Table 1. Serum proteins of *Rana catesbiana* at various stages of development.

Stage	No. of animals	Total protein (%)*	A/G†
Young, undeveloped	6	1.01	0.11
Young, triiodothyronine-treated	5	1.74	0.25
Froglet	9	2.56	0.96
Adult	5	2.56	0.86

* Nitrogen in TCA insoluble components × 6.25.
† Derived from electrophoretic patterns.

and other species of frog are being thoroughly studied and will be published subsequently.

The modifications of the serum proteins noted here during tadpole metamorphosis telescope into one species a variety of trends occurring in evolution. The frog is particularly well suited for a comparative study since, in the course of its development, it changes from an aquatic form to a terrestrial form. In addition, genetic and environmental factors can be kept relatively constant for a given species of frog at various stages of metamorphosis. These factors cannot be controlled as well in comparative phylogenetic studies.

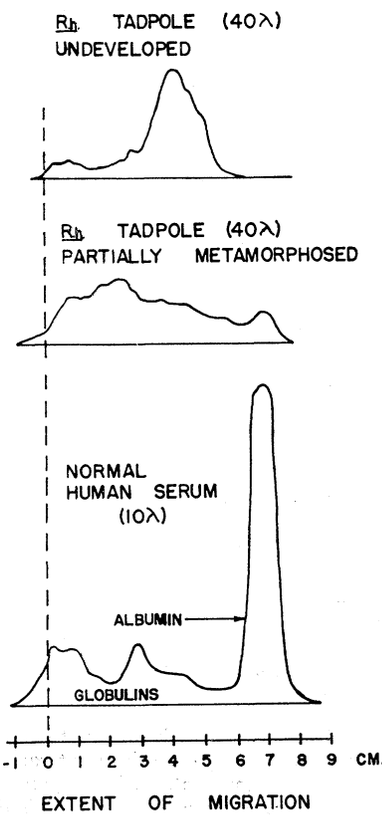

Fig. 2. Comparison of paper electrophoresis patterns of tadpoles of species *Rana hecksheri* with normal human serum pattern.

An examination of the literature on the nature of the serum proteins of widely different animals does not present a perfectly consistent picture on the character of evolutionary changes in the serum protein constituents (*5*). Although exceptions are noted, the increased complexity of animals is usually associated with (i) an increased total protein concentration (*6*), (ii) an increase in the A/G ratio, and (iii) the appearance or great increase in the concentration of a fraction or fractions with very low mobility (*7*) (corresponding to human gamma globulin). Thus, it appears that the tadpole may be reflecting its larval ontogeny with its rapidly changing serum-protein composition.

A teleologic rationale may also be constructed for the increase in A/G ratio and total protein of the tadpole during differentiation. The conservation of the body water and the maintenance of the plasma volume—properties enhanced by high plasma albumin and protein content—are certainly more critical for the terrestrial form. It is noted that a low serum protein concentration is typical of most aquatic animals (*6*).

Finally, the balance between the albumin and globulins seems to be related to the thyroid state of some organisms, with an increase in the A/G ratio as the animal progresses from hypothyroidism to euthyroidism (*8*). The increase in A/G ratio during amphibian metamorphosis might, then, reflect a response of the tadpole to endogenous or exogenous thyroid hormone.

It is hoped that the extensive study, now in progress, of the serum proteins in metamorphosing animals, aquatic forms, and a wide range of phyla will contribute to our understanding of the place of the serum proteins in comparative biochemistry (*9*).

