 1 and N. 2) and one against the male proteins (anti-mentella N. 4).

The immunization proceeded in a damp cell at +20-22°C in 1 per cent agar 'Difco' gel spread as a thin layer on the surface of an object glass. With the help of a special matrix wells in agar gel were cut and analyzed serum<sup>a</sup> were put in them. After the formation of precipitate clots was complete the preparations were photographed with the help of a special lighting set (Limansky; 1965).

The method of immunoelectrophoresis used by us is described in detail by Zilber and Abelev (1962).

### Results

The results<sup>s</sup> of the experiments are represented in fig. 2 by photographs. The 'A' photo illustrates the character of reactions between the antiserum<sup>s</sup> No.2 and the blood serum<sup>a</sup> of males

(wells No.2,4,6) and females (wells No.1,3,5) of S.marinus. In the photograph 'B' the antigens composition in the male and female blood of S.mentella is compared similarly with the precipitating anti-'marinus' No.1 serum. These data reveal the sexual differences; viz., at the background of a great number of connecting precipitate strips, showing the identity of the antigen components in the compared specimens, there are well seen the same antigens, contained only in the female blood serum <sup>both</sup> in S.marinus and S.mentella.

The association of these differences with sex is confirmed by the results of the experiment, shown in the photograph 'C'. Here the same serums, as in photograph 'B' are in the peripheral wells, while in the central well is the immune anti-'mentella' No.4, developed against the male serum. Accordingly, anti-'mentella' serum does not show any specific antigens in the female blood, but rather make visible only the common components, inherent in blood of the specimens of both sexes. As distinct are also the sexual antigen differences in the absorption experiments. Their results are shown in the photographs 'D' and 'E'. The difference is only that in the sets "A" and "B" whole antisera<sup>a</sup> were used whereas in the experiments "D" and "E" they were absorbed by the male serum proteins according to <sup>the</sup> Björklund's (1952) scheme (photo "D") and to the conventional variant of absorption (photo "E"). In the first case male blood serum of S.marinus was put in the agar slide wells, the preparation being kept in the damp cell till a complete absorption. After this, the same ingredients as in the "A" set were put in the wells. The second variant of absorption was run by adding one part of a mixture of serum from several males of S.mentella to two parts of the antiserum No.1. The mixture was placed in a refrigerator for night, and centrifuging it then we obtained the reagent, specific only against the blood antigens of the females.

On the other hand, the reactions with the absorption by antisera<sup>a</sup> show that the specific female antigen is not homogeneous, but consists of several components. It is possible to

identify not less than three strips of precipitation, as is quite distinctly seen in fig. D. The antigen<sup>s</sup> in this experiment are arranged as a compact group; two of them, recognisable near the equivalent zone, evidently prevail in quantity over the third antigen, the nearest to the central well. The pattern shown in fig. 'E' actually does not differ from the just considered, though the division of the first two precipitate strips is not so distinct. However, in the immunoelectrophoretic analyses<sup>i</sup> of the antigen composition in the female blood serum<sup>a</sup>, examined in the set 'E', the number of precipitate bows is five a wise equal to three (photo 'G'). Under the given methodical conditions the antigen<sup>s</sup> identified by the absorbed anti-marinus No. 1 serum, are characterised by the similar electrophoretic mobility.

The described experiments, in which the two absorbed anti-'marinus' serum<sup>a</sup> behave as the reagents specific only against female antigen<sup>s</sup>, prove the presence of this complex independently of whether the female belongs to the 'marinus' or to 'mentella' types of redfish. It can be therefore be suggested that <sup>the</sup> discovered difference is not of the species-specific character. Such supposition is confirmed by the experiment "F"; in the central well is situated the serum No. 1, absorb<sup>s</sup>ed by the mixture of anti-'marinus' male blood serum<sup>a</sup>, while in the periferal wells, the female serum of the 'marinus' (wells No. 2,6) and 'mentella' (No. 3,5) types of redfish. For comparison the male blood serum<sup>a</sup> were dropped in the wells No. 1 and No. 4. These data show the merging of the precipitate strips, forming by the contact between the specific antibodies and the sexual antigen<sup>s</sup> of females take place, this testifies to the identity of the compared components.

<sup>us</sup> The, our investigations demonstrate clearly that the complex of specific antigen<sup>s</sup> accompanying the sexual maturation can be revealed by the methods of double immunodiffusion and immunoelectrophoresis. They are actually present in the blood of females, the maturity of which is recognisable visually,

and, moreover are present in the female ovaries at the II stage, that is in the first maturing females. For example, in the wells No. 1 and No. 3 of 'A' and 'D' experiments are the serums<sup>a</sup> of the females at the II stage of maturity, and in the well No. 5 is the serum of the mature female at the IX-II stage<sup>x/</sup>. Not a single male among 118 specimens of all the three studied types of redfish possessed the sexual antigen $\delta$ . As to the studied females, which ever mainly at the II, II-III stages, rarely at the III and occasionally at the IX-II, it is necessary to emphasize the following: 1) among 14 'mentella' type females, caught in the East and West Greenland waters, the antigen $\delta$ s of maturation were found in 6 specimens; 2) among 31 females of the 'marinus' type, caught in the Iceland and East Greenland areas, they were found in 17 individuals; 3) among 22 females of the West Greenland 'marinus' redfish, only in 3 specimens.

