The Uptake and Rate of Clearance of ^{125}I γG Globulin in Lampreys (*Lampetra fluviatilis*)

Lampreys are considered to be the living representatives of the most primitive vertebrates. Studies directed toward the demonstration in these animals of the formation of specific antibodies after intentional stimulation, have met with little success; with most of the antigens used the antibody response was entirely absent[1-3]. Antibodies were shown to be produced in response to *Brucella abortus* by FINSTAD and GOOD[3]. Recently, MARCHALONIS and EDELMAN[4] have detected a feeble immune response in *Petromizon marinus* injected with bacteriophages. The neutralization of bacteriophages by the immune sera was found to be extremely weak in comparison with the activity observed in amphibia and mammals.

In this paper we describe the results of injecting lampreys with a soluble antigen. The uptake of the antigen and its rate of clearance was studied using human γG molecules labelled with ^{125}I.

Materials and methods. Sexually mature lampreys (*Lampetra fluviatilis*) were collected over a period of 3 years from the river Severn; groups of animals were kept in separate tanks at approximately 16 °C.

Human γG globulin was separated by DEAE-cellulose chromatography[5]; iodination with ^{125}I was carried out following the method described by MCFARLANE[6]. The autoradiography of the tissue sections and cell smears were prepared using a stripping film technique[7]; after 3 weeks the films were developed on the glass slides and the preparations were stained[8].

The presence of antibodies against γG molecules was studied using tanned human red cells coated with γG globulin[9] and Rh-positive red cells sensitized with an incomplete anti-D antibody previously shown to be associated with γG globulin[5]. Serial doubling dilutions in buffered saline of the lamprey sera were prepared; 4 volumes of each dilution were mixed with 1 volume of a 5% suspension of tanned red cells coated with human γG molecules or with 1 volume of a 5% suspension of Rh-positive red cells sensitized with anti-D.

When tanned red cells were used, the mixtures were left in test tubes at 20 °C for 90 min; the deposited cells were then examined microscopically. The final stage of the test using red cells sensitized with anti-D was carried out on opal tiles; the mixtures were examined macroscopically after 15 min.

Results and discussion. In a preliminary experiment, 10 lampreys were injected i.p. with a 4% solution of Evans blue; 3 h after the last injection the animals were killed. The macroscopic examination of the organs showed that the kidney and the last portion of the intestinal tract were uniformly stained blue; the liver, however, did not appear to contain the dye. Apart from occasional streaks on the surface of the organs, the dye did not appear to be locally concentrated. Following the injection of the dye, granules of Evans blue were observed in several peritoneal cells.

To study the in vitro uptake of the dye, cells from the peritoneal cavity of untreated lampreys were suspended in a 4% solution of Evans blue containing 20% of lamprey serum. The mixtures were left at 20, 16, 12 and 8 °C for 1 h. Only a few cells incubated at 20 °C were found to contain granules of Evans blue; the optimal uptake of the dye was observed when the cells were incubated at 12 °C.

Investigations on the uptake of human γG globulin labelled with ^{125}I were carried out using an autoradiographic technique.

One group of 4 lampreys was injected with 1.5 mg of ^{125}I labelled γG globulin (3.35 μc/mg γG globulin) into the peritoneal cavity. 3 h after the injection the animals were killed and samples of blood and tissues were collected. Cells from the peritoneal cavity were washed 3 times and smears were made on glass slides.

Evidence of selective fixation of ^{125}I γG molecules was observed in some peritoneal cells; these cells were morphologically distinguishable from other cells present in the peritoneal cavity, since they contained a polylobate nucleus (Figure 1). The amount of the labelled antigen fixed by these peritoneal cells, as judged by the number of radioactive grains present in each cell, varied within wide limits in each animal; some cells were found to be weakly labelled (Figures 1 A and B) and yet other cells in the same animal were strongly labelled (Figures 1 C and D).

The liver contained much radioactivity. Grains were present in both the parenchymal cells and macrophages. They were particularly numerous in the macrophages. Very little activity was present in striated, smooth and cardiac muscle. Sections of the gill region showed much evidence of radioactivity. The grains were most numerous

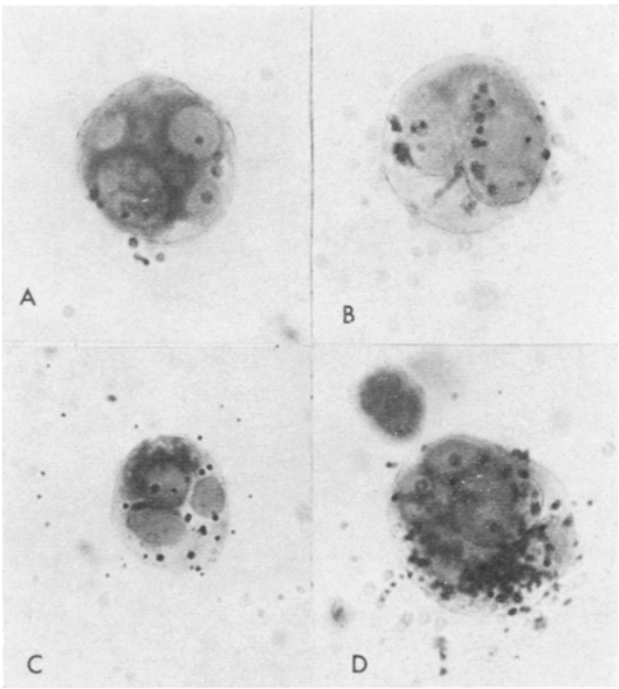

Fig. 1. Autoradiography of peritoneal cells from a lamprey injected with ^{125}I γG globulin. The cells were collected from 1 animal 3 h after the injection of the labelled antigen.

