

# CROSS-REACTIVE PROPERTIES OF ANTISERA PREPARED IN RABBITS BY STIMULATIONS WITH TELEOST VITELLINS

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## ABSTRACT

Antisera were prepared by injecting rabbits with egg or serum vitellin preparations from seven teleost species belonging to different families. The ranges of reactivity of these antisera were tested with sera from mature females of nine teleost families as well as with sera from females of spiny dogfish, Pacific lamprey, and white sturgeon. All of these antisera reacted with vitellins from all species tested from the homologous families. Antisera prepared against

rockfish and flounder vitellins cross-reacted with sera from mature females of all teleost species tested. A greater antigenic complexity in the vitellins of more taxonomically advanced species than more primitive species is indicated by the results of the reactions and absorption tests. The results are of practical importance in studies on maturity of fishes and have theoretical implications in the field of systematics.

Fishery researchers have used serological techniques with increasing frequency since 1950. Much of the work has been directed toward identification of populations either through blood-grouping techniques or studies of variable serum antigens (Cushing, 1964). Ridgway, Klontz, and Matsumoto (1962) observed a characteristic antigen in the serum of maturing and mature female sockeye salmon (*Oncorhynchus nerka*). A review of the literature and subsequent studies by our group has revealed that similar components occur not only in all the teleosts but also in all vertebrate classes where oviparity occurs (Urist and Schjeide, 1961; Drilhon and Fine, 1963). These antigens appear to have considerable practical value in investigations of maturity in female fish because of their connection with the process of sexual maturation (Ho and Vanstone, 1961; Olivereau and Ridgway, 1962; Ridgway, 1964; Utter and Ridgway, 1966).

The function of the blood serum as a transporting medium between the site of synthesis in the liver and the site of storage in the ovary appears to explain the presence of yolk components in the blood (Vanstone, Maw, and Common, 1955). The serum vitellins studied have displayed similar biochemical properties, are characterized as phospholipoproteins, and

conform to the classical description of avian vitellin as the water-insoluble fraction of the egg yolk (Jukes and Kay, 1932; Vanstone, Maw, and Common, 1955).

This report is based on data obtained through testing numerous antisera for cross-reactive properties. The antisera were prepared in rabbits against vitellins of teleosts. We intend to bring out points of both practical significance and theoretical interest.

## METHODS AND MATERIALS

### IMMUNOLOGICAL TESTS

A microslide adaptation of the double diffusion method of Ouchterlony as modified by Ridgway, Klontz, and Matsumoto (1962) was used for all serological tests. The agar medium consisted of 1.5 percent Difco agar, 0.72 percent sodium chloride, 0.60 percent sodium citrate, 0.01 percent merthiolate, and 0.01 percent trypan blue. Wells were punched in the agar at 8-mm. intervals and filled to a volume of about 0.01 cc. of reactant. Slides were evaluated after 24 hours of incubation at 37° C.

### PRODUCTION OF ANTISERA

Table 1 lists the antisera used in this study. Egg vitellin preparations were made by blending and centrifuging one part eggs with three

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TABLE 1.—Antisera used in this study

Designation	Vitellin source	Number of rabbits
Anti-CLM...	Pacific herring ( <i>Clupea harengus pallasi</i> ) eggs.....	1
Anti-SM.....	Chinook salmon ( <i>Oncorhynchus tshawytscha</i> ) eggs.....	3
Anti-CM.....	Northern squawfish ( <i>Ptychocheilus oregonensis</i> ) eggs.....	1
Anti-GM.....	Pacific cod ( <i>Gadus macrocephalus</i> ) serum.....	1
Anti-TM.....	Yellowfin tuna ( <i>Thunnus albacares</i> ) eggs.....	1
Anti-RM.....	Copper rockfish ( <i>Sebastes caurinus</i> ) eggs.....	3
Anti-HM.....	Starry flounder ( <i>Platichthys stellatus</i> ) eggs.....	5

parts 1 percent saline in a Waring Blender.<sup>2</sup> After centrifugation, the addition of 11 parts of distilled water precipitated the vitellins from the supernatant fluid. The precipitate was dissolved again in saline, reprecipitated and redissolved, and used for injections. Whole serum from a mature female Pacific cod was used to produce the anticod vitellin reagent. The resulting antiserum was absorbed at a 1:1 ratio with male cod serum before testing. Usually the vitellin-bearing materials were suspended in a bayol-aralacel mixture and injected into the rabbits intraperitoneally. Consistently uniform results were obtained when other injection procedures were used, but a greater number of injections was usually required. Single bleedings were used for testing with the exception of the reagent prepared against starry flounder vitellin which was a pool of numerous bleedings from five rabbits. The antisera produced in different rabbits injected with the same vitellin material were qualitatively very similar. This uniformity of reagent indicates that the differences reported later are not due to variations in the immune response of individual rabbits.

