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Changes in hemoglobin during metamorphosis of the Salamander

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CHANGES IN HEMOGLOBIN DURING METAMORPHOSIS OF THE SALAMANDER

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INTRODUCTION

Hemoglobin is a prime constituent of the erythrocytes of vertebrate animals. The functional importance of hemoglobin lies in its capacity for reversible combination with oxygen.

Investigations of the physical and chemical properties of hemoglobins indicate that among any given species there are varied forms of hemoglobin. The adult hemoglobin is usually of one form while the immature or fetal stage is of another. In addition, mutant variations are frequently found (Manwell, 1957).

Most studies of the adult-fetal relationships have been conducted on the mammalian system; very little has been done with the amphibians. A review of the literature shows that no work has been done on salamander hemoglobin.

The research reported here is a study of the heterogeneous forms of hemoglobin observed in one species of salamander, Ambystoma tigrinum, and the change in synthesis of hemoglobin types during metamorphosis.

Literature Review

Hemoglobin heterogeneity has been shown in many species, both vertebrate and invertebrate (Gratzer and Allison, 1960; Bangham and Lehmann, 1958; Dunlap, Johnson and Farmer, 1956).

Electrophoretic methods have shown up to four components in human adult hemoglobin (Hb A), some of which vary in quantity in pathological conditions such as thalassemia (Kunkel, et al., 1957; Schapira,

et al., 1958; Kunkel and Bearn, 1957). Some pathological conditions are due to an alteration in the amino acid sequence in one or more of the globin chains which accounts for the observed variations. Hunt (1959) distinguished human adult hemoglobin from the fetal hemoglobin on the basis of one amino acid difference in one of the two polypeptide chains.

The important functional difference between adult and fetal hemoglobins in both amphibian and mammalian systems is the higher oxygen affinity exhibited by the fetal hemoglobin (McCarthy, 1954; Barron and Bataglia, 1955; Hamada, et al., 1964). In addition to fetal hemoglobin (Hb F) Halbrecht et al. (1956, 1958, 1959) have demonstrated the presence of an "early fetal hemoglobin" termed "HbP", during the first few weeks of the development of human embryos (allison, 1955). A similar situation also exists in the goat (Helm et al., 1958). Various anuran amphibian hemoglobins have been shown, by electrophoretic techniques, to be heterogeneous (Dessauer, Fox and Ramirez, 1957; Rodnan and Ebaugh, 1957; Sydenstricker, et al., 1956).

McCutcheon (1938) found that hemoglobins with a greater oxygen affinity (presumably fetal hemoglobins) are synthesized in the mesonephros of the tadpole whereas the adult form of hemoglobin is synthesized in the spleen and bone marrow in the mature bullfrog. In addition to a developmental change, McCutcheon also found a seasonal change in the hemoglobin of the bullfrog, Rana catesbiana. In the summer months the bullfrog's bone marrow synthesizes the hemoglobin with a low oxygen affinity; during hibernation, synthesis shifts to the splenic production of a hemoglobin which has a higher affinity for oxygen.

MATERIALS AND METHODS

Salamanders (Ambystoma tigrinum) were collected from two locations in northern Utah in different stages of development. A group from Salamander Lake, on the north side of Mount Timpanogos, was collected from the first of May through the last of August and included three stages of development: adult, gilled (with legs), and tadpoles. Another group was collected from James Lake, in the Uinta Mountains, during the last week of July. This group consisted of only adult and gilled with legs; no tadpoles were obtained because of the lateness of the season. All of the gilled forms from both locations lost their gills within a week after they were brought into the laboratory. This has been attributed to the change of temperature (Kollros, 1959).

Blood Extraction Procedure Blood was collected in the following manner: the animals were injected (ip) with a heparin solution (0.5 ml/100 gms of blood). They were anesthetized with Sandoz MS-222 (1:500) and the pericardial sac was opened to form a pocket. One drop of the heparin solution was placed in the cavity and the ventricle was cut. The blood was withdrawn with a heparinized pipet and deposited in chilled tubes containing 3 drops of heparin solution.

Hemoglobin Purification All steps were carried out at a temperature of 0 to 4°C. The whole blood was centrifuged at 3,000 RPM (1500 X g) for 3 minutes and the supernatant plasma was discarded. The cells were then

washed with 0.15 NaCl solution (5 volumes per volume of compact cells). The cell solution was centrifuged for 3 minutes at 3,000 RPM (1500 X g) and the supernate was discarded. The cells were washed in this manner four times.

The washed erythrocytes were lysed with distilled water by letting them stand in 2 volumes of water per volume of compact cells for 10 minutes. The hemoglobin solution was then centrifuged at 15,000 RPM (26,000 X g) for 30 minutes. Toluene was added to the hemoglobin solution ($\frac{1}{4}$ ml. toluene per ml. hemoglobin) and shaken for 3 minutes, then centrifuged for 3 minutes at 1,000 RPM. The clear hemoglobin solution was removed and checked for purity by adding to an aliquot of solution an equal volume of saturated ammonium sulfate. No precipitate occured, indicating that all stroma had been removed.

