In all groups which were injected with Sr^{89} there was a very rapid rate of excretion of the isotope in the first 8 hours after which the excretion rate decreases slightly. On the other hand the rate of excretion following oral administration of Sr^{89} continued to increase at a rapid rate through 48 hours.

These data indicate that following the discrimination by the intestinal mucosa, the next great discriminatory barrier which Sr must overcome before skeletal deposition is that exhibited by the kidney.

Summary. The maximum concentration of Sr^{89} observed in the blood following an oral dose was observed to occur in about 2 hours and decreased to a negligible quantity in 24 hours. The rate of uptake of Sr^{89} by the bone was found to parallel the rate of increase in activity of the blood, but the former continued to increase to 14 hours post-injection time. The eyes took up radiostrontium in a similar way, but in much lower concentrations.

It was observed that the most efficient excretion was accomplished when the isotope was ingested orally, and the lest efficient elimination was observed when the isotope was administered by intraperitoneal injection. All routes of administration, except the oral route, resulted in higher activity in the urine than in the feces with the highest urinary excretion occurring as a result of intramuscular injection.

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Comparative Immunology. Active Immunization of Young Alligators with Hemocyanin.* (31761)

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Recent immunological studies from a comparative or phylogenetic standpoint have shown this area to be a relatively unexplored region of immunology, and have provided new approaches and different perspectives on old problems as well as a source of fundamentally new information(1,2). The mechanisms of immunity of poikilothermic vertebrates are of special interest because of the important role of environmental temperature(3). Furthermore, the crocodilian reptiles are believed by Good *et al*(4) to constitute a sequential step in the evolutionary development of the lymphoid system, in which the lymphoepithelial tissue found in the pharyngeal tissue of the alligator appears to be the evolutionary precursor of the tonsils of mammals. Although

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advantages of the crocodilian reptiles as experimental animals have been pointed out by Coulson and Hernandez(5), reports on the ability of crocodilians to produce antibody have been fragmentary and restricted to their response to micro-organisms(3).

Immunological response can vary with different kinds of antigens and schedules of injections. It has been necessary, therefore, before initiating a quantitative characterization of the immune response of crocodilians in terms of the effect of temperature and nature of the immunoglobulins produced, to show first that these animals produce circulating precipitin antibody of more than one type to a soluble (protein) antigen upon active immunization, and secondly, to obtain preliminary data on the production of antibody as a function of time and secondary injections.

Materials and methods. Young alligators, *Alligator mississippiensis,* weighing 900-1440 g (about 2-3 years) were used. Animals were kept in cages with access to food twice a week and water at all times. The temperature at which the alligators were kept and injected varied from 22-26°C. Alligators, not injected, served as a source of "normal" serum for controls in analysis of antibody.

Key-hole limpet hemocyanin (KLH), a molluscan respiratory protein, was used as an antigen. The KLH was isolated from live limpets sent by air-express from California and purified by differential ultra-centrifugation(6). KLH is highly immunogenic and is commonly used for immunochemical studies, a number of which have involved lower vertebrates(2).

Blood was obtained and injections made by cardiac puncture. With this method, samples of blood could be withdrawn when needed before injection of antigen without removing the needle. Prior to each injection of antigen ½ ml blood was removed, and at 5-day intervals after the last injection. Serum was prepared by allowing the blood to clot at room temperature and removal of clot by centrifugation. All serum was tested for antibody within 48 hours after the blood was drawn. No anti-KLH antibody could be detected in serum from the test alligators before they were injected with antigen.

Since no information on the immune response of alligators to protein antigens was available, the response of animals had to be observed over a period of time on an individual basis. This necessitated multiple bleedings of a single animal, and, therefore, required the use of small amounts of serum for any one analysis. Inasmuch as an aim of this research was to establish the time sequence of circulating antibody production, the inter-facial (ring) test was used, as this method can detect as low as 1 μg of antibody (6). Although the inter-facial test is primarily a qualitative one, relative amounts of antibody among the sera to be compared can be indicated by the amount the antiserum can be diluted to give detectable antibody, provided that serum is titrated with serial dilutions of antigen. Therefore, individual sera, both undiluted and diluted 1:5 with borate-saline, were titrated, and a positive response with the 1:5 diluted serum was interpreted as a rise in titer for that animal.

As is usual with anti-KLH systems, borate-saline (pH 8.6) was used to dilute serum and antigen for the inter-facial titrations(7,8). KLH dissociates at pH 8.6 into sub-units approximately ⅛ (or 1/10th) the molecular weight of the associated molecule seen at pH 6.5, and, therefore, the antibody/antigen ratios will differ at different values of pH. The equivalence points at pH 8.6 may be determined more exactly because antibody constitutes a greater percentage of the precipitate. Furthermore, at pH 6.5, KLH aggregates to some degree (5-10%), and this aggregation can contribute to a false positive ring test if unclarified serum is used.

A total of 10 alligators was injected. Two alligators (Group A) received a single injection of 10 mg KLH. Group B and C (4 alligators each) received 5 injections of 10 mg KLH each over a 9-day period. Forty days after the last injection, when no circulating anti-KLH antibody could be detected, Group C received additional injections of 2.5, 5, and 10 mg KLH on day 50, 52, and 54, respectively.