#### COLLECTION OF SERUM SAMPLES

Samples of fish serum were taken from whole blood that had been processed within 48 hours after collection; the samples were then stored at -35° C., a temperature at which the vitellin fraction appeared to be stable. Some sera had been stored as long as 8 years when tested.

#### REACTIVE PROPERTIES OF THE ANTISERA AND VITELLINS TESTED

Table 2 summarizes the data obtained

<sup>2</sup> Trade names referred to in this publication do not imply endorsement of commercial products.

through testing of sera from mature females of various fish species. All antisera were tested with the same fish sera; this testing included males as well as females from most species. The only reaction with male serum occurred between the antirockfish reagent and male rockfish serum. This reaction was very weak and was most likely the result of nonvitellin antigens present in the injected material. The reaction with male rockfish sera could not be confused with the reaction with female rockfish sera.

TABLE 2.—Cross-reactivity of rabbit antitelost vitellin sera with vitellins of fish representing various taxonomic groups<sup>1</sup>

[X = strong reaction; W = weak reaction; 0 = no reaction]

Reagent	Nonteleosts			Teleosts								
	Agnatha (1)	Chondrichthys (2)	Chondrostei (3)	Clupeid (4)	Salmonid (5)	Cyprinoid (6)	Gadid (7)	Scombrid (8)	Scorpaenid (9)	Cottid (10)	Pleuronectid (11)	Batrachoidid (12)
Anti-Clupeid (CLM).....	0	0	0	X	0	0	0	0	W	0	0	0
Salmonid (SM).....	0	0	0	0	X	0	0	0	0	0	0	0
Cyprinid (CM).....	0	0	X	0	W	0	0	0	0	0	0	0
Gadid (GM).....	0	0	0	0	0	X	0	0	0	0	0	0
Scombrid (TM).....	0	0	0	W	W	W	X	X	X	X	X	X
Scorpaenid (RM).....	0	0	X	X	X	X	X	X	X	X	X	X
Pleuronectid (HM).....	0	0	0	X	X	X	X	X	X	X	X	X

(1) Pacific lamprey, *Lampetra tridentata*; (2) spiny dogfish, *Squalus acanthias*; (3) White sturgeon, *Acipenser transmontanus*; (4) shad, *Alosa sapidissima*; Pacific herring, *Clupea harengus pallasi*; (5) sockeye salmon, *Oncorhynchus nerka*; (6) carp, *Cyprinus carpio*; northern squawfish *Ptychocheilus oregonensis*; largescale sucker, *Catostomus macrocheilus*; (7) Pacific hake, *Merluccius productus*; Pacific cod, *Gadus macrocephalus*; (8) bigeye tuna, *Thunnus obesus*; Pacific mackerel, *Scorpaenopsis japonicus*; (9) copper rockfish, *Sebastes caurinus*; (10) cabezon, *Scorpaenichthys marmoratus*; staghorn sculpin, *Leptocottus armatus*; (11) sand sole, *Psettichthys melanostictus*; English sole, *Parophrys retulus*; starry flounder, *Platichthys stellatus*; (12) northern shipman, *Porichthys notatus*.

All antisera reacted strongly with sera from mature females within the families that provided the vitellin for antibody stimulation. Because of this high degree of cross-reactivity within families, the reactions of the antisera may be considered mainly with regard to the family rather than the species from which the vitellin used for antibody stimulation originated. The arbitrary designations given in tables 1 and 2 refer to vitellin of any species of that family.

Four of the reagents also reacted strongly beyond the immediate family group; two of

them reacted distinctly with sera from mature females of all teleost species tested. Figure 1 illustrates the reactions of sera from mature