It has been shown that difficulty in analysis is encountered because of the unstable condition of the heme portion of oxyhemoglobin. In order to acertain heterogeneity due to the protein portion it is necessary to convert the oxyhemoglobin to either ferrihemoglobin or the more stable cyanomethemoglobin (Chernoff and Pettit, 1964; Allen, Schroeder, Balog, 1958; Itano and Robinson, 1957).

The hemoglobin solution was converted to the ferrihemoglobin form by dialyzing against a buffered ferricyanide solution (equal volumes of .01 M phosphate at pH 7.0 and .01 M potassium ferricyanide). In some cases the ferrihemoglobin was converted to cyanomethemoglobin by dializing against buffered sodium syanide (equal volumes of .01 M phosphate and .01 M sodium cyanide). Our work indicated there was no alteration in

heterogeneity when ferrihemoglobin was used instead of cyanomethemoglobin.

Separation of Hemoglobin Types

(a) Column Chromatography

Separations were conducted on a carboxy-methyl-cellulose column (CMC). The column was prepared by adding a slurry of CMC in .01 M phosphate buffer (pH 6) to a 6 X 50 mm. glass column. A larger column (9 X 55 cm) was prepared to compare the efficiency of the small column with a larger system (Huisman and Meyering, 1960). The columns were packed by gravity flow, then equilibrated with a .01 M phosphate buffer at pH 6.0. Elution with starting buffer proceeded until the pH of the effluent was equal to that of the influent.

The purified hemoglobin solution was placed on the column and eluted initially with .01 M phosphate buffer, pH 6.0. Phosphate buffer at pH 8.0 was gradually added which caused a gradual increase in pH of influent. The effluent passed through a micro flow cell which was placed in a Beckman "DB-G" spectrophotometer. The optical density pattern of effluent at 414 mu was recorded with a Photovolt Varicord 43 recorder.

The pH of the effluent was read and recorded manually as it passed through a Beckman micro-blood-pH-electrode assembly. Fractions were collected from each of the observed peaks and scanned spectrophotometrically from 660 mu to 340 mu in order to evaluate whether or not the peaks and shoulders observed were, in fact, hemoglobin.

(b) Electrophoresis

Electrophoretic separation of hemoglobin components was accomplished on Sepraphore III (Gelman) acetate strips. The strips were equilibrated overnight in the starting buffer. Approximately 5-10 lambda of the hemoglobin solutions were placed on the strips and were subjected

to a D.C. current. Best separation was achieved in a pH 7.2 phosphate buffer at 250 volts for 60 minutes. The strips were removed and air dried, stained in Ponceau R for at least 5 minutes, then washed in 5% acetic acid for 5-15 minutes; removed and dried. After drying, the strips were cleared with immersion oil, placed in a Gelman strip scanner and the migration patterns were plotted.

RESULTS

Column Chromatography Figures 1 through 14 represent the optical density pattern at 414 mu of the hemoglobin effluent related to the pH change. The two major components are labeled L (larval) and A (adult). The first fraction was eluted from the column between pH 6.0 and 6.1. Since the protein in this fraction decreased in concentration during organogenesis it was labeled the larval component. The second major component was eluted from the column between pH 6.4 and 7.1. It increases during maturation and is termed the adult component. This component may consist of several proteins because it appears as a cluster of peaks, but these sub-fractions could not be characterized with this technique.

The hemoglobin elution pattern obtained from the small column of CMC (Fig. 1) is essentially the same as that obtained with the large column (Fig. 2). One of the major differences between the two patterns is the shoulder on peak A (Fig. 1) which appears as a distinct peak in Figure 2. Despite this difference, the ability of the CMC column to fractionate hemoglobin is not significantly altered when the bed volumn is reduced to accommodate the small quantity of hemoglobin.

Figures 3 and 4 represent additional adult salamanders. The major portion of the hemoglobin is represented by a single peak, labeled adult hemoglobin. The larval portion is about 3% of the total. In all adult forms examined (Figs. 1 through 4) there is very little variation in the basic elution pattern. The minor differences appear primarily in the relative quantities of material in the adult component as compared to

the larval fraction. There is also a slight variation in the height of the shoulders of both peaks, particularly in peak A. In spite of these variations the number of shoulders remain constant.

Figures 5 through 8 represent the elution pattern obtained from salamanders shortly after metamorphosis. There is a difference in the relative amounts of adult and larval hemoglobin as compared to the older stage. Larval hemoglobin constitutes about 13% in the recently-metamorphosed young adult. The pattern in all specimens examined is basically the same. The minor variations present in the adult peak are the same as were shown in the mature animal. The presence of a double peak in the larval component (Figs. 7 and 8) is found in post metamorphic, adult, and gilled but not in the tadpole samples.