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References and Notes

1. Previous papers from this survey: E. Frieden and B. Naile, *Science* **121**, 37 (1955); J. L. Dolphin and E. Frieden, *J. Biol. Chem.* 217, 735 (1955); E. Frieden and H. Mathews, *Arch. Biochem and Biophys.*, in press. This work was aided by grants from the U.S. Public Health Service (C-3006) and the National Science Foundation (G-2068).
2. Sterox SE is a polymeric thioether available in experimental quantities from the Monsanto Chemical Company, Boston 49, Mass. The presence of Sterox markedly improves resolution as the result of sharpening the bands in the electrophoretic pattern. It functions as a non-ionic detergent and presumably reduces protein-protein interaction without altering the nature and the charge of the protein molecules. In the presence of Sterox SE, A/G ratios are obtained from the electrophoretic patterns of normal human serum which agree with ratios obtained by sulfite precipitation methods within 2 percent (J. Downs and K. Lunan, private communication).
3. The Research Council of the Florida State University generously provided the Spinco "Analytrol" and electrophoresis unit.
4. We use the terms *albumin* and *globulin* with some reservation. In doing so we refer to fractions with mobilities corresponding to the mobilities of human albumin and human globulins under our experimental conditions.
5. H. F. Deutsch and M. B. Goodloe, *J. Biol. Chem.* **161**, 1 (1945); D. H. Moore, *ibid.* **161**, 21 (1945); H. F. Deutsch and W. H. McShan, *ibid.* 180, 219 (1949); H. C. Dessauer and W. Fox, *Nature* 126, 225 (1956).
6. Prosser *et al.*, *Comparative Animal Physiology* (Saunders, Philadelphia, Pa., 1950), p. 104.
7. A. M. Schechtman, in *Biological Specificity and Growth* (Princeton Univ. Press, Princeton, N.J., 1955). Schechtman reports that the chicken is unable to form antibodies until 4 to 5 weeks after hatching. This is associated with an absence of gamma globulin in the very young chick.
8. J. H. Leatham and R. D. Seely, *Endocrinology* 42, 150 (1948). These workers report a decrease in A/G ratio in thyroid-deficient mice.
9. Work has been done by A. Riggs [*J. Gen. Physiol.* 35, 23 (1951)] on hemoglobins in tadpole and adult bullfrogs. The changes noted for serum proteins in this paper indicate that more extensive modifications in protein structure are involved with the serum proteins than with the hemoglobins during metamorphosis.

13 June 1957

Chlorination of Poliovirus

The inactivation of viruses by chlorination is of interest to sanitarians, since it is the usual method for the disinfection of water supplies and sewage. Although the effects of chlorine on viruses have received their share of attention in the past, quantitative data illustrating rates of inactivation of animal viruses have not been presented. The experiments discussed here indicate that inactivation of poliovirus by chlorine, under the conditions described, may follow a course not strictly linear. When water suspensions of poliovirus were exposed to chlorine for various lengths of time, the change in infectivity titer was not necessarily constant.

Suspensions of polioviruses type 1 (Mahoney), type 2 (MEF_1), and type 3 (Saukett), grown on HeLa cell cultures, centrifuged at low speed, and partially purified to minimize chlorine demand by adsorption onto, and elution from, Dowex-1 resin and dialysis, were added to chlorine-demand-free water, buffered at *p*H 7.0, to give 300 to 10,000 50-percent tissue-culture infectious doses ($TCID_{50}$). They were dosed with chlorine water to yield a free available chlorine residual (at the end of 1 minute at room temperature) of 0.17 to 0.23 ppm. Six-milliliter samples were withdrawn at intervals for determination of residual chlorine, and 1-ml samples, for estimation of infectivity titer. Samples for infectivity titers were added to 0.25 ml of 0.1*N* sodium thiosulfate to stop the action of the chlorine. Infectivity titers were estimated by inoculating HeLa cell cultures, in duplicate, with undiluted or

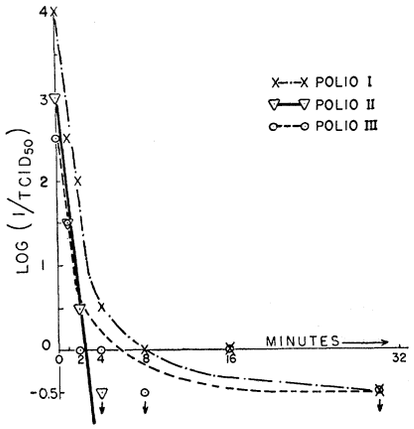

Fig. 1. Infectivity titers of polioviruses at intervals after exposure to chlorine at *p*H 7.0 at 25 to 28°C.

tenfold dilutions of sample and observing them for degeneration over a 6-day period. Residual chlorine was determined as the free available and combined available residuals, by means of the orthotolidine-arsenite method (*1*).

Infectivity titers after exposure to chlorine for various intervals are shown in Fig. 1. The change in titer of poliovirus type 2 was constant, resulting in an inactivation curve that is linear; the change in titer of polioviruses 1 and 3 was constant only during the first few minutes of exposure, resulting in a curve that is linear for the early part of the inactivation only.

The linear response of poliovirus 2 to chlorine in amounts that did not inactivate poliovirus 1 and 3 with similar regularity suggests that the latter are more resistant to chlorine than is poliovirus 2. This is indicated also by the time required for complete inactivation (infectivity titer < 0.0) of the three viruses. From 15 to 30 minutes were required for complete inactivation of polioviruses 1 and 3; 4 minutes were required for poliovirus 2—a time required, under similar conditions (*2*), to inactivate Coxsackie A_2. Whether these differences are characteristic of the virus types tested or

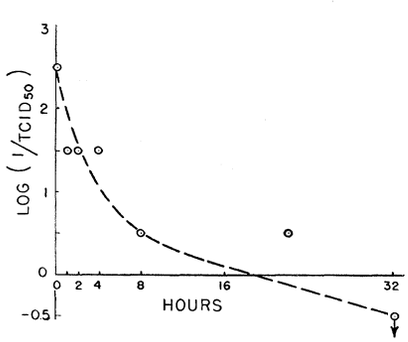

Fig. 2. Inactivation of poliovirus type 1 (Mahoney) by formaldehyde (1/4000 dilution) at *p*H 7.0 at 35°C in chlorine-demand-free water.

are dependent on the particular conditions chosen may be made clear by further studies.

