INTRASPECIFIC DIFFERENCES IN SERUM ANTIGENS OF RED SALMON DEMONSTRATED BY IMMUNOCHEMICAL METHODS

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ABSTRACT

The sera of most Alaskan red salmon (*Oncorhynchus nerka* Walbaum) were found to contain at least 14 antigenic components when tested by the double diffusion precipitin analysis method of Ouchterlony with antisera prepared in rabbits. Two of these components, designated antigens I and II, were missing from 116 of 126 blood samples from red salmon taken in Asian waters. In contrast, only 31 of 905 blood samples from red salmon of American origin lacked these components. Intermediate frequencies of occurrence of these antigenic components were found in blood samples from red salmon caught in the central North Pacific and Bering Sea. The results are considered indicative of the intermingling of Asian and American stocks of red salmon in these areas.

An additional antigen (or antigens) was found which occurs in the sera of only mature or maturing female red salmon.

CONTENTS

INTRODUCTION

As part of the research program of the International North Pacific Fisheries Commission, serological or immunochemical methods have been applied to the problem of distinguishing geographic races of Pacific salmon. Research directed toward discovering differences in cellular antigens has been described in two previous reports (Ridgway, Cushing, and Durall, 1958) and (Ridgway and Klontz, 1960). The application of such differences in cellular antigens to populations sampled on the high seas has been hampered because of the difficulty of obtaining, preserving, and transporting fresh whole blood from fish caught in Asian and high-seas areas without hemolyzing the cells. Consequently, an extensive search was made for intraspecific differences in the soluble antigens present in serum. It was considered that if such antigenic differences were present, they would probably be detectable in the samples of whole frozen blood available when one of the modern immunochemical methods of diffusion precipitin analysis was used.

This paper will report on research which led to the discovery of such intraspecific differences in soluble blood antigens of red salmon (*Oncorhynchus nerka* Walbaum) and which has been applied to the problem of distinguishing the continental origin of red salmon taken on the high seas.

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REVIEW OF LITERATURE

Rapid developments have been made in the past few years in the study of intraspecific differences in soluble antigens, and the inclusion here of a short review of pertinent literature is considered essential.

The extent of intraspecific antigenic heterogeneity discovered in serum components of man and other animals is considerably less than that in cellular antigens. Until recently, differences in soluble blood-

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group substances were the only intraspecific differences in soluble antigens known to occur. In man, the presence or absence of soluble substances in saliva and serum, corresponding in specificity to the major blood-group antigens of the individual, were found to be controlled by a pair of allelic genes called the secretor and nonsecretor genes (Schiff and Sasaki, 1932). Subsequently, other genetically controlled factors known as the Lewis factors were found to be involved in the specificity of these substances (Grubb and Morgan, 1949). A soluble, serologically reactive substance known as the J-substance was found to be present in the sera of some cattle (as well as on the erythrocytes of some) and its presence or absence was demonstrated to be under genetic control (Stormont, 1949; Stone and Irwin, 1954).

In addition to such differences in soluble blood-group substances, recent studies have demonstrated the existence of intraspecific heterogeneity in the serum or milk proteins of some of the better-studied domestic animals and of man. In some cases, this heterogeneity has been demonstrated serologically and, in others, through the use of electrophoresis.

The occurrence of genetically controlled differences in the beta-lactoglobulins of cow's milk has been reported by Aschaffenburg and Drewry (1955). Paper electrophoresis of the fraction of their milk soluble in 20-percent sodium sulfate showed that cows had either or both of two beta-lactoglobulins. Blumberg and Tombs (1958) compared the frequencies of the beta-lactoglobulin types of Nigerian and Icelandic cattle with those of British cattle and found the frequencies did not differ significantly among populations. These authors also found polymorphism in the alpha-lactalbumins of Nigerian cattle by paper electrophoresis. Some Nigerian cattle had a single alpha-lactalbumin with the same mobility as that in British cattle; others had two alpha-lactalbumins, one with a higher mobility than the other. British and Icelandic cattle lacked this more-mobile alpha-lactalbumin.

The existence of six electrophoretically different phenotypes in the serum beta-globulins of cattle and evidence for genetic control of these characters were described by Ashton (1957, 1959). Ashton (1958) also presented evidence for the existence of eight phenotypes in the beta-globulins of sheep. Smithies and Walker (1955) found differences in the beta-globulins of humans by starch gel electrophoresis and demonstrated the differences were genetically controlled. Sutton *et al.* (1956) demonstrated significant differences in the frequencies of these characters between Caucasians and Africans. Thompson *et al.* (1954) studied the sera of seven strains of mice and found that one contained a distinct, genetically controlled beta-globulin.