Furthermore, studied by us were also 24 females of 'giants', caught in August 1965 in the West Greenland waters. By their large size they might be expected to have reached maturity, yet their ovaries were at the II stage. Accordingly we succeeded to reveal the sexual antigen complex only in 4 fish, and it consisted of only two components, instead of three.

Among the 46 studied females of 'giants' and West Greenland 'marinus' we stated the presence of the sexual antigen $\delta$  only in 7 or 15,2 per cents, which is far less than its frequency in females of 'marinus' from the Iceland - East Greenland Area (54,8 per cent). The discovered difference is statistically significant ( $P < 0,001$ ). It is therefore possible to suggest that very slow rates of maturation are characteristic of the 'giants' and 'marinus' redfish, caught off West Greenland shores.

Owing to the comparatively small volumes of the collected material a thorough discussion of these data is not possible for the time being. Nevertheless, they seem to be deserving attention, since the cytophysiological and biochemical data

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<sup>x/</sup> We have adopted the maturity scale, developed for redfish by Dr.V.P.Sorokin (Polar Institute).

(Altukhov, Nefyodov & Pajusova; 1967; Altukhov & Nefyodov, 1967) point directly to the hybrid<sup>b</sup> nature of the West Greenland 'marinus' populations and of 'giants'; moreover, the biological observations testify the juvenale nature of the significant part of these fish, mainly of females. Up till now such conclusions has been based upon the materials obtained by a visual examination of the sexual glands and identification of maturity coefficients (Zakharov, 1962). Now investigations of such kind may be conducted at the molecular level, moreover the discussed immunological data agree with the biological observations. Further accumulation of data would probably confirm our hypothesis that the juvenale nature of the considerable part of the West Greenland 'marinus' population of females, including the 'giants', is connected with their hybrid<sup>b</sup> origin, hence, with a certain degree of physiological incompatibility between the 'marinus' and 'mentella' types, these species seeming to be in progress of formation.

At the same time, although further investigations in this direction are necessary, the examined immunological data satisfactorily solve the main problem of the present work; viz., to prove the immunological express-method of an early determination of the sexual maturation of females in the redfish populations of the North Atlantic. Besides the data obtained by us, increase the number of the fish species in the blood of which the sexual antigen complex can be identified.

#### Conclusion

The immunological analysis has revealed significant sexual differences in the antigen blood serum composition of the genus Sebastes from the North Atlantic. It has been found that <sup>in</sup> the blood of the mature and first maturing females there is a specific antigen complex, absent in males. The method of double immunodiffusion in agar gel is proposed to be used for an early determination of the sexual maturation of the specimens in the redfish female population.

Literature

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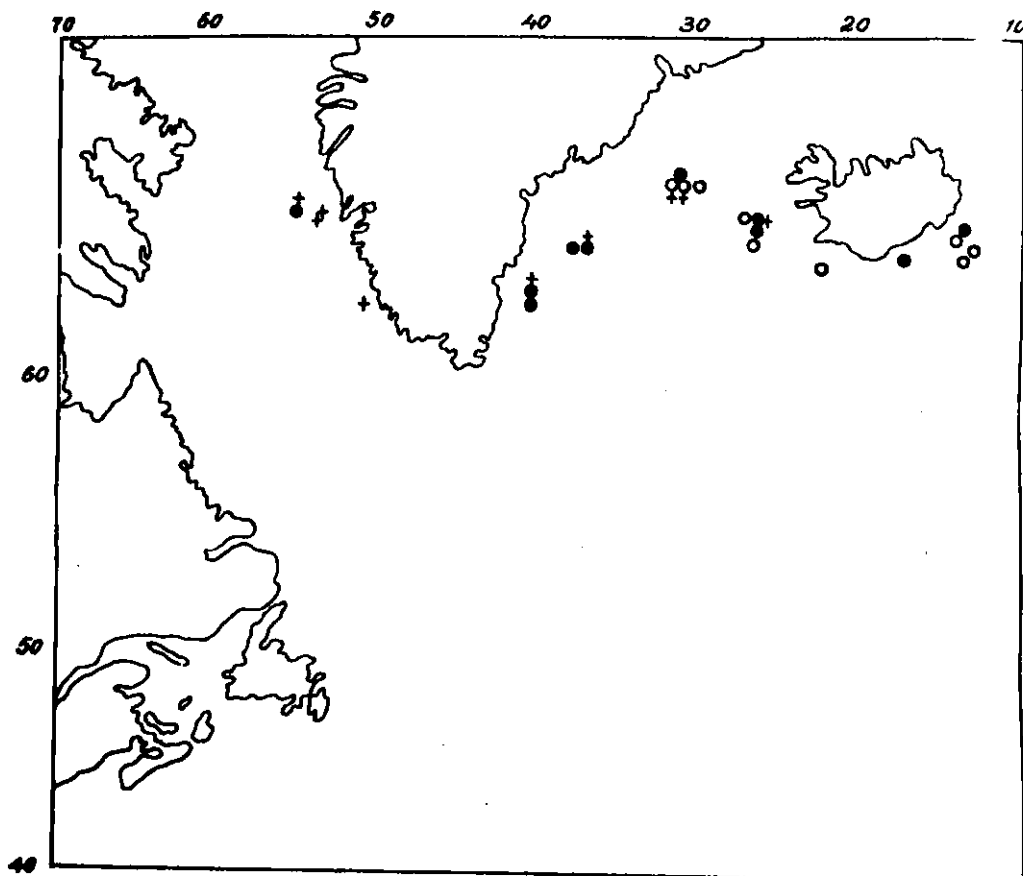


