Hemoglobin: Molecular Changes during Anuran Metamorphosis

Abstract. *Anuran metamorphosis is accompanied by changes in the sedimentation patterns of the lysates of red blood cells of several species.* Rana grylio *and* Rana catesbeiana *tadpoles have hemoglobins that sediment at 4.3*S *(molecular weight, 68,000) and gradually produce a heavier 7.0*S *component (estimated molecular weight, 136,000) during metamorphosis to the adult frog. There is extensive change in the amino acid compositions of these hemoglobins; these changes may account for observed changes in electrophoretic mobility.*

The functional differences between tadpole and frog hemoglobins are well known (*1*, *2*) and have been recently summarized (*3*). Some of the differences in the chemical nature of tadpole and frog hemoglobins have only recently been investigated. Previously differences in electrophoretic mobility, rate of alkali denaturation, biosynthesis, and titratable SH groups have been noted (*4*, *5*). This report shows additional significant molecular changes of tadpole and frog hemoglobins.

In several species, metamorphosis is accompanied by a transition from an exclusive hemoglobin with a sedimentation constant of 4.3*S* in the tadpole to a mixture in the frog of hemoglobins that sediment at 4.3*S* and 7.0*S* plus a small amount upon occasion of a component sedimenting at 10 to 11*S*. Thus far, the 7.0*S* component appears to be a unique double hemoglobin molecule which is separate and noninterconvertible with the more typical 4.3*S* hemoglobin of molecular weight 68,000. Though multiple amphibian hemoglobins were reported earlier (*6*) in several European species and the principal 4.3*S* hemoglobin reported in *Rana catesbeiana* tadpoles (*1*), no correlation of the changes in sedimentation constants or molecular weights during metamorphosis has been made heretofore.

Table 1. Comparison of the properties of tadpole and frog hemoglobins.*

Property	Tadpole	Frog
a. pO_2 for 50% saturation	4 mm	14 mm
b. Bohr effect†	None	Typical
c. Heme spectrum	Same	Same
d. Tryptophan "fine structure" (about 290 mμ)	Definite peak	Weak shoulder
e. Methemoglobin formation	Resistant	Less resistant
f. Alkali denaturation	Resistant	Less resistant
g. Paper electrophoretic mobility	11 cm	7.5 cm
h. Starch block electrophoretic mobility	Fast	Slower
i. Sedimentation coefficients	(2.7); 4.3	4.3; 7.0 (*10*)
j. Average molecular weight	68,000	127,000
k. Amino acid analysis:		
Acidic in 68,000*g*	97	75
Basic in 68,000*g*‡	48	60
Ratio	2.00	1.25
1/2 Cystine	0–1	7–8

* Data for properties a, b, and c, see references (*1*) and (*2*); data for e, f, and g, see (*4*); data on other properties are from present work. Properties a, b, c, and g from *R. catesbeiana;* c to k from *R. grylio.* † A typical Bohr effect refers to the decrease in oxygen binding affinity as the *p*H is decreased. ‡ Histidine is omitted from this calculation since it is assumed to be essentially uncharged at *p*H 8.6.

The animals used in this investigation were tadpoles, froglets, and adults of the *R. grylio, R. pipiens, R. catesbeiana,* and *Xenopus laevis* (adult only) species. The red blood cell lysates were obtained essentially as described previously (*4*). The frogs were bled, and the red blood cells were then washed with isotonic saline solution (*p*H 7.8; ionic strength, 0.13), and lysed by freezing and thawing at least three times. The lysate was then centrifuged at 20,000*g* for 30 min. The supernatant solution was used full strength for the electrophoresis experiments and diluted to about 7.5g/1 for analyses in a Spinco model E ultracentrifuge. All molecular weights were calculated by the Archibald approach to equilibrium method (*7*), and all sedimentation constants were corrected to water at 20°C. Electrophoresis of these lysates of red blood cells was carried out on a starch block of nonhydrolyzed starch granules in a barbital buffer (Spinco buffer B-2), *p*H 8.6, and ionic strength of 0.04, with a constant current of 10 ma for 24 to 48 hours. After this time, the bands of hemoglobin were removed from the starch bed, transferred to a sintered-glass funnel, and layered with saline which quantitatively displaced all the red protein. After dialysis against 0.13 ionic strength saline (*p*H 7.8) or the addition of the calculated amount of sodium chloride, the hemoglobin solutions were ready for use.