Different genetically controlled serological types of human gamma-globulin were demonstrated by Grubb and Laurell (1956), and differences in the frequencies of these types were found between the Swedes and Eskimos.

Oudin (1956), using immune isoprecipitins induced by injecting rabbit immune precipitates incorporated in Freund's adjuvant, found serological differences between the gamma-globulins of individual rabbits. The differences were demonstrated by Oudin's (1946, 1952) method of agar diffusion precipitin analysis. Dray and Young (1958, 1959) confirmed the findings of Oudin and extended the observations to differences in other antigenic components. They induced immune isoprecipitins by the injection of whole serum in adjuvants and conducted tests by agar diffusion, immuno-electrophoresis, and passive cutaneous anaphylaxis. Differences were found in components with the mobilities of alpha-, beta-, and gamma-globulins. The extent of heterogeneity was sufficient to allow separation of 90 rabbits into 30 groups. Another example of a genetically controlled intraspecific difference in serum proteins is the hereditary disease of man, congenital agamma-globulinemia, which was first reported by Bruton (1952). It is apparent that ample evidence exists for intraspecific differences among the serum antigens of the better-studied vertebrate animals.

There have been only a few previous reports of the serological distinction of lower taxonomic units of fish based on differences in serum antigens. Taliev (1941, 1946) found serological differences between races of *Coregonus autumnalis migratorius* (Georgi) and forms of wild and domesticated carp (*Cyprinus carpio* L.). Bargetzi (1958), using the agar diffusion technique of Ouchterlony, found some differences between the sera of two forms of Neuchatel Lake coregonids *Coregonus fera* Jurine and *Coregonus macrophthalmus* Nüsslin.

Intraspecific differences in the soluble antigens of micro-organisms have been known for a long time. For example, Tempel (1957) used the Ouchterlony method of agar gel diffusion to demonstrate serological differences between physiological races of *Fusarium oxysporum*. Many additional studies of intraspecific differences in the soluble antigens of micro-organisms could be cited.

METHODS

OUCHTERLONY METHOD OF DOUBLE DIFFUSION PRECIPITIN ANALYSIS IN AGAR

A modification of Ouchterlony's (1949) method of double diffusion precipitin analysis in agar has been used throughout the course of this work. Since this method is somewhat specialized, a brief and diagrammatic description of it and its theoretical basis is presented. The interested reader will find additional descriptions and discussions of this method in the papers of Ouchterlony (1949, 1953a, 1953b); Oudin (1952); Wodehouse (1956); and Feinberg (1957).

The method consists of allowing the antigen solutions and the antiserum to diffuse from small cups toward each other through a thin layer of semisolid agar medium that contains saline, buffer, and a preservative. As the components diffuse from their cups through the agar medium, opposing gradients in their concentrations are formed. Along some perpendicular line, between the antigen and antibody wells, the antigen molecules encounter their specific antibody molecules in an optimal proportion of concentrations and precipitation occurs. After a given period of diffusion, the position of this line of precipitation for each antigen-antibody system is dependent on the initial concentrations of the particular antigen in the solution being tested, the diffusion rate of the antigen, and the concentration of the specific antibody in the antiserum being used. Since each of these factors can vary between antigen-antibody systems, separate lines can be formed for each antigen-antibody system present.

The precipitation reaction is most easily understood in terms of Marrack's (1938) lattice theory of antigen-antibody reactions. According to this theory (presented diagrammatically in Figure 1) and a considerable amount of immunochemical evidence, the antigens and antibodies that participate in precipitation reactions are multivalent; that is, they possess more than one reaction site. Present evidence indicates that antibodies possess two reaction sites while antigens possess two or more, frequently many. The reaction between a soluble antigen and its specific antibody is thought to occur in two stages. The primary reaction is a rapid molecular interaction which results in the formation of relatively small complexes. The secondary reaction, which occurs when the ratio of antigen and antibody concentration is optimal, is a slow interaction of the primary complexes to form insoluble aggregates.