The experiments described illustrate the rapidity (in minutes) with which chlorine inactivates viruses in a medium free of other oxidizable substances in comparison with time required (in hours) for inactivation under similar conditions by such common inactivating agents as formaldehyde (Fig. 2). They point out, conversely, and as recorded previously (*2*), that the chlorine residuals found to be sufficient (*3*) for bacterial disinfection are not similarly effective as viral disinfectants.

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2. N. A. Clarke and P. W. Kabler, *Am. J. Hyg.* 59, 119 (1954).
3. C. T. Butterfield *et al.*, *Influence of pH and Temperature on the Survival of Coliforms and Enteric Pathogens when Exposed to Free Chlorine, Public Health Repts.* 58, 1837 (1943).

1 July 1957

Binding of Histamine and Antihistamine to Bovine Serum Albumin by Mediation with Cu(II)

In the course of our work directed toward the evaluation of physical chemical data regarding protein-histamine-antihistamine interactions (*1*) we have been successful in forming a stable complex, *in vitro,* between copper(II)-bovine plasma albumin and histamine. The Cu(II)-bovine plasma albumin was also found to form a stable complex with the commercial antihistamine, Antistine (*2*).

The physiological role of histamine in allergy has been postulated (*3*) to involve the binding of histamine to a protein; however, recent *in vitro* experiments (*4*) have failed to show any measurable interaction between histamine and a series of purified animal proteins. The complexing of cupric ions to bovine plasma albumin has thus been interpreted as providing a site on the protein capable of binding histamine.

The technique of equilibrium dialysis was used to determine the extent of interaction between the Cu(II)-bovine plasma albumin and the binding molecule, histamine or antihistamine. The protein was crystalline bovine plasma albumin (*5*). The protein was dissolved in a phosphate buffer, placed in Visking 18/32 cellulose casing, and purified by out-dialysis at 0.0°C for 48 hours with the phosphate buffer. This buffer was prepared with reagent grade monobasic potassium phosphate and dibasic potassium phosphate. An ionic strength of 0.2 was maintained throughout the study. Buffer *p*H values of 6.95 and 8.90 could be obtained by using the proper proportions of the buffer constituents. Final protein concentrations were determined by drying aliquot portions of the purified protein solution at 105°C and correcting for the amount of buffer salt.

The copper-proteinate was prepared by adding the purified protein solution to a standard solution of cupric chloride and diluting it with buffer to a known volume. The concentration of copper in the standard solution was determined by electrolytic deposition.

The binding studies were conducted at 0.0°C. Ten milliliters of the copper-proteinate of known concentration was placed in the semipermeable membrane, which was then immersed in 20 ml of ligand solution. These cells were allowed to equilibrate for 24 hours. Simultaneously, blank cells were run in which the membrane contained only buffer, but they were immersed in the same ligand solutions. After equilibration, the difference in concentration between the blank cell and that containing the proteinate was directly proportional to the amount of ligand bound by the proteinate. Histamine concentrations were determined spectrophotometrically by means of a method developed in this laboratory (*6*). Antistine concentrations were determined spectrophotometrically at a wavelength of 240 mμ.

The calculation of the maximum number of moles of a given substance which

Table 1. Interaction of histamine and Antistine with Cu(II)-bovine plasma albumin at 0.0°C. (*A*) Represents the molar concentration of unbound ligand in equilibrium with the proteinate, and *r* represents the moles of ligand bound per mole of proteinate.

$(A) \times 10^5$	*r*	$(A) \times 10^5$	*r*
Histamine, pH 6.95			
4.94	0.273	18.0	0.671
7.06	0.347	19.0	0.980
9.22	0.454	22.4	1.351
13.8	0.621	42.5	6.250
Histamine, pH 8.90			
4.19	0.294	9.31	0.719
6.00	0.460	10.00	0.775
7.35	0.588	14.00	0.877
8.46	0.617	15.30	0.934
Antistine, pH 6.95			
3.03	0.155	10.25	0.357
5.67	0.191	12.14	0.529
7.46	0.253	19.96	0.689
8.04	0.315	22.57	0.917