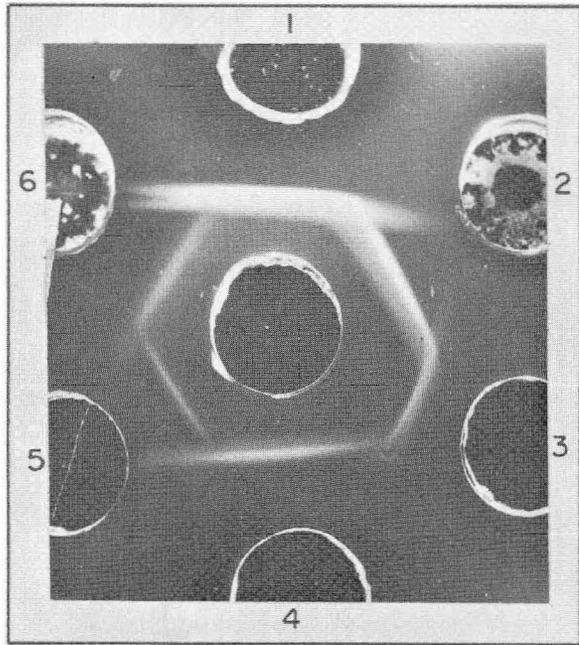


FIGURE 1.—Reactions of sera from mature females of six teleost species with rabbit anti-RM serum. Peripheral wells contain sera from 1, copper rockfish; 2, sand sole; 3, bigeye tuna; 4, Pacific cod; 5, carp; 6, sockeye salmon.

female teleosts of six different families when tested with the anti-RM reagent. The strongest reaction was with the female rockfish serum. The degree of cross-reactivity regularly decreased through the somewhat distantly related salmonoids and cyprinoids. Even in these groups, however, the reaction was clear.

Table 3 gives the results of absorptions of the anti-RM reagent with the fish sera of figure 1. It is evident from both figure 1 and table 3 that the anti-RM reagent contains antibodies of numerous specificities.

Figure 2 presents a more detailed examination of the relationship between SM and RM vitellins. It is evident from figure 2a that the RM vitellin has at least three distinct com-

ponents. The SM vitellin cross-reacts completely with the antibodies directed against one of these components. Two components are visible in figure 2b that react with the anti-SM antiserum. The RM vitellin cross-reacts partially and very weakly with the antibodies directed against one of these components.

TABLE 3.—Results of absorptions of rabbit antirockfish vitellin (RM) serum with sera of mature female teleosts

[X=strong reaction; W=weak reaction; 0=no reaction. See figure 1]

Fish sera used for absorption	Fish sera tested					
	Rockfish	Sole	Tuna	Cod	Carp	Salmon
Unabsorbed.....	X	X	X	X	X	X
Rockfish.....	0	0	0	0	0	0
Sole.....	X	0	W	0	0	0
Tuna.....	X	0	0	0	0	0
Cod.....	X	X	X	0	0	0
Carp.....	X	X	X	X	0	X
Salmon.....	X	X	X	X	0	0

Comparisons similar to those presented in figure 2 were made between the broadly cross-reactive antisera (anti-RM and anti-HM), the less cross-reactive antisera (anti-CLM, anti-SM, anti-CM, and anti-GM), and the corresponding vitellins. All results were similar. The weak or negative reactions of the RM and HM vitellins with the less cross-reactive or group-specific antisera contrasted with the strong reactions of the anti-RM and anti-HM antisera with the vitellins which elicited the less cross-reactive antisera present an interesting serological phenomenon. The cross-reactive antibodies of the anti-RM and anti-HM antisera appear to have a considerably greater avidity for the vitellins which elicit group-specific antisera than the group-specific antibodies have for the RM and HM vitellins.

Both the homologous and cross-reaching heterologous antisera gave uniform results where tested with a larger number of individuals. Randomly selected sera from 48 sockeye salmon were tested with anti-SM, anti-RM, and anti-HM reagents; 48 halibut sera were tested with the anti-RM and anti-HM reagents. Results were identical regardless of the antiserum used, including two weak but positively reacting halibut sera.