One of the advantages of the Ouchterlony method

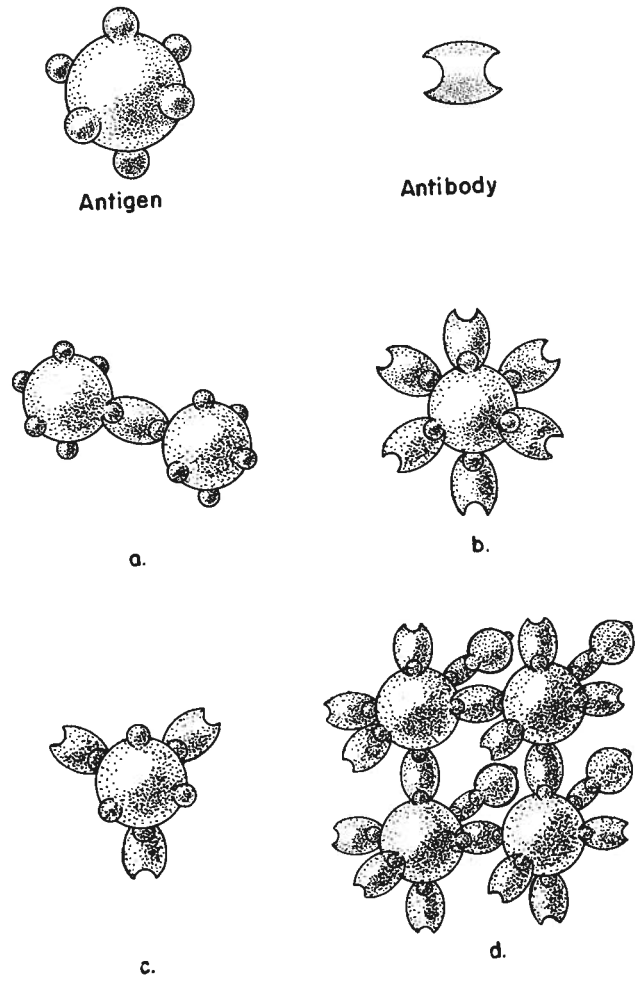

FIGURE 1. Diagrammatic representation of the lattice theory of antigen-antibody reactions. a. Soluble primary aggregate formed in excess antigen. b. Primary aggregate formed in excess antibody. c. Primary aggregate formed at zone of optimal proportions. d. Insoluble lattice formed by interaction of the primary aggregates shown in c.

is that the antigens present in different samples of known or unknown antigenic composition can be compared directly. The samples to be compared are placed in adjacent cups equidistant from the antiserum cup. The types of comparative reactions as demonstrated by Ouchterlony are illustrated in Figure 2. Identical antigens from the two samples form lines which fuse (Fig. 2a). If antigens are related but not identical the lines partially fuse resulting in the formation of a "spur" (Fig. 2b). Unrelated antigens diffusing from adjacent cups form lines which cross (Fig. 2c). Finally, if no antibodies in the antiserum are directed against the antigens in a particular sample, no lines form (Fig. 2d, right well).

these lines (antigens) were missing from all eight of the 1955 samples from the Okhotsk Sea but present in 10 of 11 samples taken at Brooks Lake on the Naknek system in 1955. These results were confirmed and extended in tests on samples taken in subsequent years. The two antigenic characters, both of which were missing from most blood samples from Asian areas and either or both of which were present in most blood samples from American areas, were designated antigens I and II. A test for these characters is illustrated in Figure 4.

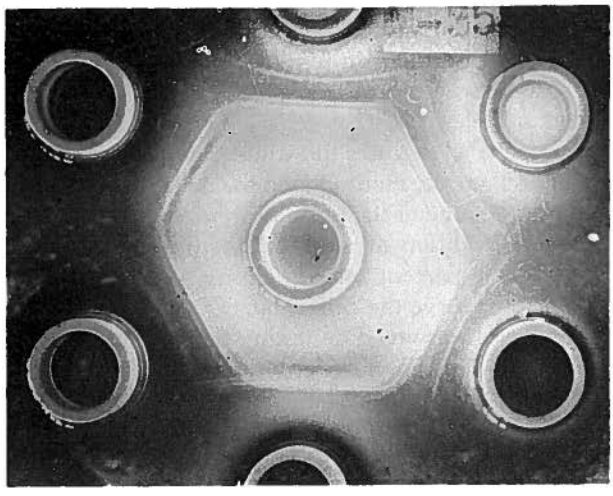

FIGURE 4. Ouchterlony plate demonstrating antigenic differences in red salmon serum samples. The outermost lines, present in positions 1, 2, 3, and 5, and missing from positions 4 and 6 (starting at the top and counting clockwise), are due to components designated antigens I and II.

