study of *D. willistoni* hemocytes utilized Yeager's[21] descriptive terminology and no effort was made at that time to determine which cells in *D. willistoni* accumulate melanin precursors as do the crystal cells in *D. melanogaster*[5]. After the discovery of the *Bc* mutant[8,9], we examined the hemocytes of a number of *Drosophila* species, including *D. willistoni*[22] (manuscript submitted prior to seeing Srdić and Gloor's article). Using an alternate method we concur with Srdić and Gloor[2] that the spheroidocytes of *D. willistoni* and the crystal cells of *D. melanogaster* share the common feature of accumulating melanin precursors. Note that these cells are morphologically distinguishable when examined by light microscopy; hopefully, biochemical comparisons and electron microscopy will reveal the nature of the differences between these cells (studies in progress). The inclusions in the crystalloid cells of *D. willistoni*, however, are unique from those in the crystal cells of *D. melanogaster* and there is no morphological evidence to support homology of these cell types as concluded by Srdić and Gloor[2]. It should also be pointed out that in this study[22] we examined the hemocytes of sibling species of *D. melanogaster*, and our observations do not support utilization of crystal cell inclusions as a characteristic for distinguishing *D. melanogaster* from *D. simulans* as recommended by Srdić and Gloor[2]. We find variety in the size and number of paracrystalline inclusions in both species; furthermore, the variation in crystal cell inclusions in *D. melanogaster* has been adequately demonstrated in the literature[3-6]. Srdić and Gloor[2] misquoted our observations on the staining reactions of the paracrystalline inclusions in *D. melanogaster;* after Carnoy fixation the inclusions did not show a positive reaction with Millon[4].

In conclusion, *D. melanogaster* with its extensive collection of mutant strains is the species of choice for establishing basic concepts of hemocyte relationships and functions. Available evidence supports the categorization of the larval hemocytes of this species into 2 general classes: plasmatocytes (and their variants) whose properties involve surface adhesion either for phagocytosis or encapsulation, and crystal cells containing melanin precursors and hemolymph phenol oxidase activity. Suggested homologies between the blood cells of this species and hemocytes in other insects are feasible without modifying this nomenclature which has been in use since 1956[3].

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Blood clearance of *MS2* bacteriophage in *Salmo trutta:* a paradoxon

J. G. O'Neill

Department of Life Sciences, Trent Polytechnic, Nottingham NG1 4BU (England), 19 November 1979

Summary. A primary challenge of *MS2* bacteriophage was cleared from the blood of the teleost *Salmo trutta* within 2.5 days. In contrast the clearance of a 2nd challenge required an extra 3.5 days and the induction of the secondary antibody response was delayed, though immune memory was observed later.

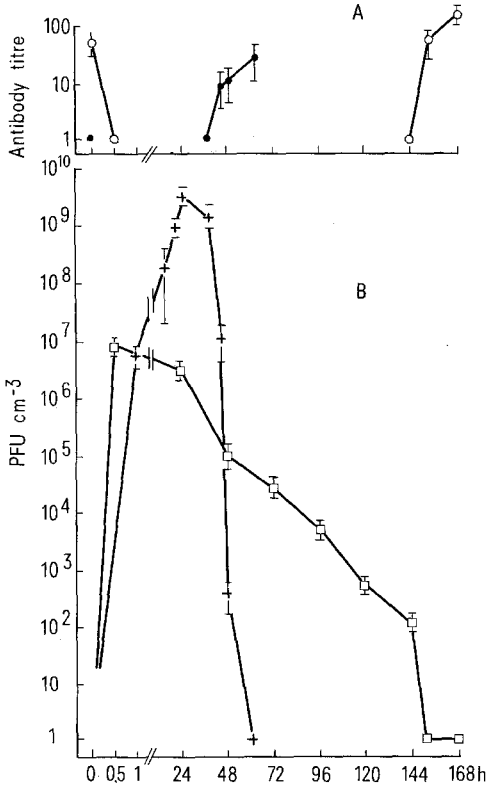
One characteristic of a classical secondary immune response is the enhanced clearance from the bloodstream of the 2nd immunogen challenge, which is then followed by the early induction of enhanced antibody titres. Such an enhanced secondary clearance was observed in mice using the bacteriophage *X174*[1] and a similar response was found with the bacteriophage T^2 in carp[2], though in the latter case no neutralisation antibody was detected on clearance. In an earlier examination of the humoral antibody response of brown trout to the bacteriophage *MS2* an initial suppression of antibody levels was observed following a 2nd challenge[3], and even increased clearance times were noted with certain inoculation schedules. However, immune memory and enhancement were demonstrated by the subsequent secondary antibody response in the trout. Similar results have been reported for carp on secondary challenge with soluble immunogens[4-6]. The following work was thus initiated to examine the clearance of *MS2* bacteriophage from the blood of brown trout, and the subsequent induction of neutralisation antibody, on primary and secondary challenge.