Results. All 10 alligators gave a positive response, and all but 2 animals lived through

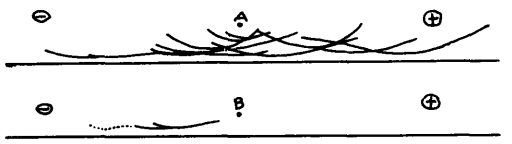

FIG. 1. (A) Immunoelectrophoretic pattern of normal alligator serm *vs* rabbit anti-alligator; (B) immunoelectrophoretic pattern of alligator anti-hemocyanin (KLH) serum *vs* hemocyanin (KLH). The dotted precipitin band (B) was observed in serum of 1 of 4 animals examined. The solid lines in (B) were observed in serums of all 4 animals. No precipitin band was observed in immunoelectrophoresis of normal alligator serum *vs* hemocyanin. Immunoelectrophoresis carried out in LKB immunophor set in borate buffer (pH 8.6, ionic strength 0.1) for 1 hr at 250 v.

the whole experimental period. Positive ring tests were observed only with undiluted serum (KLH diluted 1/2000) from the 2 alligators receiving only one injection (10 mg KLH). Antibody could not be detected in serum from these animals diluted 1:5. Circulating antibody was first observed 20 days after the injection, and continued to be observed for 45 days in one animal.

Circulating antibody also was first observed 20 days after the last injection in all the 8 animals receiving 50 mg KLH, although 2 animals showed detectable antibody by the 15th day. However, in these 8 animals, circulating antibody could be detected in serum diluted 1:5 (KLH diluted 1/8000) after 25 days. Thereafter, the titer decreased until no antibody could be detected by the 45th day after injection.

At this time, the second series of injections was given to the 4 animals of Group C with samples of blood taken before each injection. On the second day after receiving 2.5 mg KLH, circulating antibody could be detected in serum diluted 1:5 (KLH diluted 1/8000) in 3 of the 4 animals and in undiluted serum (KLH diluted 1/2000) from the fourth animal. The titer did not appear to rise further with the second and third injections, and it then fell again after about 40 days.

Sera (undiluted) taken from the Group C animals were examined by immunoelectrophoresis with KLH (diluted 1/2000) as the test antigen. Two precipitin bands, with different electrophoretic mobilities, corresponding to a γ_1-globulin (β_2) were observed in all 4 animals. One animal contained a third precipitin component with a mobility corresponding to a γ_2-globulin (Fig. 1). Baril *et al* (9) reported that the γ-globulin fractions of alligator serum contain 7S and 19S components.

Discussion. It is apparent that alligators, like several other poikilotherms, can be actively immunized with KLH, a protein antigen, and the circulating antibody consists of at least 2 kinds of immunoglobulin that differ in electrophoretic mobility and perhaps size. Evans *et al*(3) suggested that on the basis of reported responses by turtles, tortoises, and lizards, reptiles, in contrast to mammals, do not show an anamnestic response, even though they may produce more than one type of immunoglobulin. The crocodilian reptiles differ from the above reptiles from a phylogenetic standpoint. They constitute an offshoot from the stock from which birds evolved, and except for the birds, they are the only living creatures closely related to the group of dinosaurs(10). In this respect, although Grey(11) reports only 4 antigenic components in turtle serum when examined by immuno-electrophoresis, we readily observe at least 10 components in the serums of alligator, caiman, and crocodile. The presence of lymphoepithelial tissue in the pharyngeal tissue of the alligator would suggest that an anamnestic response might be observed in the alligator(4). If we define immunologic memory as a more efficient response when again stimulated with small doses of antigen after the antibody from the initial stimulation has disappeared from the serum, then our results, although primarily qualitative, indicate that alligators possess an immunological memory. On the assumption that the inter-facial test can detect 1 μg of antibody, then a rise of at least 50 μg of antibody/ml of serum was detected 2 days after the animals received a secondary injection of 2.5 mg KLH, as compared to the 20-day period following the initial injections of 50 mg KLH. No conclusions can be drawn as to whether this response reflects a "release" of circulating antibody or the production of new antibody, nor whether the antibody primarily is of the 7S or 19S variety.

Despite discrepancies in experimental findings, it would appear that the initial antibody produced in poikilotherms constitutes a rapidly sedimenting one with γ_1-globulin electrophoretic mobility, and therefore, is the one most commonly observed. Even though γ_2-globulins are observed, they appear to lack specific antibody activity when specific activity has been found in the γ_1-globulins(12,13). Uhr *et al*(14) report that in the frog and goldfish, antibody activity is associated with γ_2-globulins. As has been pointed out by workers in this field, the known variations in the immune response to different antigens, antigen dosages, and time of immunization are factors that may contribute to the differences observed. Our finding of at least 2, and possibly 3, electrophoretic components in the alligator with specific antibody activity may reflect an evolutionary trend, but, it may also reflect the injection schedule followed.

Summary and conclusions. Alligators can be actively immunized with hemocyanin, a protein antigen, and the circulating antibody consists of at least 2 kinds of immunoglobulin that differ in electrophoretic mobility and perhaps size. Results indicate that alligators, in contrast to other reptiles, may possess a primitive type of immunological memory. Although the production of circulating antibody by the alligator was not unexpected, the nature of the immune response and of the anti-KLH immunoglobulins in the alligator appear to differ enough from that reported for other poikilotherms to warrant further study, especially from an evolutionary standpoint.

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Atherosclerosis in Denervated and Intact Carotid Arteries in Rabbits.* (31762)

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Evidence indicating that the autonomic nervous system, both through the adrenal medulla and directly through its innervation of adipose tissue, is involved with lipid metabolism, chiefly the mobilization of free fatty acids (FFA), has been well reviewed(1-3). Stimulation of nerves innervating adipose tissue has been shown to increase FFA release (4). The process of atherosclerosis occurs in vascular channels which possess a rich adrenergic innervation. Do these sympathetic nerves exert any appreciable influence on the

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