Gilled salamanders (Figs. 9 through 12) show a further difference toward an increase in the relative amount of larval hemoglobin, in some cases approaching 50%.

The number of samples of tadpole hemoglobin was limited, so only two samples were examined (Figs. 13 and 14). The larval peak is predominant in both. Possibly because of the limited amount of blood available in the tadpole, the shoulders previously noted are not detected in peak A.

Fractions from each peak were collected and scanned with the spectrophotometer from 660 to 340 mu. The pattern (Fig. 15) is typical for all fractions examined. The absorption maxima observed are characteristic of cyanomethemoglobin, with lambda max. at 540 mu and 414 mu in the beta and gamma bands and no absorption in the alpha band.

The area under each peak (Figs. 1 through 14) was measured to find the relative percent of hemoglobin present in both the adult and larval peaks. These values were tabulated (Table 1) and plotted (Fig.19) for better comparison of hemoglobin heterogeneity with the stage of development.

TABLE 1

PERCENT COMPOSITION OF HEMOGLOBIN
AT VARIOUS STAGES OF DEVELOPMENT

	Individual		Group Average	
Stage	Larva	Adult	Larva	Adult
Adult	0.5%	99.5%	3.0%	97.0%
	8.5	91.5		
	2.5	97.5		
	2.5	97.5		
	0.5	99.5		
	3.5	16.5		
Post Gilled	11.0%	89.0%	13.5%	86.5%
	11.6	88.4		
	5.0	95.0		
	17.0	83.0		
	19.9	80.1		
	15.0	85.0		
Gilled	37.5%	62.5%	38.3%	61.7%
	48.7	51.3		
	36.7	63.3		
	30.0	70.0		
	40.5	59.5		
	36.0	64.0		
Tadpole	98.1%	1.9%	98.3%	1.7%
	98.5	1.5		

Electrophoresis Electrophoretic separation of salamander hemoglobin (Figs. 16, 17 and 18) at pH 7.2, indicates there are at least three components of salamander hemoglobin. Figure 16 is the pattern obtained from an adult animal. The major hemoglobin portion is concentrated into two bands, one remaining at the origin and the other migrating a very short distance from the origin. The third component moves much faster and comprises the minor percentage of hemoglobin at this stage. It seems likely that the fast moving minor component represents larval hemoglobin (Compare Figs. 1 through 4 with Fig. 16).

The hemoglobin obtained shrtly after metamorphosis (Fig. 17) also has three distinct bands. There is a higher concentration in the faster moving fraction and a slightly reduced quantity in the slower moving and stationary bands (Compare Figs. 5 through 8 with Fig. 17)

The gilled salamander (Fig. 18) shows a very significant increase in the amount of material in the fast moving component and a very distinct decrease in the pigment remaining at the origin (Compare Figs. 9 through 12 with Fig. 18).

No electrophoretic pattern was obtained for tadpole hemoglobin due to the limited quantity of material available.

The electropherograms reveal that salamander hemoglobin is heterogeneous, consisting of two or more components which vary in relative amounts during maturation.

DISCUSSION

The volume of blood that can be obtained from a small larval salamander is quite limited, so it was necessary to use a chromatographic column with a reduced bed volume for this study. The concentration of the hemoglobin solution varied from 0.5 gms% to 2.0 gms%, which is very dilute. A literature review indicated that carboxy-methyl-cellulose (CMC) would probably give the best separation pattern. Because of the weak reaction between the protein and the resin there would be a minimal amount of alteration of the hemoglobin molecule (Prins, 1959).

The elution pattern indicates that the small column was as effective in hemoglobin separation as the large column (Fig. 1 and 2). Therefore, a small column could be used with confidence.

Salamander hemoglobin occurs in a heterogeneous form as indicated by the various peaks on the chromatograms. Spectrophotometric analysis of each peak exhibited the same absorption pattern and there was no shift of the lambda max. from fraction to fraction. This would indicate that the heterogeneity observed was not due to incomplete oxidation of the oxyhemoglobin to the methemoglobin-cyanide, but rather to differences in the globin moieties.

Several adult salamander's hemoglobin samples were then chromatographed and the elution patterns were very similar in all cases (Figs. 3 and 4), with minor variations in the hemoglobin concentration in the minor peaks. These minor peaks and shoulders show a fairly consistent

pattern within the various samples, but these patterns could not be correlated with organogenesis.

The pattern of peaks and shoulders was also similar in the groups obtained from the two geographically isolated regions. Therefore, it does not provide evidence of evolutionary divergence.

Electropherograms (Figs. 16,17 and 18) show a shift in hemoglobin type with development. In the adult form the greatest concentration of material designated Adult (A), is at the origin and in the slow moving band. Just the opposite is noted in the gilled (Fig. 18) where the greater concentration of hemoglobin is in the faster moving component labeled Larval (L). The post gilled stage (Fig. 17), which is intermediate between the adult and gilled forms, shows hemoglobin distribution intermediate between the adult and youngest stage. It seems likely, because of the shift in pattern with development, that the rapidly moving component represents the same hemoglobin component as that designated as larval in the chromatographic pattern.