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in the connective tissue stroma and round the cartilage at the base of the gill. Less activity was present in the gill filaments. Within the gill area the radioactive grains appeared both in the intercellular tissue and in the cytoplasm of mononuclear cells. The kidneys showed light, but diffuse and widespread stromal radioactivity. Numerous grains were present within the renal tubular epithelial cells. Some of the sub-serosal mononuclear cells contained much activity. The intestine contained radioactive grains in the submucosal connective tissue; the majority of activity appeared related to mononuclear cells. In the pharynx some grains were present in mononuclear cells but generally little activity was seen in this tissue. The pharyngeal epithelium contained no grains.

In order to see if the fixation of ^{125}I γG molecules increased in animals previously treated with the antigen, 5 lampreys were injected with 0.4 mg of γG globulin, twice at an interval of 1 week. Two of these animals died soon after the second injection. 6 days after the last injection the remaining 3 lampreys and 3 untreated animals were injected with 2 mg of ^{125}I γG globulin (1.5 μc/mg γG globulin). 3 h later the lampreys were killed and samples of blood and organs collected. When the uptake of the labelled antigen by the peritoneal cells was compared in these 2 groups of animals, no differences in the distribution of the labelled antigen were observed; in fact, the percentage of the cells labelled and the number of grains in each cell were similar.

To study the rate of clearance of γG molecules 1 group of untreated animals and 1 group of lampreys pretreated with the antigen were injected with ^{125}I γG. Each group contains 5 lampreys; the animals of the second group were injected i.p. 3 times at intervals of 3 days with 0.4 mg γG globulin; 4 days after the last injection these animals and 5 untreated lampreys were injected i.p. with ^{125}I labelled γG molecules. One animal in each group was killed 1, 6, 24, 48 and 72 h after the injection of the labelled antigen; samples of blood were taken.

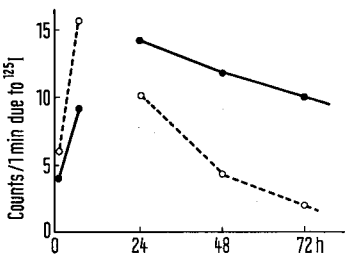

Fig. 2. Rate of clearance of ^{125}I γG in lampreys previously injected with γG globulin (open circles) and in untreated animals (black circles). Radioactivity due to ^{125}I is expressed as 1000 cpm × 0.1 ml serum (see Table).

Rate of clearance of ^{125}I γG in lampreys

Lamprey No.	Sex	Weight (g)	h after injection of ^{125}I γG	cpm
Pre-treated				
L 22	F	31.8	1	6,100
L 23	F	42.6	6	15,900
L 24	M	40.2	24	10,200
L 25	M	36.6	48	4,300
L 26	F	38.1	72	2,100
Untreated				
L 17	F	32.5	1	3,900
L 18	F	39.5	6	9,200
L 19	M	42.4	24	14,300
L 20	M	34.5	48	11,800
L 21	F	36.0	72	10,100

Lamprey pre-treated with γG globulin and untreated were injected with ^{125}I γG and killed at various intervals after the injection.

The Table shows the results of testing the radioactivity of the lamprey sera; the Table also indicates the sex and weight of the animals. It was found that the levels of labelled γG globulin fell more rapidly in the pretreated animals than in the untreated lampreys (Figure 2); in fact, 72 h after the injection of the labelled antigen the residual radioactivity, expressed as a percentage of the maximal activity observed in each group, fell to 12% in the pretreated lampreys and to 70% in the untreated animals.

These findings suggested that antibodies against γG globulin were produced in the animals repeatedly injected with the antigen; however, attempts to detect circulating antibodies were unsuccessful. 12 lampreys were injected 3 times at intervals of 1 week with 0.4 mg of γG globulin previously heated at 63 °C for 30 min; 4 animals died during the treatment; the remaining 8 lampreys were killed 8 days after the last injection.

Antibodies against human γG globulin were not detected in the sera using tanned red cells of the lampreys previously injected with the antigen, coated with γG globulin or Rh-positive cells sensitized with γG anti-D. Negative results were also obtained when these sera were tested using the single diffusion and double diffusion in agar gel[10].

FINSTAD and GOOD[3] have investigated in *Petromizon marinus* the capacity to clear bovine albumin and γ-globulin; after at least 2 antigenic stimulations no rapid clearance of these 2 antigens was observed. It seems possible that the different doses of albumin and γ-globulin injected may be responsible for the persistence, over prolonged periods, of the antigens in the circulation of the *P. marinus* studied.

Our observation that the rate of clearance on γG molecules is increased in lampreys pretreated with the antigen, although circulating antibodies appear to be absent, is in good agreement with the results published by NELSTROP et al.[11], who observed an increased rate of clearance of bacteriophage in *P. marinus* previously injected with the antigen, in the apparent absence of humoral antibodies[12].

Zusammenfassung. Lampreten wurden mit menschlichem ^{125}I-markierten, γ-Globulin injiziert, und die Aufnahme und Abgabe des Antigens wurden untersucht. Der Ort der Aufnahme des Antigens wurde autoradiographisch bestimmt.

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