Fig. 1. The places of sampling the redfish blood:  
circles = S. mentella; black circles = S. marinus;  
crosses = "giants".



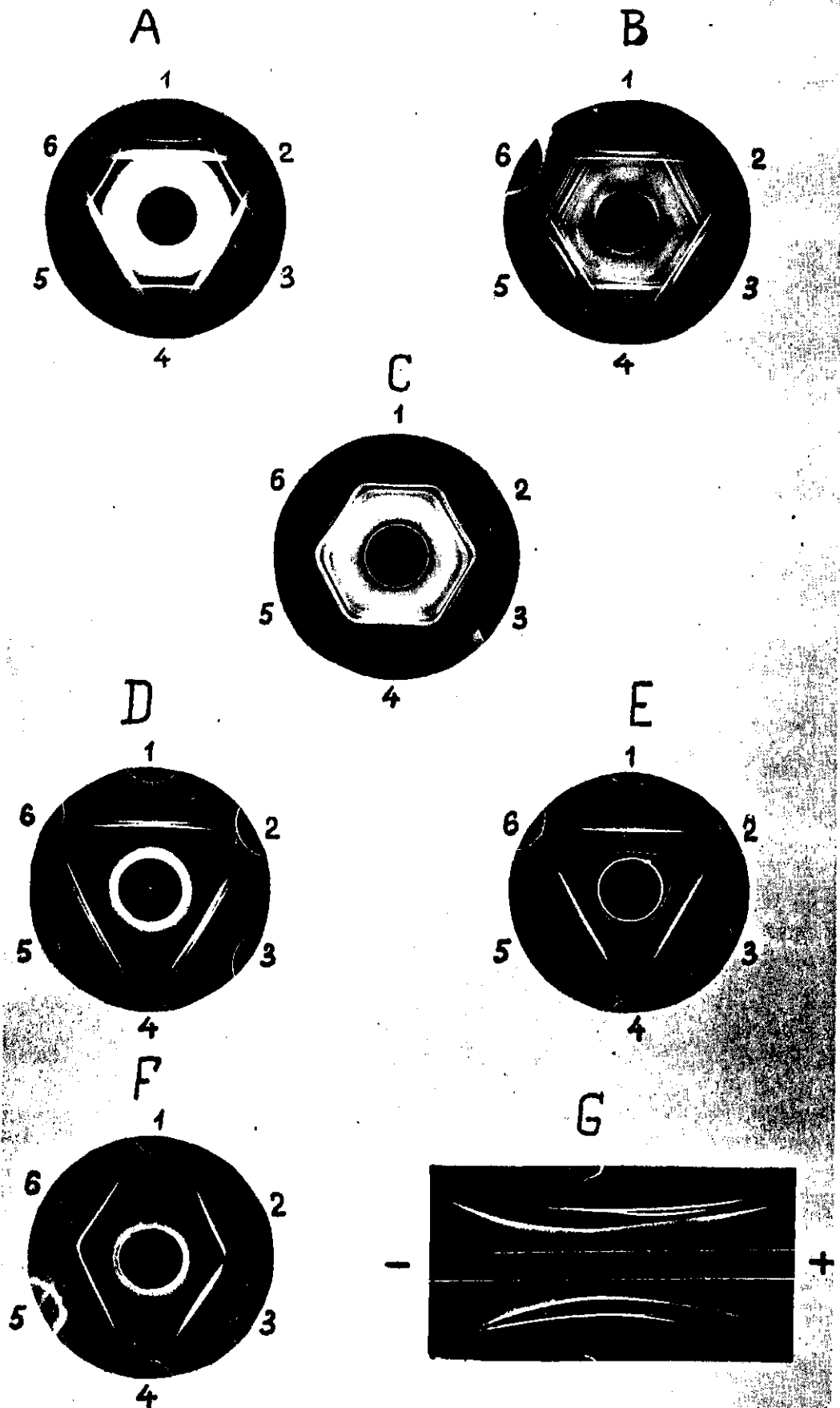


Fig. 2. The antigen differences in blood sera of male and female redfish of the genus Sebastes. Elsewhere in the central wells the antisera, in the peripheral antigens. The other descriptions see in the text.