It is essential to note, in the electropherograms, the presence of at least three distinct components in all three developmental stages. These may reflect some of the heterogeneity noted in the minor peaks of the chromatograms.

The most notable feature that emerges from a study of the elution patterns and electropherograms of the various stages is the shift from one form of hemoglobin (Hb Larval) in the larval stages to another form (Hb Adult) in the adult stage. Hemoglobin in the tadpole is 98.3% of the larval type and 1.7% of the adult variety. The gilled salamander,

a slightly older stage, has approximately 38.3% larval hemoglobin and 61.7% adult. Shortly after the salamander loses its gills the ratio is shifted even further to an average of 13.5% larval; 86.5% adult. Mature adults, at least a year after metamorphosis, show approximately 3% larval hemoglobin and 97% adult type. It seems apparent that these changes in hemoglobin production reflect changes in genetic control of protein synthesis.

In order to acertain the possible difference between the adult and larval form it is necessary to consider the type of reactions which occur in the experimental methods employed. Carboxy-methyl-cellulose, at an acid pH, will bind positively charged particles, represented in this experiment by hemoglobin. By gradually increasing the pH the more weakly bound particles, which would be the most negatively charged particles would be removed first. The more positively charged particles would be eluted later as the pH is increased.

When employing electrophoresis a slightly basic pH of 7.2 is used and the results are similar to those previously obtained at pH 8.6 for frogs (Herner and Frieden, 1961). At this pH (7.2) the negative charges are contributed by the second carboxyl group of aspartic and glutamic acids (Scheinberg, 1958). Since the charge is the same on all hemes the separation must be due to the difference in amino acid content of the globins. The adult form, having less aspartic and glutamic acids, would be more positively charged, therefore causing a greater rate of migration toward the cathode. The aspartic and glutamic acid concentration would be greater in the fetal form which accounts for the slow mobility at this pH.

It has been noted that in most mammalian fetus' the principal hemoglobin (Hb F) has an affinity for oxygen that is better adapted to intra-uterine life than is the hemoglobin of the adult (Hb A). It is proposed that the similar shift in hemoglobin types in the salamander reflects adaptations to an aquatic, gill-breathing existance at first, changing gradually to a terrestrial, lung-breathing environment. No tests have been made to substantiate this hypothesis.

SUMMARY AND CONCLUSIONS

The results of this investigation indicate that a change in the production of hemoglobin from a larval to an adult type occurs in the metamorphosing salamander. The tadpole erythrocytes contain less than 2 percent of the adult hemoglobin. With an increase in age there is a gradual increase in the adult type and a decrease in the larval hemoglobin form. The time of greatest change is between the tadpole and the gilled stage, when the production of the adult hemoglobin increases from less than 2 percent to an average of 61 percent. This is correlated with a change from gill to lung breathing. By the time the gills are lost the hemoglobin is 86 percent of the adult type, and one year later, in the completely mature animal the proportion of adult type is over 95 percent of the total.

PLATE I

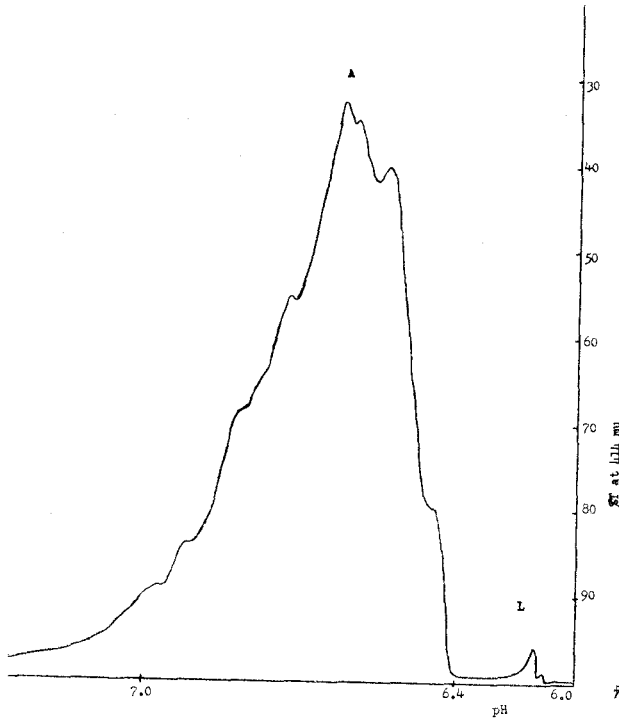

Fig. 1. Adult salamander hemoglobin elution pattern.