Representative sedimentation patterns are shown in Fig. 1. Both *R. grylio* and *R. catesbeiana* showed a single 4.3*S* component (plus occasionally a slower moving component) in the tadpole. As the tadpole developed to the intermediate stages where the ratio of the lengths of the hind leg to the tail was more than 1.25, a component at 7.0*S* appeared which became predominant as

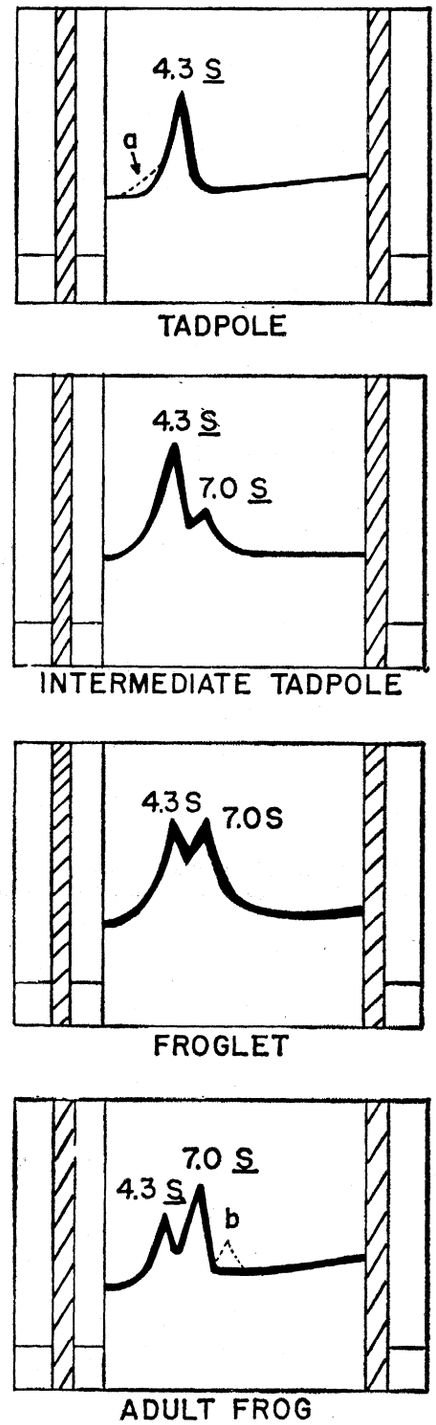

Fig. 1. Drawings made from photographs of sedimentation patterns during metamorphosis of *R. grylio.* Note the gradual decrease of the 4.3*S* component and the development of the 7.0*S* component as metamorphosis proceeds. The intermediate shown above is a tadpole with a hind-leg length to tail length ratio of 1.25; the froglet corresponds to an animal having just completed metamorphosis, that is, its tail is completely resorbed. Letters *a* and *b* denote the 2.7*S* and 10- to 11*S* components referred to in the text. Conditions: *p*H 7.8, ionic strength 0.13, concentration about 7g/liter, t = 64 minutes, 59,780 rev/min at T = 20°.

metamorphosis progressed. *R. pipiens* tadpoles had only the 4.3*S* component in its red blood cells and did not show the heavier 7.0*S* component; the adult *X. laevis* had only the 4.3*S* component initially, but apparently formed a limited amount of the 7.0*S* hemoglobin when the lysate of the red cells was stored under refrigeration for several days. Average molecular weights indicated 68,000 for the hemoglobin of *R. grylio* tadpoles and 68,000 and 136,000 for the two principal components for the frogs of this species.

Certain of the red blood cell components detected in the ultracentrifuge were separated by starch-block electrophoresis in sufficient quantities for further study and identification. The small amount (less than 10 percent) of the 2.7*S* and the predominant (more than 90 percent) 4.3*S* ultracentrifuge components of the red cells of the *R. grylio* tadpole corresponded respectively to the slower and faster moving bands visible on starch-block electrophoresis of the lysate. The 4.3*S* and 7.0*S* components of the red cells from the *R. grylio* adult corresponded to the faster and slower moving electrophoretic bands, respectively. When present in the cells of the adult, the 10*S* to 11*S* component is eluted with the band of 7.0*S* material. With respect to relative electrophoretic mobility, the slower (2.7*S*) tadpole component migrates at about the same rate as the faster (4.3*S*) frog component.