Brown trout (*Salmo trutta*, L.), 1 year old and 127–136 g, were maintained in polyethylene aquaria with a through-flow of aerated and chlorine-free tap water, at a temperature of 15.5 ± 0.5 °C. Each fish was i.p. inoculated with $7.7 \times 10^9 \pm 8.6 \times 10^8$ plaque-forming units (PFU) of *MS2* bacteriophage in 0.1 cm^3 of sterile saline[3]. A 2nd inoculum of $9.2 \times 10^8 \pm 6.2 \times 10^7$ PFU *MS2* in 0.1 cm^3 saline was administered 49 days after the first. Blood samples of not more than 0.05 cm^3 were taken by caudal venipuncture at intervals after the inoculations and the sera were assayed in duplicate for live *MS2* and neutralisation antibody. The fish were gently netted and anaesthetised in 1 g:200 dm^3 solution of tricaine methanesulphonate (Sandoz) before all inoculation and venipuncture procedures.

The bacteriophage *MS2* (picornovirus, group 1 RNA-phage) was grown using the Petri-plate and 'soft agar overlay' method of Eisenstark[7], with *Escherichia coli* K12 as

the host. The bacteriophage was isolated from the bacterium by ultrasonic disruption (20 kHz at 6 nm) followed by 1 low-speed (20 min, 3000× g) and 2 high-speed (120 min 40,000× g) centrifugations at 4 °C. The bacteriophage pellets were deaggregated in sterile saline using ultrasonic disruption and stored at 1 °C. A semi-micro viral plaque neutralisation assay procedure was modified from the Petri-plate method of Adams[8] and the neutralisation antibody titre was described as the dose of serum required to produce 50% inactivation of the bacteriophage (SD_{50})[9]. Live bacteriophage in the sera could also be detected and titred by the same method by omitting the stage at which the serum dilutions were incubated with a known number of viral particles. The full procedure has been described elsewhere[3].

The primary and secondary i.p. inoculations of *MS2* were observed to enter the bloodstream of the trout rapidly (figure). At +24 h after the primary challenge the meaned peak titre of live *MS2* in the sera was 3.1×10^9 PFU cm^{-3}. This represented a substantial proportion, about 80%, of the inoculated *MS2*. The 2nd challenge produced a smaller peak titre of 7.9×10^6 PFU cm^{-3} which may have been caused by the presence of detectable levels of antibody in the now *MS2*-sensitized fish. The primary inoculum was cleared from the sera within 48–60 h, though 1 fish had cleared the *MS2* 38 h after inoculation. At +44 h 2 fish were producing detectable levels of antibody and at +62 h all the fish were producing an antibody response. The clearance of the 2nd *MS2* challenge was much slower than that of the primary challenge and required 144–152 h for complete clearance in all the fish. At the time of total *MS2* clearance antibody titres were detected in all the trout and the rate of secondary antibody production was enhanced.



Blood clearance of *MS2* and induction of antibody in *S. trutta*. *A* Mean antibody titre (SD_{50}) values ±2 SE after primary (●) and secondary (○) challenge. *B* Clearance curves of a 7.7×10^9 PFU *MS2* primary (+) and a 9.2×10^8 PFU *MS2* secondary (□) challenge, ± SE. Each point is the mean of results from 5 fish.

The clearance of the primary challenge of *MS2* from the bloodstream of trout resembled that observed in mice[1] and guinea-pigs[10] using the bacteriophage *X174*. The 2 bacteriophage species were eliminated within 2–2.5 days after their inoculation. In both mammal and trout the elimination of the bacteriophage represented the appearance of small quantities of neutralisation antibody. However, for trout the clearance of a 2nd inoculum of *MS2* required 3.5 days longer than that required to clear the primary inoculum. This was in distinct contrast to the enhanced clearance of *X174*, observed in sensitized mammals[1,10], in which the bacteriophage was eliminated within 2–24 h of challenge. Although the clearance of the 2nd *MS2* challenge was suppressed in the trout the subsequent antibody response was enhanced.

The presence of specific neutralisation antibody, in the *MS2*-sensitized trout, was probably responsible for reducing the proportion of the 2nd *MS2* challenge entering the bloodstream, and would in part account for the initial antibody suppression observed in fish by other workers on secondary challenge[3–6]. However, the antibody titre present in the trout on secondary *MS2* challenge was low enough to combine with a proportion of the inoculum only. The clearance of the remaining *MS2* indicated that, apart from any suppression of phagocytic activity, the induction of the secondary antibody response was delayed. As in mammals[11] the clearance of small viral particles, like *MS2* and *X174*, is dependent on the release of humoral antibody and the 'immune elimination' of the opsonised immunogen.

The mechanisms involved in the control of the immune response in vertebrates are complex. These controls and feedback mechanisms in mammals are based on local interactions of lymphocyte and macrophage populations and are also mediated by soluble factors[12]. Immunogen-antibody complexes, formed when sensitized individuals are rechallenged, will interact with these mechanisms to produce immune suppression in higher vertebrates. Immune complexes can produce a direct feedback action on either antibody producing lymphocytes[13] or associated macrophages[14], though a direct action of the immunogen may also initiate T-cell suppressor activity[15]. In the consideration of immune suppression at the level of antibody production the greater susceptibility of heavy molecular weight (HMW) antibody production to suppression in mammals[16] has an added significance in teleost fishes which only produce HMW antibody.

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