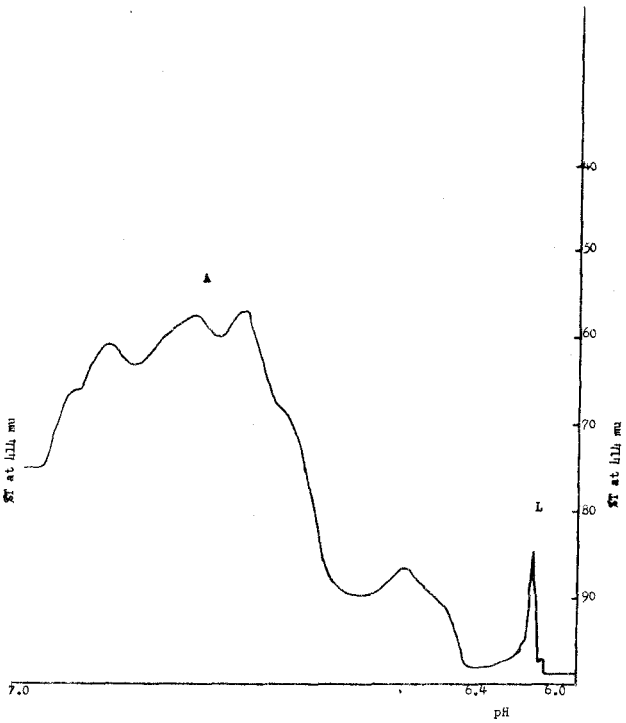

Fig. 2. Adult salamander hemoglobin elution pattern

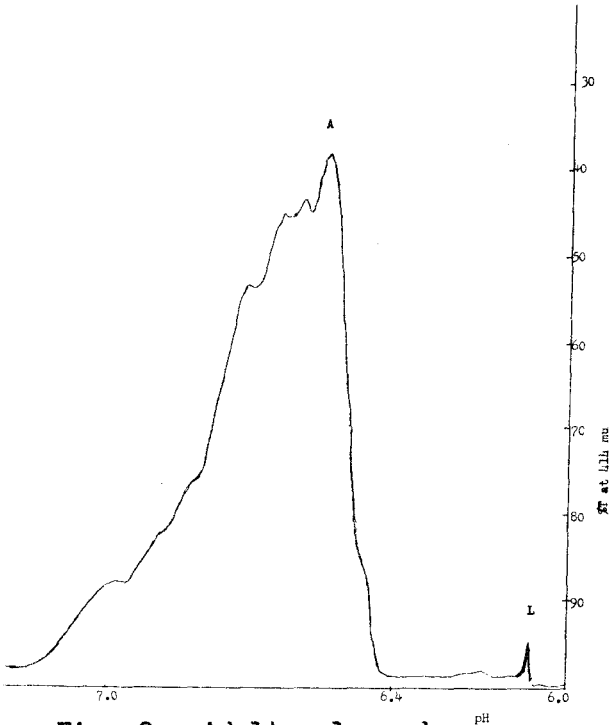

Fig. 3. Adult salamander hemoglobin elution pattern

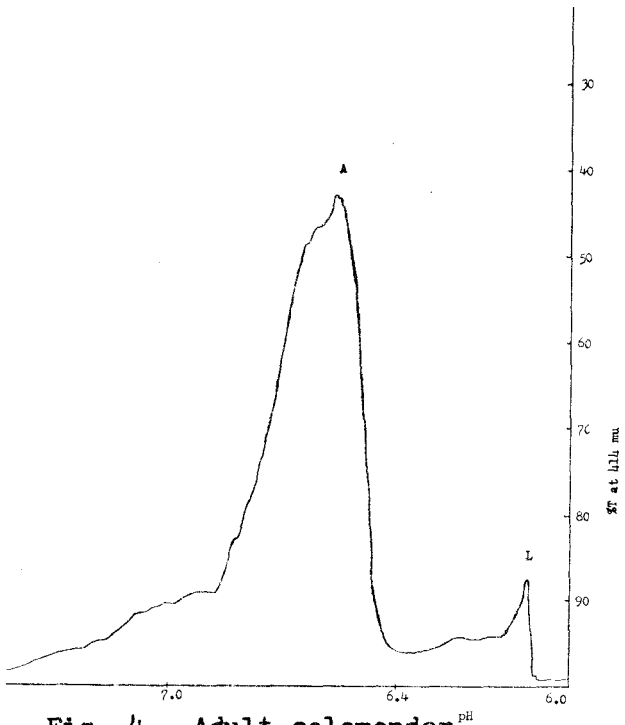

Fig. 4. Adult salamander hemoglobin elution pattern

PLATE II