In our standard testing procedure for antigens I and II, the reaction of each sample was compared with those of a known positive sample and a known negative on the same plate. Independent readings were made by two trained persons. The agreement between readings was excellent, with most antisera used. For example, 294 tests were run on one antiserum with only 8 differences (2.7 percent) between the independent readings of two individual readers. Another antiserum was used to make 462 tests with only 18 differences (3.9 percent) between the independent readings. Samples scored differently by two individual readers were observed further and retested until complete agreement was obtained. If an antiserum did not give sufficiently clear-cut reactions for good agreement between individual readers, the tests were repeated with another antiserum. For example, 390 tests were made with one antiserum with 39 differences (10 percent) in the two independent readings. As a consequence, the tests were repeated with another antiserum, which gave consistent results.

We found the presence of antigens I and II to be independent of sex. Some evidence is available that indicates these antigenic characters are independent of sexual maturation, diet, and environment. The characters were present in 14 of 15 immature 3-year-old red salmon (*O. nerka*) from the Columbia River, reared in captivity and fed standard hatchery production diets. This frequency is consistent with that found in maturing red salmon migrating up the Columbia River.

TABLE 1. Incidence of antigens I and II in blood samples of red salmon taken in American areas, 1955–58.

Year and area	Number lacking antigens I and II	Number with antigens I or II
1955 and 1956:		
Bristol Bay and Kuskokwim	4	82
Karluk	1	27
Cook Inlet	0	18
Ketchikan	0	7
Rivers Inlet	1	9
Skeena River	0	9
Fraser River (Cultus Lake)	1	9
Columbia River	3	11
Subtotal	10	172
1957:		
Kuskokwim	0	28
Bristol Bay	2	82
King Cove	0	10
Karluk and Red River	0	50
Cook Inlet	3	17
Prince William Sound	1	16
Southeastern Alaska	0	40
Skeena River	0	4
Fraser River	0	17
Quinault River	2	8
Columbia River	4	56
Subtotal	12	328
1958:		
Salmon Lake (Nome)	2	23
Bristol Bay	0	192
King Cove	0	25
Karluk	4	68
Cook Inlet	0	25
Prince William Sound	2	12
Southeastern Alaska	1	70
Fraser River	0	9
Subtotal	9	424
Total	31	924
Percent	3.2	96.8

Antigens I and II are lost from the serum of salmon sometime between 1 week before spawning and death, which is not surprising considering the marked physiological degeneration Pacific salmon undergo at this time. These antigenic characters are stable in frozen blood samples held up to 18 months at temperatures of −12° to −35°C. Samples held for longer periods show increasingly weaker reactions when tested for antigens I and II.

INCIDENCE OF ANTIGENS I AND II IN BLOOD SAMPLES FROM AMERICAN AND ASIAN AREAS

The results of tests for antigens I and II on samples obtained from American and Asian areas from 1955 through 1958 are presented in Tables 1 and 2. The American samples are divided into two groups: one from areas north of the Fraser River, the other from the Fraser River and areas south. Within these groups the frequency of individuals possessing antigens I or II or both did not differ significantly between years.

TABLE 2. Incidence of antigens I and II in blood samples of red salmon taken in Asian areas, 1955–58.

Year and area	Date	Number lacking antigens I and II	Number with antigens I or II
1955:			
Okhotsk Sea:			
53°32′ N., 155°03′ E	July 22	8	0
1956:			
Okhotsk Sea:			
51°09′ N., 154°17′ E	July 15	3	0
51°24′ N., 153°53′ E	16	5	0
51°09′ N., 153°19′ E	16	7	0
51°19′ N., 152°40′ E	23	8	0
Subtotal		23	0
1957:			
Northwest Pacific:			
49°58′ N., 159°59′ E	July 5	10	0
50°52′ N., 158°21′ E	13	11	2
Subtotal		21	2
1958:			
Okhotsk Sea:			
51°09′ N., 153°40′ E	June 25	9	2
50°55′ N., 153°15′ E	July 17	14	2
East Kamchatka:			
58°52.5′ N., 167°15′ E	June 19	12	2
58°56′ N., 166°57′ E	20	9	2
59°18′ N., 170°38′ E	July 31	20	0
Subtotal		64	8
Total		116	10
Percent		92.1	7.9

The northern group consisted of 835 individuals, 814 or 97.5 percent of which possessed antigens I or II or both. The southern group consisted of 120 individuals, 110 or 91.7 percent of which possessed antigens I or II or both. This difference, although small, is significant (chi square 11.30, with 1 degree of freedom).

The differences in frequency of occurrence of anti-

TABLE 3. Incidence of antigens I and II in blood samples of red salmon taken on United States research vessels, June and July 1957.

