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Temperature Effect on Antibody Production and Immunological Memory, in Carp (*Cyprinus carpio*) Immunized Against Bovine Serum Albumin (BSA)

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Summary. A group of carp were immunized with bovine serum albumin. The fish, kept at low temperature (12°) after initially being kept at high temperature (25°) for a short period following the first antigen stimulation, showed a rising titre of antibody. In contrast no circulating antibody was found in carp kept at 12°. This rising titre occurred whether they were transferred to low temperature before or after appearance of first circulating antibodies. The anamnestic response which occurred in carp kept at high temperature may also take place, under certain conditions, at low temperature.

INTRODUCTION

Antibody production by carp immunized with soluble antigen is inhibited when they are kept at environmental temperatures lower than 14° (Fijan and Cvetnic, 1964, 1966; Avtalion, 1968). The first circulating antibodies to bovine serum albumin (BSA), as detected by Avtalion (1968) using the passive haemagglutination method, occurred on the 10th day after immunization. When immunized carp were transferred from an optimal temperature of 25° to 14°, on the 8th day after immunization and before appearance of circulating antibodies, they were able to produce antibodies at these relatively low temperatures. Consequently, we suggested that production of antibodies at low temperature would be possible if fish were previously in natural contact with antigens, or exposed experimentally, to these antigens, at high ambient temperature (Avtalion, 1968). This suggestion is confirmed in the present investigation.

MATERIALS AND METHODS

Carp of 250–300 g weight were used. They were all immunized in the same manner by injections into the caudal muscle of 0·4-ml quantities of an emulsion of Freund's complete adjuvant containing 10 mg BSA (Bovine Plasma Albumin Fraction V, Armour Pharmaceutical Co. Ltd, Eastbourne, England). This schedule of immunization was the most efficient (Avtalion, unpublished. The carp were kept in 750-litre tanks, with a continuous flow of water and air. The water temperature was kept constant at 25°$\pm$1° or at 12°$\pm$1°. Each fish received 5 g of dry fish food containing 15 per cent protein twice a week. The fish were bled by cardiac puncture. The sera were diluted 1 : 8 or 1 : 16 in physiological saline

and immediately tested for the presence of anti-BSA antibodies by passive haemagglutination (Stavitsky, 1964). A known positive carp serum was included as control with each test run. The sera were then stored individually at −20°. The sera of each fish were retested at the end of the experiment.

The fish were divided into four groups, three of five carp each and one of twelve carp. Each fish was individually marked.

RESULTS

The first group was continually kept at 25° and the second group at 12°. The third and fourth group were kept at 25° when injected with the antigen. They were transferred to 12°; the third group at the 8th day after the antigenic stimulation and before appearance of detectable antibodies, the fourth group at the 15th day, at which time antibody synthesis had already begun. From the first group three fish survived until the 254th day after the first injection at which time no antibodies to BSA were detected in their blood. They were then given a secondary stimulation with 5 mg BSA in Freund's complete adjuvant. We noted in this case that antibody production began immediately after the injection, and that the maximal antibody titre obtained was higher than in the primary response (Fig. 1).

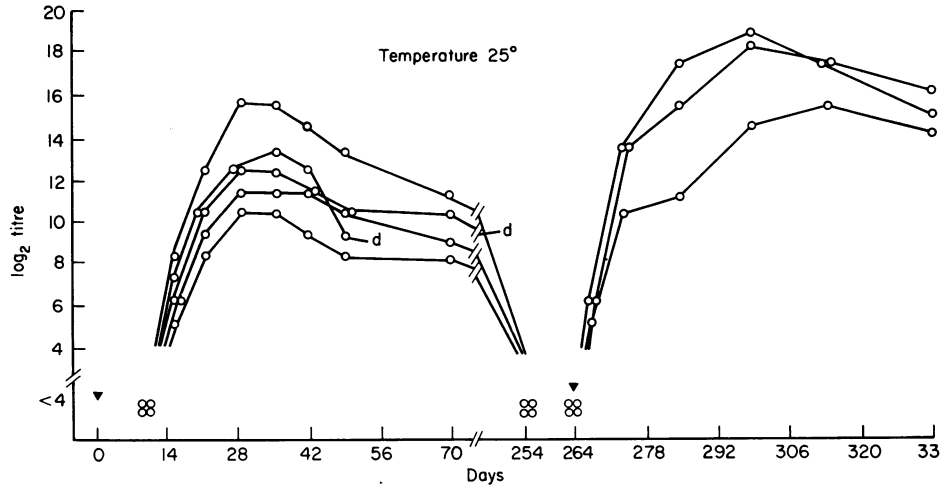

FIG. 1. Primary and secondary responses to bovine serum albumin in carp continually kept at 25°. d, Dead; ▼, antigenic stimulation; ○, antibody titre of individual carp as detected by the passive haemagglutination method.

The second group, which was kept continually at 12°, served as control group. No antibodies to BSA were detected in carp kept at this temperature. The third group, which was kept at 25° for 8 days and then at 12°, showed a rising titre of antibody at this relatively low temperature. One hundred and four days later, only three fish survived in this group. After the secondary stimulation, there was a decrease in antibody titre, probably due to the formation *in vivo* of antigen–antibody complexes. This was followed by an anamnestic response with rising antibody titres (Fig. 2).

The last group, which was kept at 25° for 15 days and then placed at 12° after appearance of first circulating antibodies, showed a rising titre of antibody despite the low temperature (Fig. 3).