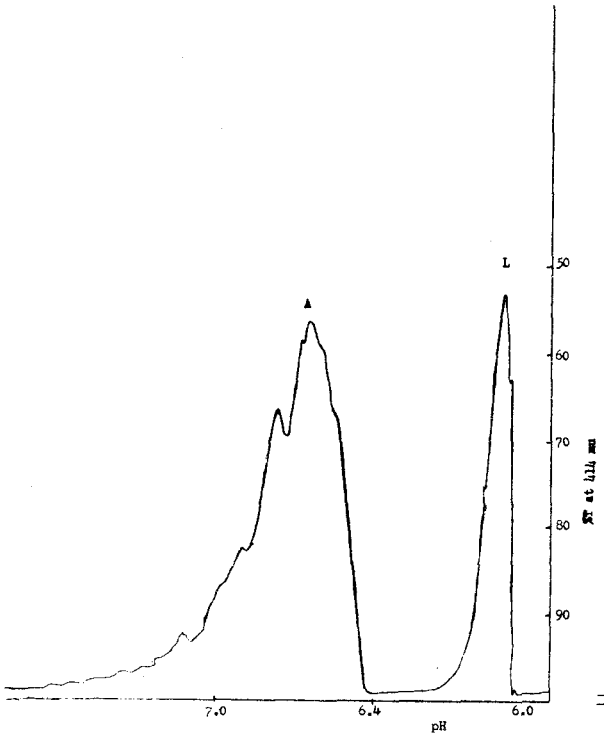

Fig. 5. Post Gilled salamander hemoglobin elution pattern.

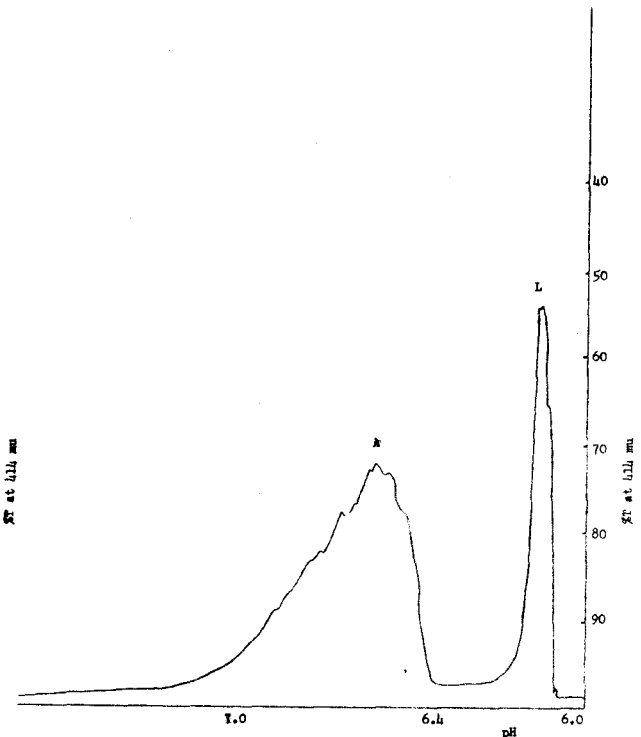

Fig. 6. Post Gilled salamander hemoglobin elution pattern.

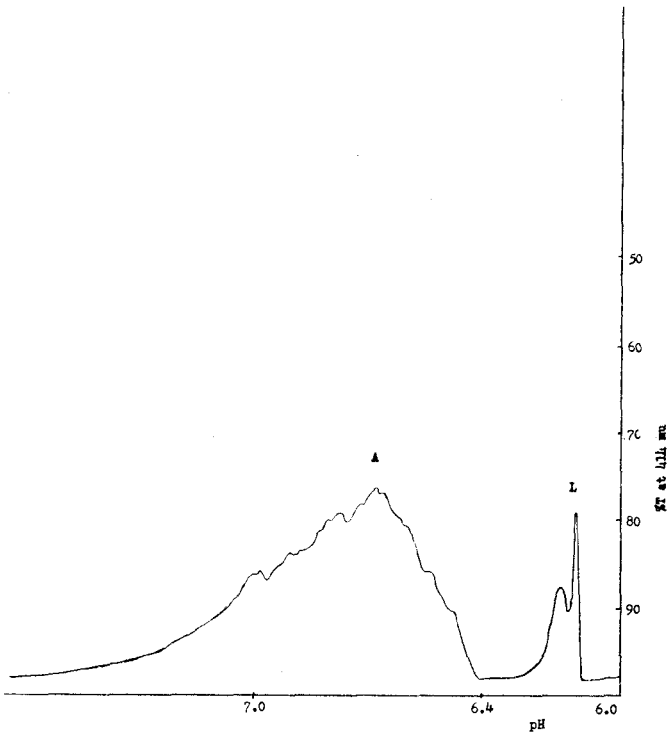

Fig. 7. Post Gilled salamander hemoglobin elution pattern.