Vessel and set	Date	Location		Number with antigens I or II	Number in sample
		Latitude	Longitude		
GROUP A:					
Pioneer:					
1	June 10	50° N.	175° E.	12	20
2	11	50° N.	175° E.	6	18
3	12	50° N.	175° E.	11	17
4	14	53° N.	175° E.	12	22
5	20	53° N.	175° E.	7	10
6	22	53° N.	180°	4	9
Attu:					
4	June 8	53° N.	175° W.	19	36
7	15	56° N.	175° W.	20	31
Pioneer:					
7	June 29	56° N.	175° W.	1	5
8	30	56° N.	175° W.	4	11
9	July 3	53° N.	175° W.	1	1
10	4	53° N.	175° W.	1	4
11	6	50° N.	175° W.	0	1
13	8	51° N.	176° W.	11	18
Total				110	202
Percent				54.5	
GROUP B:					
Pioneer:					
14	July 16	56° N.	175° E.	2	4
15	19	53° N.	175° E.	3	3
17	22	50° N.	175° E.	3	3
18	23	52° N.	175° E.	2	2
19	24	53° N.	175° E.	1	1
20	25	54° N.	175° E.	1	1
21	26	55° N.	175° E.	1	1
22	27	56° N.	175° E.	1	1
23	27	56° N.	175° E.	1	1
24	28	56° N.	175° E.	3	3
Paragon:					
2	July 22	50° N.	175° W.	2	5
6	27	56° N.	175° W.	1	1
7	28	56° N.	175° W.	1	1
8	30	53° N.	175° W.	1	1
Total				23	28
Percent				82	

TABLE 4. Incidence of antigens I and II in blood samples of red salmon taken by United States research vessels, August 1957.

Vessel and set	Date	Location		Number with antigens I or II	Number in sample
		Latitude	Longitude		
GROUP C:					
Pioneer:					
28	Aug. 11	53° N.	175° E.	2	5
29	12	53° N.	175° E.	3	7
30	13	54°13′ N.	175° E.	0	2
31	14	56° N.	175° E.	4	5
32	17	53° N.	175° E.	6	9
33	18	53° N.	175° E.	4	8
36	23	51°30′ N.	175° E.	5	9
37	24	53° N.	175° E.	5	6
Paragon:					
11	Aug. 6	51°30′ N.	175° W.	7	12
12	7	51° N.	175° W.	14	26
13	8	50° N.	175° W.	10	27
14	9	50° N.	175° W.	14	38
15	12	53° N.	175° W.	2	2
19	17	53° N.	175° W.	0	1
20	18	53° N.	175° W.	1	1
21	20	50° N.	175° W.	6	17
22	21	50° N.	175° W.	12	20
23	22	51° N.	175° W.	7	21
24	23	51°30′ N.	175° W.	4	6
Total				106	222
Percent				47.7	

gens I or II or both among the various Asian samples are not significant. Among 126 Asian red salmon samples only 10 or 7.9 percent possessed these characters. Since we have not yet been able to develop an antiserum that will readily distinguish between these two antigens in every case, we report the presence of either or both of them as positive and their complete absence as negative.

INCIDENCE OF ANTIGENS I AND II IN BLOOD SAMPLES FROM HIGH-SEAS AREAS

In 1956, nine red salmon blood samples were collected in the eastern Bering Sea (54°–60° N., 160°–170° W.) by the biologist on the chartered vessel MV *Tordenskjold*. Eight of these samples were positive for antigens I or II or both and thus similar to our American standards from shore stations in that area.

In 1957, many blood samples were taken by the American biologists on the motor vessels chartered for research in the North Pacific and Bering Sea. All samples taken on the MV *Attu* after June 15 were found to be unsuitable because of failure of the refrigeration system; consequently, high-seas samples were available only from the area bounded by 175° W., 175° E., 50° N., and 55° N. These samples are divided into three groups, A, B, and C, according to time of collection. These groups are also characterized by the proportion of mature red salmon in the catch and the number of red salmon caught. The results are presented in Tables 3 and 4.

Most red salmon sampled in groups A and B were mature, but the majority of the fish sampled in group C were immature (from evidence of age and gonadal development). The catches of red salmon were good in groups A and C but poor in group B.[1]

Within each of these groups the proportion of individuals possessing antigens I or II or both does not differ significantly among sets or with longitude. The proportion of individuals possessing these characters does differ significantly between groups (chi square 13.9, with 2 degrees of freedom), indicating considerable variability with time.

