NATURAL ANTIBODIES IN THE BLOOD SERUM OF FRESH-WATER FISH

By K. A. BISSET, *Department of Bacteriology, University of Birmingham*

INTRODUCTION

The opinion that infections of aquatic animals may be caused by saprophytic water bacteria has frequently been expressed. Some of the relevant literature has been reviewed by the author (Bisset, 1947*a*). It has been shown that the degree of infection produced by these bacteria in fresh-water fish decreases as the temperature rises and increases as it falls (Bisset, 1946, 1948*a*), and it has been suggested that this phenomenon is due to changes in the immune response of the fish at different temperatures. Laboratory experiments have shown that antibody production in amphibia is inhibited at low temperatures (Bisset, 1947*b*, 1948*b*).

Although some evidence has been offered, complete proof of the identity of the parasitic bacteria with the water bacteria, which they resemble closely, has not been established, and the present work is intended to indicate the presence, in fish and frogs, of serum agglutinins specific for the water bacteria of their environment, as well as for the bacteria derived from their own body cavities. It is also intended to show that the seasonal variation in antibody titre, which was postulated to explain the observed changes in intensity of bacterial parasitism, does in fact occur under natural conditions.

TECHNIQUE

Perch (*Perca fluviatilis*), trapped in Whitacre reservoir, were brought alive to the laboratory in fry tanks, and were examined without delay. The batches varied from twelve to thirty fish, and were taken at weekly intervals.

At first it was attempted to measure the antibody content of the fluid expressed from the minced muscles of the fish, but this was found to precipitate spontaneously at room temperature, producing a heavy, flocculent deposit. It was, however, found possible to obtain blood from the pericardial cavity. The fish was stunned by a blow on the head, and the pericardial cavity was opened while the heart continued to beat. The ventricle was punctured, and blood pipetted from the cavity, where it collected quite rapidly. If the heart failed too soon, a small amount of blood could be obtained from the sinus venosus, which became dilated. The blood cells separated rapidly and a clear serum was obtained.

Frogs were bled by decapitation.

OBSERVATIONS

The first batches of fish were obtained towards the end of May. An antigen was prepared of a killed suspension of bacteria cultured from the peritoneal cavities of the same batch of fish. A similar suspension of bacteria from their opercular cavities was also prepared. The bacteria from the two sites appeared identical, as had previously been observed (Bisset, 1948*a*), and the pooled sera of the fish agglutinated with both suspensions to a titre of 1 : 10.

The sera of subsequent batches of fish were tested against these suspensions and against similar ones prepared from themselves. The pooled sera of fish obtained in the middle of June gave agglutination at 1 : 25 against all these antigens, and by the beginning of July the titre had risen to 1 : 60. The sera of the latter batches of fish were also tested against mixed suspensions of cultures of bacteria derived from the water of the reservoir, with which they gave agglutination to the same titre.

Sera from one early and one late batch were tested against suspensions of *Salmonella typhi* and *S. paratyphi* A, which they failed to agglutinate.

From late May to early July the water temperature had remained fairly constant, at about 15° C., which is thus shown to be above the critical temperature for antibody production. To verify this point a batch of twelve fish was taken, of which six were examined upon arrival in the laboratory, and six were placed in an aerated tank at 20° C. and examined after 3 days. The agglutinin titre of the six which had been examined on arrival was 1 : 25, which was the normal at the date of the experiment; the water temperature was 15° C. The titre of the six fish which had been kept in the tank was also 1 : 25. It may be deduced, therefore, that the progressive rises in titre which occurred in the course of the summer were due mainly to the length of time which the fish had spent at a temperature high enough for progressive development of immune response. The low titre of agglutinins found at the beginning of the warm season was presumably due to the effect of the low winter temperatures (Bisset,

https://doi.org/10.1017/S0022172400036408 Published online by Cambridge University Press

1948*b*). In February the temperature of the reservoir was as low as 3° C.

The bacteria isolated from both fish and water were typical water bacteria. They included Gram-negative bacilli, Gram-positive cocci and non-sporing Gram-positive bacilli, all of low fermentative power.

Confirmatory observations were made upon twelve frogs which had been kept for about a fortnight in an open-air tank containing 1 in. of water which had deliberately been permitted to become dirty, and was heavily contaminated with fluorescent bacteria. A few drops of peritoneal fluid, aspirated from each frog with a sterile hypodermic syringe, were inoculated upon agar, and produced a heavy growth of bacteria, culturally similar to those from the water, in every case. This was a much higher incidence of infection than would have been expected in frogs which had been kept in clean water (Bisset, 1947*b*, 1948*a*).

The sera of these frogs agglutinated at 1 : 15 with suspensions of bacteria isolated from the peritoneal fluid and from the water, but gave no agglutination with *Salmonella typhi* or *S. paratyphi* A.

CONCLUSIONS

The presence in fresh-water fish and frogs of serum agglutinins specific for the bacterial parasites of these animals and also for the water bacteria, which the parasitic forms so closely resemble, provides additional evidence that the two are, in fact, identical, and that the water bacteria are capable of invading the tissues of aquatic animals.

The rise in agglutinin titre, during the first part of the summer, serves to verify the author's previous conclusions, that the decrease in bacterial parasitism which occurs during the summer months is due to an increase in the immune reactions of the fish (Bisset, 1948*a*).

SUMMARY

1. Fresh-water fish possess serum agglutinins specific for the bacteria which parasitize their peritoneal and opercular cavities, and also for the bacteria inhabiting the water in which they live.

2. The titre of these agglutinins is low in spring and rises during the summer months, in a manner which corresponds to the summer decrease in infection.

3. Similar agglutinins are found in frogs.

4. These observations provide additional evidence that water bacteria are capable of parasitizing aquatic animals.

REFERENCES

Bisset, K. A. (1946). The effect of temperature on non-specific infections of fish. *J. Path. Bact.* **58**, 251.

Bisset, K. A. (1947*a*). Bacterial infection and immunity in lower vertebrates and invertebrates. *J. Hyg., Camb.*, **45**, 128.

Bisset, K. A. (1947*b*). The effect of temperature on immunity in amphibia. *J. Path. Bact.* **59**, 301.

Bisset, K. A. (1948*a*). Seasonal changes in the normal bacterial flora of fresh-water fish. *J. Hyg., Camb.*, **46**, 94.

Bisset, K. A. (1948*b*). The effect of temperature upon antibody production in cold-blooded vertebrates. *J. Path. Bact.* **60**, 87.

(*MS. received for publication* 15. VII. 48.—Ed.)