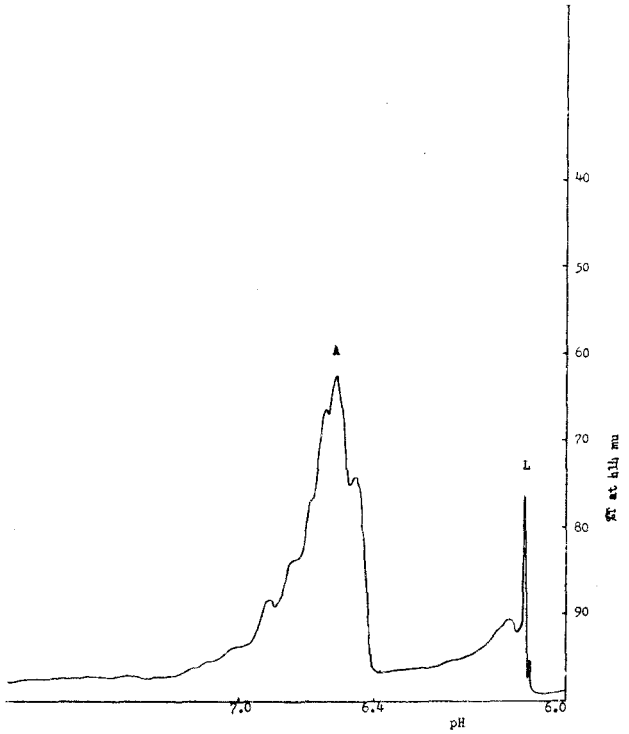

Fig. 8. Post Gilled salamander hemoglobin elution pattern.

PLATE III

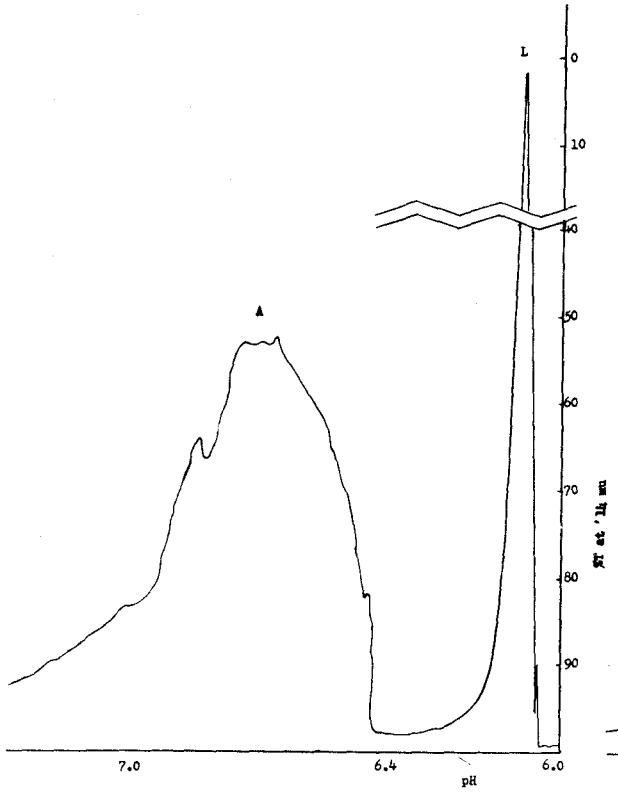

Fig. 9. Gilled salamander hemoglobin elution pattern.

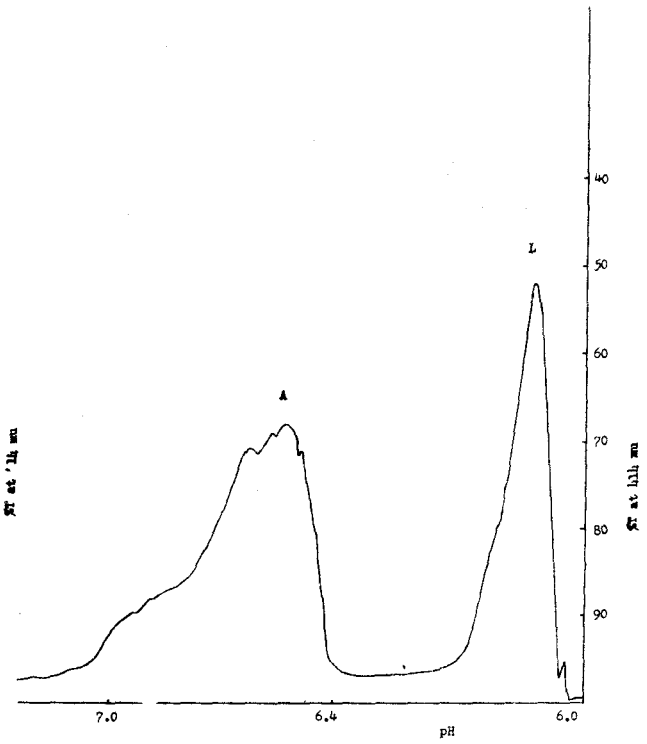

Fig. 10. Gilled salamander hemoglobin elution pattern.