Since the proportion of individuals possessing antigens I or II or both did not differ significantly within each group, the samples were combined to obtain estimates of the proportion of red salmon possessing these characters within the space and time boundaries sampled. In group A, consisting of samples taken between June 8 and July 8, were 202 individuals, of which 110 or 54.5 percent possessed antigens I or II or both. In group B, consisting of samples taken from July 16 to July 30, were 28 individuals, of which 23 or 82 percent possessed these antigenic characters. In group C, consisting of samples taken from August 6 to August 23, were 222 individuals, of which 106 or 47.7 percent possessed antigens I or II or both.

Because of the high incidence of antigens I and II in red salmon samples of American origin and a correspondingly low incidence in samples of Asian origin, estimates of the incidence of these two antigens in populations sampled on the high seas are also estimates of the proportion of red salmon of American origin present in the area during the periods of sampling. These estimates could be corrected for the amount of overlap; however, the correction does not greatly change the value of the estimates and seems rather extravagant considering the number of individual samples available from the various sets. It would thus appear from the data in Tables 3 and 4 that there was considerable intermingling of American and Asian stocks of red salmon in the high-seas areas

[1] Catch data are available in the Annual Report for 1957 of the International North Pacific Fisheries Commission (1958).

TABLE 5. Incidence of antigens I and II, in blood samples of red salmon taken by United States and Japanese research vessels, late May, June and July, 1958.

Vessel and set	Date	Location		Number with antigens I or II	Number in sample
		Latitude	Longitude		
Pioneer:					
2	May 28	53°00′ N.	178°15′ E.	2	6
3	30	55° N.	174°00′ E.	3	5
4	31	54° N.	173°00′ E.	6	12
5	June 1	53° N.	172°00′ E.	2	4
Attu: 5	June 7	53°07′ N.	175°02′ E.	0	1
Pioneer: 6	8	51°35′ N.	173° E.	5	6
Attu:					
6	June 9	54° N.	178° W.	0	1
7	16	53°30′ N.	170°02′ W.	1	8
Pioneer: 8	June 18	54° N.	180°	1	3
Wakashio Maru:					
No data	June 18	56° N.	172°30′ W.	11	16
No data	19	56° N.	172°50′ W.	4	6
Attu:					
10	June 24	55°04′ N.	169°24′ W.	7	16
11	25	55°58′ N.	170°04′ W.	2	4
Pioneer:					
11	July 6	51°17′ N.	175°53′ W.	7	13
12	7	50°30′ N.	175° W.	1	2
13	8	49°30′ N.	175° W.	1	1
Attu: 18	July 11	52°04′ N.	178°38′ W.	3	4
Pioneer:					
15	July 12	50°59′ N.	178°50′ W.	7	7
16	13	50°18′ N.	177°28′ E.	4	5
Attu:					
20	July 13	53°31′ N.	180°	0	3
21	14	54° N.	178° E.	2	3
Pioneer:					
17	July 14	49°39′ N.	176°11′ E.	1	1
18	15	49° N.	175° E.	5	7
19	16	50° N.	175° E.	1	4
20	17	51° N.	175° E.	2	3
21	18	52° N.	175° E.	2	3
24	21	52°07′ N.	176°55′ E.	1	1
Attu:					
23	July 16	54° N.	175° E.	1	1
24	17	55° N.	175° E.	0	2
27	20	54° N.	174°58′ E.	0	2
Pioneer: 29	July 27	52°58′ N.	175°13′ W.	0	2
Total				82	152
Percent				53.9	

TABLE 6. Incidence of antigens I and II in blood samples of red salmon taken by United States and Japanese research vessels, August 1958.

Vessel and set	Date	Location		Numder with antigens I or II	Number in sample
		Latitude	Longitude		
Pioneer:					
31	Aug. 5	51°30′ N.	175° W.	8	16
32	6	50° N.	175° W.	13	23
33	7	49° N.	175° W.	4	10
34	9	49° N.	170° W.	3	4
35	10	50° N.	170° W.	3	9
36	11	51° N.	170° W.	1	2
Attu:					
33	Aug. 16	52° N.	165° W.	7	11
34	17	51° N.	165° W.	2	8
35	18	50° N.	165° W.	7	10
Total				48	93
Percent				52	
Wakashio Maru	Aug. 7	55° N.	164° E.	6	25
Percent				24	

sampled in 1957.

The precision of the estimates presented is dependent on several factors. Among these factors are the representativeness of the samples obtained for the actual populations of red salmon present in the area during the sampling periods and the representativeness of the Asian and American reference samples for the actual populations involved in the high-seas areas. Since it may not be practical or even possible to evaluate these factors directly, the research program of the International North Pacific Fisheries Commission is designed to allow the testing of such estimates by several independent methods. These methods include tagging and the study of morphological and scale characteristics. We expect that in the future additional, as yet undiscovered, serological characters will also be available for use.

