The soluble fraction has been shown to retain its antigenic potency for at least 3 months when stored in the liquid state at 4° C. The fraction may be lyophilized but so far the method used has given rise to losses of activity of up to 50 per cent.

Further investigations are to be made to evaluate the product in pharmacological and immunological tests, and also to purify it still further.

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Secondary Immune Response to Bacteriophage *T1* in the Shore Crab, *Carcinus maenas*

COMPARATIVE investigations of the clearance of injected bacteriophage from the circulation of several species of animal have been carried out. Clearance from the chicken, frog and from certain elasmobranch, holostean and teleost fish was very rapid and was associated with the development of neutralizing antibody[1,2]. In the cyclostomes *Petromyzon marinus* and *Eptatretus stoutii* clearance was relatively slow, and little or no neutralizing ability developed in the serum of injected animals[2]. The insect *Samia cecropia* cleared 90 per cent or more of the injected dose of bacteriophage from the hæmolymph between the second and the tenth hour, but no neutralizing activity appeared to develop[3]. Accelerated secondary clearance has been demonstrated in the chicken, the frog and in some fish, but was not tested in the insect. The following preliminary experiments were carried out to investigate primary and secondary clearance of bacteriophage in other invertebrates as part of an investigation in comparative immunology.

Bacteriophage *T1* (10^3 'phage particles) was injected into the hæmocœle of three shore crabs (*Carcinus maenas*) which were maintained in sea water at 16°–18° C. Hæmolymph samples were taken 30 min after injection and then at fortnightly intervals. In the two instances in which the bacteriophage was completely cleared from the circulation, a further dose of 10^3 particles was injected and again the clearance was followed in fortnightly samples.

Samples of hæmolymph were allowed to clot, centrifuged and the supernatant tested for viable bacteriophage by the following pour plate technique. The hæmolymph was serially diluted in nutrient broth and 0·1 ml. of one or more suitable dilutions was mixed with 0·1 ml. of a 4 h culture of *Escherichia coli B*. The mixture was pipetted into 0·5 ml. of melted nutrient agar (0·7 per cent) at 50° C which was then poured evenly over the surface of a 1 per cent nutrient agar plate. After overnight incubation at 37° C the number of plaques was counted and from this the number of viable bacteriophage particles was calculated. Each selected dilution of hæmolymph was titrated in this way 5 times and the mean of the results taken.

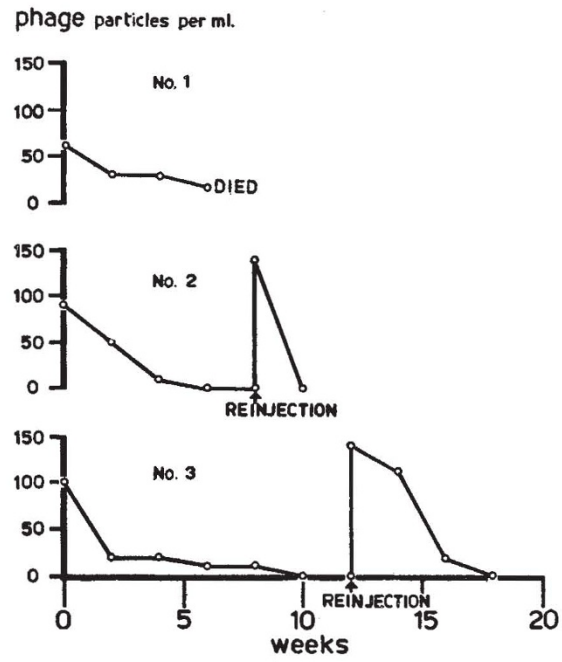

Fig. 1

Fig. 1 illustrates the decrease in bacteriophage particle counts in the three animals. Primary clearance occurred in 42 and 70 days in animals 2 and 3, respectively. Animal 1 died before complete primary clearance, but the 'phage particle counts were following a similar course to those in the other two animals. Secondary clearance was more rapid, animals 2 and 3 clearing completely in 14 and 42 days, respectively. Thus in the two instances in which secondary response was measured the rate of clearance increased by factors of 3·0 and 1·6.

Hæmolymph from crabs 2 and 3 was tested for neutralizing activity against bacteriophage *T1* after secondary clearance of 'phage from the circulation and again 2 and 4 weeks later. No activity could be demonstrated. Although this observation suggests that the accelerated secondary clearance is mediated by a cellular rather than by a humoral mechanism, the possibility that undetected humoral factors are involved cannot be discounted.

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RADIOBIOLOGY

Soluble Liver Proteins Fractionation by Zone Electrophoresis on 'Pevikon C-870'

LIVER proteins have been separated by agar-gel electrophoresis into thirteen[1] and fifteen[2] separate moieties. The use of 'Pevikon *C*-870' (PVK) as supporting medium for electrophoresis has numerous advantages[3–5], and an analysis of soluble proteins in rabbit liver separated by PVK-block electrophoresis has yielded the results reported here. PVK (Stockholms Superfosfat Fabriks Aktiebolag) blocks measuring 50·0 cm × 24·0 cm × 1·6 cm; veronal