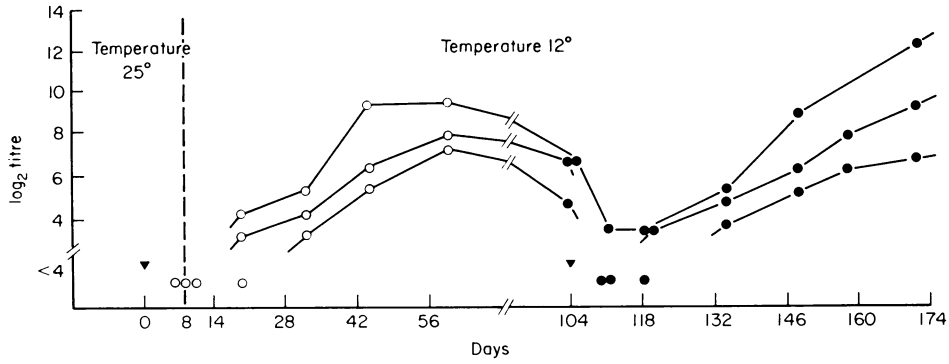

FIG. 2. Carp kept at 25° for 8 days and then transferred, before appearance of first circulating antibodies, to 12°. ▼, Antigenic stimulation; ○, antibody titre of pooled sera of four fish as detected by the passive haemagglutination method; ●, antibody titre of the individual surviving carp.

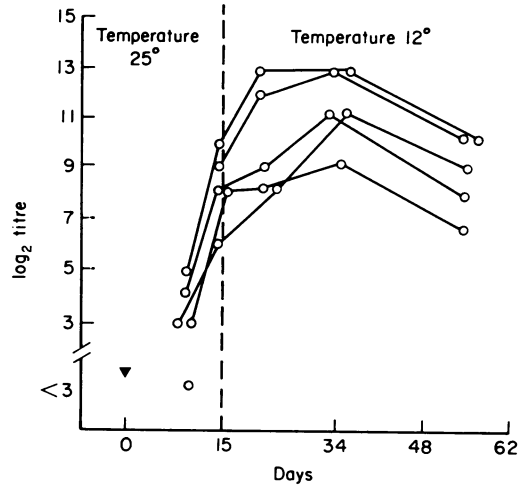

FIG. 3. Carp transferred from 25° to 12° after beginning of antibody synthesis. ▼, Antigenic stimulation; ○, antibody titre of individual carp as detected by haemagglutination method.

DISCUSSION

Our suggestion that antibody production could take place at low ambient temperature in fish which were previously exposed to antigens at high temperature is confirmed by the present results.

This suggestion has two implications: (1) that fish are able under certain conditions to respond immunologically at low temperature; and (2) that fish possess immunological memory.

It is known that immune response is depressed in the lower vertebrates in the cold season of the year and when they are experimentally kept at low temperature (Hildemann,

1962; Good and Papermaster, 1964; Ridgway, Hodgins, and Klontz, 1965). Inhibition of antibody synthesis in carp immunized with soluble antigen occur when they were kept at 14° (Avtalion, 1968) or at 12° as shown in the present work. A similar finding was reported by Fijan and Cvetnic (1964, 1966) in carp immunized with calf serum.

However, we found that carp kept at low temperature after initially being kept at high temperature for a short period following the first antigenic stimulation, with soluble BSA, showed a rising titre of antibody. This occurred whether they were moved to low temperature before (Fig. 2) or after (Fig. 3) appearance of first circulating antibodies. This also seems to be true for bacterial antigens. Gee and Smith (1941) have reported that carp immunized against bacteria and held at 20–23° for a period of 7 weeks after first antigenic stimulation, continued to produce rising titres of specific antibodies even after being transferred to 10°.

It is noteworthy that these facts are in contradiction to Bisset's hypothesis (1948). Bisset, working primarily on frogs, suggested that at low temperatures it is the release of antibodies into the blood rather than their synthesis which is inhibited.

Bisset found that when frogs were transferred from high temperature (20°) to low temperature (8°) their antibody titre was at once reduced and restored when returned to 20°. Although most of Bisset's experiments were with frogs, he notes the following: 'Experiments suggest that similar phenomena occur in nature in fresh-water fish'. Finally he concluded that in '*cold-blooded animals* the second stage of antibody formation (namely production of antibodies and their release in the circulation) is the more affected by temperature.'

Preliminary experiments in our laboratory indicate that even with frogs release of antibodies at cold temperatures does occur. However, further studies are necessary before we can come to a definite conclusion. At any rate, the experimental conditions in Bisset's and in our studies are considerably different and could explain the difference in results.

What is clear from our present work, and from that of Gee and Smith is that both synthesis and release of antibodies can take place in carp at low temperature.

The second implication was that fish which had acquired immunity against antigens would be capable of anamnestic immune response, for a long period of time, at both high and low temperatures.

The ability of fish to display anamnestic immune response is related to their phylogenic development stage (Good, Finstad, Pollara and Gabrielsen, 1965). Clem and Sigel (1965) have shown that anamnestic response exists in the marine teleost Margates, *Haemulon album* and Snappers *Lutjanus griseus*, although they lack the 2-mercaptoethanol resistant immunoglobulins of the IgG type. These immunoglobulins were reported in carp by Finstad and Good (1965). In the present work, as illustrated in Figs. 1 and 2, carp immunized with soluble BSA gave a secondary response at high and, under certain conditions, at low temperature. Consequently we may add to our above conclusion that immunological memory exists in carp and is probably established during the first 8 days after immunization.

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