Because of bad weather and lower availability of red salmon, fewer blood samples were taken by American research vessels in 1958 than in 1957. The results of tests for antigens I and II on the 1958 blood samples taken in the North Pacific and Bering Sea areas by United States and Japanese research vessels are presented in Tables 5 and 6. Of 152 samples taken in late May, June, and July, 82 or 53.9 percent possessed antigens I or II or both (Table 5). Of 93 samples taken from immature red salmon in August south of the Aleutian Islands from 175° W. to 165° W. and from 49° N. to 52° N., 48 or 52 percent possessed antigens I or II or both (Table 6).

The proportion of individuals possessing these antigenic characters does not differ significantly among sets nor among groups when the samples are grouped in several ways according to time or longitude. This lack of difference may be due to the small numbers

of individuals sampled in each set and thus be more apparent than real.

Blood samples were taken from a group of 22 red salmon on the Japanese research vessel *Wakashio Maru* in the Bering Sea. Sixteen of the fish were obtained on June 18, at 56° N., 172°30′ W., and an additional six were obtained on June 19 at 56° N., 172°50′ W. Of these 22 samples, 15 or 68 percent were positive for antigens I and II. Although the difference is not significant, the incidence is somewhat higher than the average in the samples taken by American vessels (Table 3) and is indicative of a possible concentration of salmon of American origin in the area sampled.

Most of the red salmon from which the 25 blood samples were taken on the Japanese research vessel *Wakashio Maru* at 55°N., 164°E. on August 7, 1958, were immature. Because of the lateness of the season and the immaturity of the fish, this sample cannot be considered an Asian standard. Of these 25 fish, 6 or 24 percent were positive for antigens I or II or both, a significantly higher incidence than in the other Asian samples (chi square 4.14, corrected for continuity 1 d.f.). Among the possible explanations for the higher incidence are the following:

First, the sample may be one of 5 out of 100 which would not fall within the 95 percent confidence intervals. Secondly, the fish may represent some, as yet unsampled, Asian area which has a higher incidence of antigens I or II or both than those sampled. A third possibility, which is of considerable importance in the Protocol problem of the International North Pacific Fisheries Commission, is that immature red salmon from American areas may migrate in measurable numbers as far west as 165°E. late in the season. Data presently available do not exclude this possibility.

SEX-AND-MATURITY-CORRELATED ANTIGEN IN SERA OF FEMALES

In addition to the area-correlated differences in the serum components of red salmon, an antigenic component probably unique to mature or maturing females was found in their sera (Fig. 5). This character was present in the serum of only 6 males of 504 checked. These exceptions were probably due to errors in labeling the samples. Most samples possessing this character were females with gonads greater than 10 grams in weight. This antigenic character was also present in high concentration in the eggs of salmon. Similar components have been demonstrated in the sera of laying hens (Sasaki, 1932) and mature female carp (Uhlenhuth and Kodama, 1914). The characters involved have been extensively studied in chickens and found to be phosphoproteins of the vitelline fraction, synthesized in the liver under hormonal control (Vanstone, Maw, and Common, 1955).

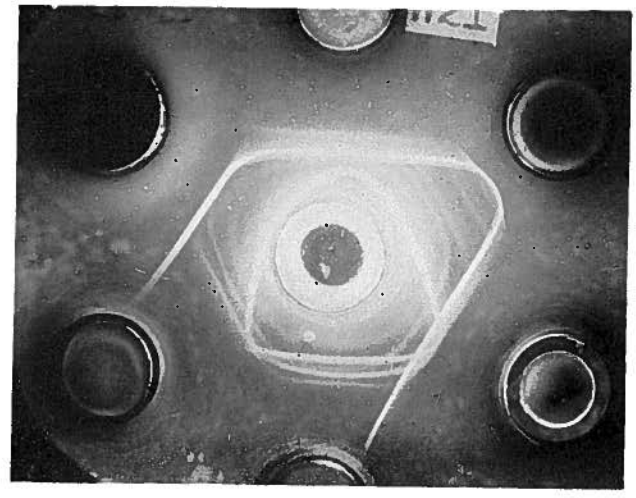

FIGURE 5. Ouchterlony plate demonstrating antigenic differences in red salmon serum samples. The line present in positions 1, 2, 3, and 6 and missing from positions 4 and 5 (starting at top and counting clockwise) is due to antigenic components found in the sera of mature or maturing female salmon and in their eggs.

