Antibody Response to Bacteriophage ΦX 174 in Non-Mammalian Vertebrates.* (27691)

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Immunization of several mammalian species with a variety of antigens results in the consecutive appearance of 2 major types of specific antibody molecules in the serum. Of these, the first to be produced are molecules of 19S sedimentation constant; while later, 7S antibody molecules appear(1-6). The purpose of this study was to investigate whether or not these 2 types of antibody molecules are also produced in lower vertebrates.

Materials and methods. The preparation and purification of bacteriophage ϕX 174 has been described(5). Unless otherwise stated, the phage was diluted in saline and 0.1-0.2 ml were used for the immunizing injections. Two chickens were injected intravenously with both 3×10^8 phage and 125 μg of diphtheria toxoid (Mass. Dept. of Health, KP59a, 50 Lf/ml) twice during the first week and again 1 and 3 months later. One chicken survived for the 3 months. Blood was obtained from the wing vein 1-2 weeks after each immunization. Five bullfrogs (*Rana catesbeiana*) were immunized subcutaneously with 10^{10} phage and were kept at room temperature in 1-2 inches of water. Only one frog survived after 17 days. During the following 3 months it was immunized every 2-4 weeks with 10^{10-11} ϕX intraperitoneally and subcutaneously. Two of these injections were given with the phage-in-saline emulsified in complete Freund's adjuvant (Difco). The frog was bled 7 times from the heart during the 3 months of observation. Eighteen goldfish (*Carlassius auratus*) 7-10 inches in length were kept in fresh water in a 50-gallon tank. Before immunization, the temperature of the water was raised to 30°C during a 3-day period. All were injected intraperitoneally with 10^{10} ϕX. At the end of 3 weeks 5 had survived. Two of these were immunized intraperitoneally every 2-4 weeks during the 5-month period of observation with a total of approximately 10^{12} ϕX of which one-half was given emulsified in complete Freund's adjuvant. The temperature of the water was raised to 32°C for the last month in order further to stimulate antibody production. The 2 fish were each bled from the heart 8 times during the 5 months of observation. The neutralizing capacity (K) of each serum was determined by the standard phage antibody titration(5,7) before and after incubation with 0.1 M 2-mercaptoethanol at 37°C for 30 minutes(8). Some of these sera were subjected to ultracentrifugation in a saline density gradient(9) and the samples obtained from each serum were pooled so as to yield a rapidly and a slowly sedimenting fraction. In a normal human serum separated under similar conditions the rapidly sedimenting fraction contained most of the 19S γ-globulin and the slowly sedimenting one was essentially free of 19S proteins and rich in albumin and 7S γ-globulin. Several goldfish and frog sera were also subjected to starch block electrophoresis (10). Anti-ϕX titrations were performed on the fractions which migrated with the γ_2, γ_1, β, α_2 globulins and albumin of a simultaneously separated human serum. More precise definition of these proteins was limited by the small amounts of serum available. Diphtheria antitoxin was determined by the toxin-neutralization assay in rabbit skin(11). T_2 bacteriophage, which was used for testing the specificity of anti-ϕX sera, was obtained through the courtesy of Dr. E. Lennox.

Results. The results of immunization of one frog and a representative chicken and goldfish are shown in Table I. Within 3 weeks following a single injection of 10^{10} bacteriophage in saline, each species produced anti-ϕX serum levels of K = 10-100 (approximately 10^4 above the minimum amount that can be detected). In contrast, no detec-

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TABLE I. Physico-Chemical Characteristics of Anti-ΦX Molecules.

Time after immunization	Treatment of serum	Serum antibody (K)* Chicken	Frog	Goldfish
2-4 wk	Untreated	9.24	96.0	10.45
	2-mercaptoethanol	.585	53.4	1.90
	Ultracentrifugation—rapidly sedimenting	14.8	106.0	—
	slowly "	<3.60‡	67.5	—
	Electrophoresis†—β	—	2.52	.235
	γ_1	—	6.57	3.19
	γ_2	—	11.5	4.98
2 mo	Untreated	2.98	—	54.7
	2-mercaptoethanol	1.40	—	2.15
	Ultracentrifugation—rapidly sedimenting	—	—	49.2
	slowly "	—	—	41.8
	Electrophoresis†—β	—	—	<.103‡
	γ_1	—	—	5.06
	γ_2	—	—	32.0
3-5 mo	Untreated	19.2	81.5	195.6
	2-mercaptoethanol	18.7	18.4	<4.31‡
	Ultracentrifugation—rapidly sedimenting	4.50	28.6	<4.31‡
	slowly "	28.0	147.0	174.2
	Electrophoresis†—β	—	.103	.196
	γ_1	—	2.20	9.08
	γ_2	—	14.03	15.75

— = not done.