Amino acid analyses were made on the globins from the best available preparations of tadpole and frog hemoglobins. For the tadpole, analyses were obtained on two pooled lysates which had been centrifuged at 20,000*g* and one pooled sample of electrophoretically purified hemoglobin with comparable results. For the frog, amino acid data are averaged from analyses of the supernatant solution from the centrifugation at 20,000*g* of the washed, lysed red blood cells of three adults. The frog hemoglobin analyses thus include the 4.3*S*, 7.0*S*, and any 10*S* to 11*S* hemoglobins and any other component present in these lysates. Other protein components present are believed to be negligible. The globins were precipitated with a mixture of HCl and acetone, and hydrolyzed by refluxing with 6*N* HCl for 18 hours. Amino acid analyses expressed as residues per 68,000*g* by the Moore-Stein technique (*8*) for tadpole and frog were, respectively: arginine 18, 26; lysine 30, 34; histidine 29, 41; aspartic acid 53, 39; glutamic acid 44, 36; methionine 2, 5; and ½ cystine 0 to 1, 7 to 8. There was a significant increase in dibasic amino acids and a decrease in dicarboxylic amino acids in the transition of tadpole to frog. Also very striking is the increase of half-cystine residues in general agreement with the observations of Riggs (*5*). The changes in amino acid composition are in accord with the experimentally observed differences in rates of migration of these hemoglobins on both starch and paper (see Table 1, properties g, h, and k). It is evident that there is an extensive alteration in amino acid composition of hemoglobin chains during metamorphosis.

Spectrophotometric studies confirmed the absence of any differences in the visible region of the spectrum (*4*). Frog red blood cell lysates resisted methemoglobin formation less readily than did lysates from tadpole cells under identical conditions of storage. A large increase in absorption at 540 mμ compared to 570 mμ and the appearance of the 625 mμ peak characteristic of methemoglobin was observed. The ultraviolet spectrum shows a decrease in the intensity of the tryptophan band at about 289 to 291 mμ during metamorphosis accompanied by a shift to longer wave length characteristic of fetal to adult hemoglobins (*9*).

The now numerous differences in tadpole and frog hemoglobins are summarized in Table 1. Our data would indicate there are at least two principal hemoglobins in the adult *R. grylio* and one in the tadpole. In these experiments the 7.0*S* component does not appear to be interconvertible with the 4.3*S* fraction as evidenced by separate sedimentation experiments with varying protein concentration and increasing ionic strength. The sedimentation patterns for adult *R. grylio* and *R. catesbeiana* are the same whether observed one hour or five days after bleeding, in contrast to adult *X. laevis* samples. Furthermore, the individual components isolated electrophoretically have never given evidence of dimerization or splitting to form the component removed by electrophoresis even after storage for more than 30 days at 5°C. Since there are two or more hemoglobins, this poses some interesting questions regarding their biosynthesis; for example, whether the 7.0*S* hemoglobin, which presumably is an octamer, contains any peptide chains in common with any of the chains of the 4.3*S* tetramers (*10*).

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Geothermal Brine Well: Mile-Deep Drill Hole May Tap Ore-Bearing Magmatic Water and Rocks Undergoing Metamorphism

Abstract. *A deep geothermal well in California has tapped a very saline brine extraordinarily high in heavy metals and other rare elements; copper and silver are precipitated during brine production. Preliminary evidence suggests that the brine may be pure magmatic water and an active ore-forming solution. Metamorphism of relatively young rocks may also be occurring within accessible depths.*

A geothermal well has been drilled recently in Southern California into a geologic environment that can only be described in spectacular terms. The well was drilled for geothermal power near Niland, close to the Salton Sea in the Imperial Valley, during the winter of 1961–62. It is 5232 feet deep (*1*) and is the deepest well in the world today in the high-temperature hot spring areas. In the lower half of the hole, temperatures are too high to measure with available equipment, but they are at least 270°C and may even reach 370°C. For