In salmon, this antigenic character was detectable in samples taken in early June from 4-year-old Columbia River red salmon reared in captivity that would not spawn until October. Only a small drop of blood is required for determining its presence. Its applicability in determining whether fish caught on the high seas are maturing is under investigation.

DISCUSSION

The results of the research reported in this paper demonstrate the utility of specialized serological or immunochemical methods in some of the most important problems of fishery biology, racial identification, and sexual maturation. The methods described are also being applied to species identification problems and work is progressing on the use of other specialized serological methods in racial or subpopulation identification problems. Especially important are the blood-grouping methods discussed in two previous papers (Ridgway, Cushing, and Durall, 1958; Ridgway and Klontz, 1960) from this laboratory and in the works of Hildemann (1956), Cushing and Durall (1957), and Sindermann and Mairs (1959). The accumulation of basic knowledge about blood groups and other serological characteristics of fish is, unfortunately, a

slow process, as only a relatively small number of investigators are studying the commercially important groups of fish from this standpoint.

The methods presently available require fresh or fresh-frozen samples. When methods of preservation more convenient for field use are developed, blood grouping and other immunochemical methods should find increasingly practical application in modern fishery biology.

The preparation of sufficient antisera with the necessary specificity was one of the most important problems in the present study. Several methods of preparing antisera with increased potency and specificity are presently being studied. An important consideration in producing antisera to detect racial differences in the serum antigens of fishes is to use only the sera of males for injection. If sera of mature females are used for injection, antibodies are produced against the antigenic components present only in the serum of mature females. Reactions with these components complicate the tests and may confuse or mask racially correlated differences.

The best antisera produced in the present study for the detection of differences between American and Asian red salmon were obtained by injecting sera of red salmon from areas in the southern part of this species' range in America (Cultus Lake and Adams River). These antisera would react with antigen I and II components of northern American samples but not with any corresponding components in Asian samples. When antisera were prepared with serum from red salmon of Bristol Bay origin, considerable cross-reactivity with corresponding components in the Asian samples was obtained. Probably a complex series of overlapping specificities is involved, analogous to that found in the A-B-H-*Lewis* soluble blood-group substance of humans (Brown, Glynn, and Holborow, 1959).

If methods can be perfected for the production of sufficiently potent and specific antisera, studies into the chemical nature of the compounds involved and the mechanism of their inheritance are possible.

Concerning the nature or cause of the difference between individual red salmon samples possessing antigens I or II and those lacking them, the most likely possibility, and one leading to useful information concerning the continental origin of samples taken on the high seas, is that the differences are due to differences in the genetic constitution of the individual red salmon. The much higher incidence of the characters in blood samples from red salmon of American origin than in those from red salmon of Asian origin would reflect a much higher frequency of the determining gene or genes in the American populations. Such differences in gene frequencies between reproductively isolated populations have been demonstrated in many animal species (Dobzhansky, 1951) and would be expected to result from the reproductive isolation of salmon populations imposed by the precision of their home-stream migrations (Scheer, 1939), their adaptation to their area of origin (Davidson and Hutchinson, 1938), and the precise chronology of the spawning migrations of each race (Killick, 1955).

The possibility that antigens I and II are two relatively unstable antigens lost from some of the samples because of selective deterioration, brought about by some difference in the sampling methods or conditions, seems unlikely because individual samples taken in the same area and under conditions as nearly identical as possible differ in their antigen-I and -II reactions. Also, improper preservation of samples is usually detectable in the agar-diffusion test because of a general diffuse reaction, with lines due to antigens other than I and II missing or weak.

The problem of representativeness of samples has been discussed briefly under Results. Studies of this nature are, of course, dependent on adequate and representative sampling; and adequate, representative sampling of the races of salmon in the North Pacific and Bering Sea is difficult.

It should also be pointed out that a substantial proportion of the red salmon samples taken east of 180° in the Bering Sea were scored negative for antigens I and II. This is inconsistent with the general belief that nearly all such fish are of American origin and probably indicates that some complicating factors are resulting in false negative tests for these antigens. The nature of these factors is under investigation.

For these reasons, estimates made in this paper should be considered preliminary and are only estimates of average values for rather extensive areas and time intervals.

SUMMARY

The sera of most Alaskan red salmon contained at least 14 antigenic components when tested with rabbit antisera by the double diffusion precipitin analysis method of Ouchterlony.

Two antigenic serum components, designated antigens I and II were missing from 116 of the 126 blood samples of red salmon collected in Asian areas in