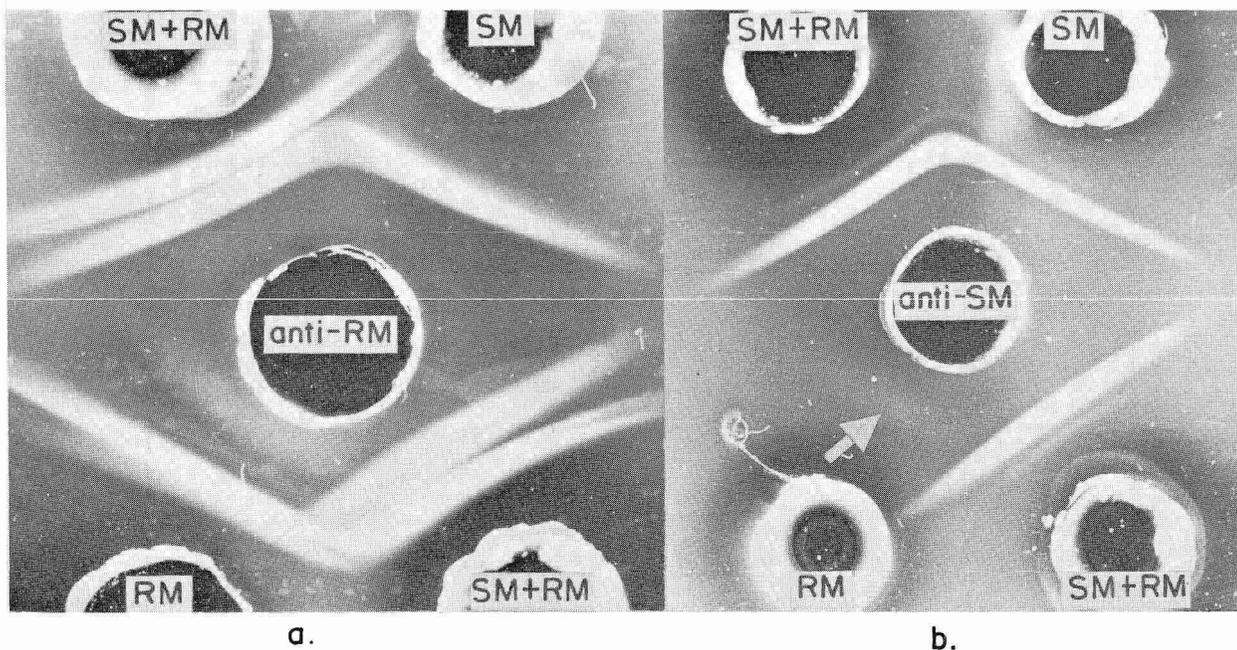


FIGURE 2.—The relation between SM and RM vitellins detected (a) by rabbit anti-RM serum and (b) by rabbit anti-SM serum. Arrow in (b) indicates the

partial cross reaction between RM vitellin and the anti-SM reagent.

#### PRACTICAL APPLICATIONS FOR STUDIES OF MATURITY

The broad cross-reactive range of antisera produced against the vitellins of rockfish and starry flounder is of practical importance. It is likely that these reagents react with serum vitellins from mature female teleosts at least through the taxonomic range of this study. An investigator wishing to include serological data in maturity studies may therefore use a single reagent throughout a range of teleost species rather than produce different antisera for relatively limited taxonomic groupings. The necessity of obtaining vitellin-bearing material for immunizations from species where such materials would be difficult to obtain or process is also eliminated.

The data suggest that vitellins of the most taxonomically advanced species stimulate the highly cross-reactive antisera. Some theoretical implications of this apparent trend are discussed below. As a practical consideration, however, it appears that vitellin from Perciform or closely allied species may be most likely to stimulate antisera which have broad cross-reactive properties.

#### SYSTEMATIC CONSIDERATIONS

Nuttall (1904) in an early immunological study, observed that the quantities of precipitates formed by specific antigen-antibody interactions decrease as the taxonomic relationships become more distant from the materials used in antibody stimulation. The present study agrees generally with this observation. As illustrated by figure 1, the broadly cross-reactive antisera reacted most strongly with the homologous vitellin and least strongly with the most distantly related cyprinid and salmonid vitellins. A notable exception is the reaction of sturgeon vitellin with anti-cyprinid vitellin, an antiserum which fails to react with vitellins of numerous, more closely related, groups. The other exceptions include the weak cross-reactions of antisalmonid vitellin with rockfish vitellin but not with herring or carp vitellin, and the similarly weak cross-reaction of anti-clupeid vitellin with rockfish vitellin but not with salmon vitellin.

Fine, Buffa, and Drillhon (1964) found a component in mature female marine lampreys analogous to the teleost vitellins described in this report. The spiny dogfish egg, unlike those

of many sharks, is provided with an abundance of yolk material. A Saline extract of the dogfish yolk material was tested in addition to the sera from numerous adult dogfish; this extract also failed to react with any of the antisera used in this study. The lack of reactivity observed here with yolk materials from females of dogfish or lampreys appears to reflect the phylogenetic gap between the teleosts and these more primitive vertebrates.

The vitellin substances of the advanced teleosts that stimulate production of the broadly cross-reactive antisera appears to be biochemically and antigenically more complex than those of the more primitive teleosts. It is evident from figure 1 and table 3 that only a small fraction of the total number of anti-RM antibodies react with SM vitellin; the major antigenic vitellin component of SM is detected, however, by the anti-RM reagent (Figure 2). Possibly the vitellin antigens of more primitive teleost species have been retained in certain advanced species without extensive modification during the evolution of additional vitellin substances.

This study further demonstrates the usefulness of serological methods to determine maturity in oviparous vertebrates. The results are also of significance in systematics. The existence of antigens in the sera of maturing females which do not occur in the sera of males and immature females must be taken into account in studies that attempt to apply serology to problems of taxonomy. These antigens themselves, as was demonstrated here, also offer additional materials for more detailed examinations of systematic relationships.

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