* Antibody was not detected in pre-immunization sera.

† Defined by a simultaneously separated normal human serum.

‡ No neutralization observed during the assay. The calculation of K is based on the possibility that a 10% neutralization may have occurred without detection.

table diphtheria antitoxin was produced in the chicken during a 3-month period despite 3 intravenous injections of toxoid in saline (not shown in Table I). This observation confirms the excellent antigenicity of this bacteriophage.

In all 3 species studied antibody activity was found chiefly in the fraction with an electrophoretic mobility corresponding to human γ-globulin and only trace amounts, probably representing contaminating γ-globulin were found in the β-globulin fraction.

In the chicken, the predominance of antibody activity one month after immunization was in the rapidly sedimenting fraction and was destroyed by 2-mercaptoethanol. By 3 months, antibody activity was predominantly in the slowly sedimenting fraction and was 2-mercaptoethanol-resistant. Such a response is analogous to that previously reported in mammals(1-6).

In the frog, at 2½ weeks, about 60% of the neutralizing activity was in the rapidly sedimenting fraction; by 3 months approximately 80% of the neutralizing activity was in the slowly sedimenting fraction. However, the anti-ϕX activity of both sera was partially sensitive to treatment with 2-mercaptoethanol.

In the goldfish, antibody activity was evenly distributed between rapidly and slowly sedimenting fractions at 2 months, but at 5 months was almost entirely in the slowly sedimenting fraction. Antibody activity of both early and late sera was almost entirely lost after treatment with 2-mercaptoethanol.

Both the 2-4-week and 3-5-month sera from the frog and goldfish shown in Table I were tested for the specificity of their ϕX-neutralizing activity using an immunologically unrelated phage, T_2. No neutralization of T_2 was detected.

Thus, in the chicken, as in mammals, the rapidly sedimenting molecules are inactivated by 2-mercaptoethanol while the slowly sedimenting ones are not significantly changed by this sulfhydryl reagent. However, in the goldfish, antibody activity of all sera is virtually abolished by 2-mercaptoethanol treatment, and, in the frog, it is much reduced, thereby suggesting a difference in structure between frog and goldfish gamma-globulins

and 7S gamma-globulins from other species that have been studied previously.

Discussion and summary. After a single injection of 10^{8-10} bacteriophage ϕX 174, the chicken, frog and goldfish were shown to produce approximately the same levels of neutralizing, rapidly sedimenting, γ-globulin antibodies as those previously obtained in analogously immunized mammals(5,6). Repeated injections of bacteriophage in the frog and goldfish, at intervals of 2-4 weeks, did not elicit an anamnestic antibody response. However, higher levels of antibody, mainly in the slowly sedimenting γ-globulin fraction were produced after immunization with bacteriophage in complete Freund's adjuvant, and, in the case of the goldfish, after further elevation of the environmental temperature to 32°C. Thus, in 3 classes of non-mammalian vertebrates a change was observed in the sedimentation properties of antibody γ-globulins produced during immunization. This change appeared similar to the replacement of 19S by 7S antibodies in the circulation of immunized mammals. These findings suggest that the mechanisms responsible for this phenomenon were present in the most recent common ancestors of terrestrial vertebrates and bony fish and that formation of rapidly sedimenting antibody is an integral and important part of the immune mechanism.

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Hemostatic Effects of Heterologous Platelets in Thrombocytopenic Rats.* (27692)

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Transfusions of fresh homologous platelets reduce or eliminate the abnormal diapedesis of red blood cells in thrombocytopenic animals, as determined by studies of lymph composition(1,2). Since the effects of platelets on permeability of small blood and lymph vessels to red blood cells(3) may represent a function separate from the coagulation-promoting and "hemostatic plug" formation activities, the species-specificity of platelets was investigated in this respect. The effect of fresh human platelets on the increased passage of red blood cells into the lymph of x-irradiated thrombocytopenic rats was compared to that of fresh rat platelets.

Methods and materials. Female Wistar rats weighing 250 to 300 g were studied. Thrombocytopenia was induced by exposure to 875 r total body x-irradiation.† Seven to

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† 250kV, 15 m.a., Target-mid-body level distance distance-88cm. 18r/min. 0.25 mm Cu and 1 mm Al filters.