Table 1. Distribution of lysogeny among *Streptomyces*. Plus sign indicates free phage present; minus sign, free phage absent; zero, not tested.

Strain	Free phage in	
	Autolysate	Phage-induced culture
S. griseus		
WAc-31	−	−
WAc-34	−	−
WAc-86	+	−
WAc-104	+	+
1945	+	−
1947	+	+
S. coelicolor		
WAc-16	−	0
WAc-133	−	0
WAc-135	−	0
S. cyaneus		
WAc-45	−	−
S. olivaceus		
WAc-11	+	+
Unidentified isolates		
247-A	+	+
247-M	+	+
247-Q	−	0
247-T	−	0
247-4	+	−
247-10	+	−

Some temperate phages were induced by the virulent phage, WSP-2 (*2*). These temperate phages were purified on hosts which WSP-2 could not attack. No other means of induction is known at present. By these three methods, nine of 17 strains tested were found to be lysogenic (Table 1). The presence of detectable phage in an autolysate was variable. One of a set of replica cultures frequently produced no free phage, whereas the other culture yielded as many as 10^5 bacteriophages per millimeter. Several of the temperate phages had similar host-ranges (Table 2).

Lysogeny is widespread among the *Streptomyces*. Many strains not now considered lysogenic will probably be found to contain temperate bacteriophage. Some of the temperate phages are able to attack the lysogenic strain from which they were derived. Apparently, lysogeny does not always confer complete resistance to the homologous temperate phage.

Table 2. Host-range of temperate *Streptomyces*-phages. Plus sign indicates lysis; minus sign, no effect.

Phage from	Hosts, WAc-				Host
	11 or 104	34	45	86	1945
WAc-104	+	−	−	+	−
1947	+	−	−	+	−
247-A	+	−	−	+	−
247-4	+	−	−	+	−
WAc-86	+	+	−	+	−
1945	+	+	−	+	−
WAc-11	+	−	−	+	+
247-M	−	−	−	+	−
247-10	+	+	+	−	+

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12 June 1957

Changes in Serum Proteins in Amphibian Metamorphosis

In a continuing survey of some of the biochemical changes which occur during amphibian metamorphosis (*1*), we have observed some remarkable alterations in the serum proteins during the transition of the tadpole to the frog. Significant changes in both the amount and the distribution of the proteins have been noted.

Our method of study involved utilization of a Spinco paper electrophoresis unit with Veronal buffer, *p*H 8.6, ionic strength 0.075, and containing 0.2 percent of Sterox SE (*2*). Blood samples were obtained by puncture of the conus arteriosus with a glass needle. Serum from the clotted blood was applied to Whatman 3-mm filter paper strips, in 10, 20, and 40 lambda portions, depending on the protein concentration of the serum. After electrophoresis (16 hours, 5 ma, 78 v) the strips were dyed with bromophenol blue, and patterns were prepared with a Spinco "Analytrol" (*3*).

Typical electrophoretic patterns of serum, obtained from the bullfrog, *Rana catesbiana*, at various stages in its life-cycle, are shown in Fig. 1. Additional data are summarized in Table 1. The serum of the young bullfrog tadpole showed a very low proportion of albumin (*4*) (about 10 percent of the total serum protein). The total serum protein concentration was also quite low (1.01 percent), as was the albumin/globulin (A/G) (*4*) ratio (0.11). After treatment with triiodothyronine, the relative percentage of albumin increased to about 16 percent (A/G, 0.25). The total serum protein concentration rose to 1.74 percent. Some displacement in the mobility of several protein fractions was also noted. A slow-moving fraction, with a mobility similar to human gamma globulin, was found present in the pattern for the tadpole treated with triiodothyronine. A fraction with a slightly greater mobility than this was present in the pattern for the froglet (a tadpole immediately after spontaneous metamorphosis). This latter fraction became pronounced in the adult frog.

The full extent of the changes which occur during the normal metamorphic period is seen in the data for froglets. In the froglet and the adult frog, the total serum protein concentration more than doubles over the level found in the young, undeveloped tadpole (1.01 percent to 2.56 percent), and the relative percentage of albumin increases to comparable values of 46 percent for the adult frog and 49 percent for the froglet.

Even more dramatic changes in serum proteins occurred during the development of the swamp frog, *Rana hecksheri*. As is indicated in Fig. 2, the serum pattern for most young *R. hecksheri* tadpoles showed no observable albumin fraction. As metamorphosis proceeded, an apparent (*4*) albumin fraction appears (A/G = 0.13). We have noted that a serum albumin fraction was also evident after induced metamorphosis with thyroid hormones. In the adult *R. hecksheri* frog the relative concentration of serum albumin reaches about 35 percent. The many interesting questions raised by the nature of the developmental changes in the protein components of serum in these

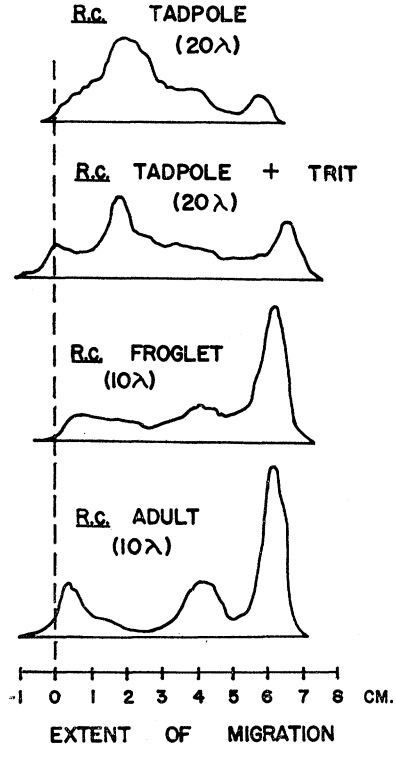

Fig. 1. Paper electrophoresis patterns of the bullfrog (*Rana catesbiana*) at various stages of development. Triiodothyronine-treated tadpoles were injected with 0.010 ml triiodothyronine ($10^{-8}M$) per gram of body weight 4 to 6 days (kept at 23° ± 1°C) prior to bleeding. The volume of serum used is indicated in parentheses.

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.

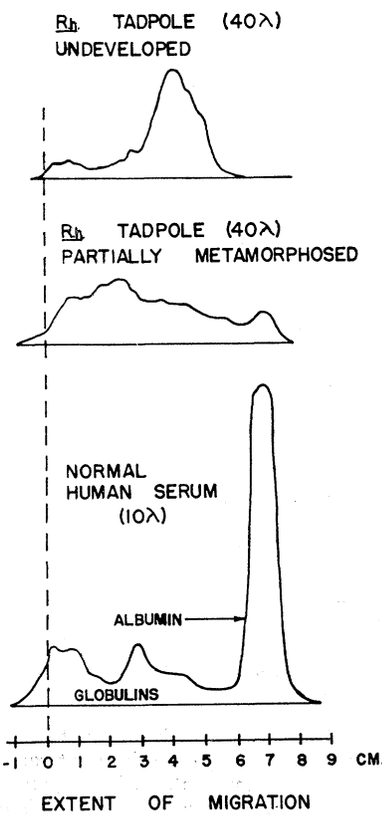

Fig. 2. Comparison of paper electrophoresis patterns of tadpoles of species *Rana hecksheri* with normal human serum pattern.

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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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.
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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