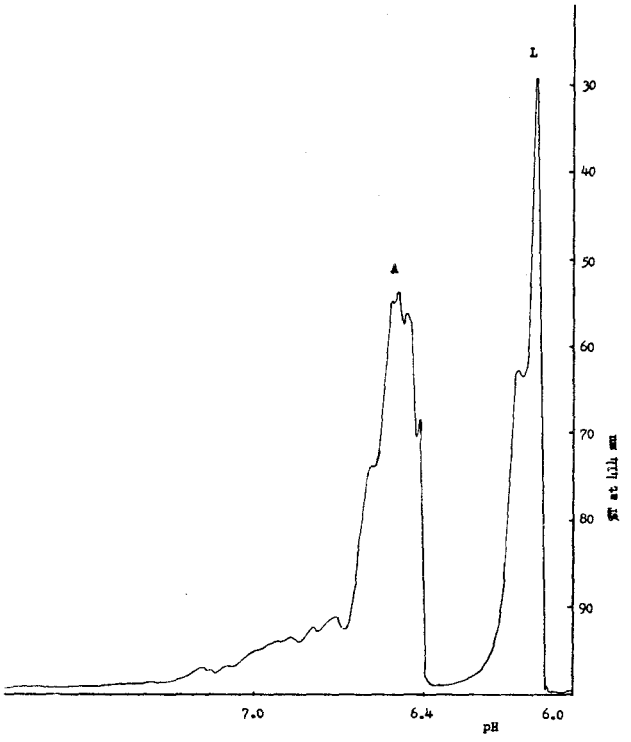

Fig. 11. Gilled salamander hemoglobin elution pattern.

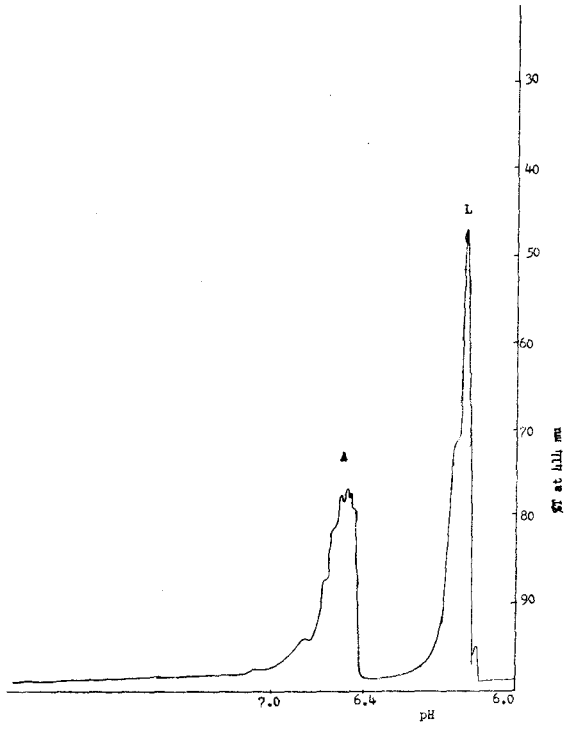

Fig. 12. Gilled salamander hemoglobin elution pattern.

PLATE IV

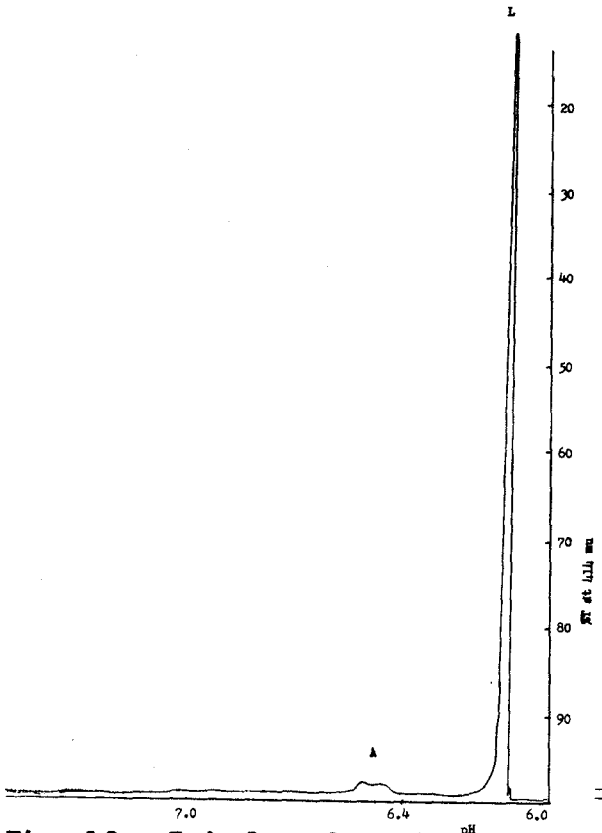

Fig. 13. Tadpole salamander hemoglobin elution pattern.

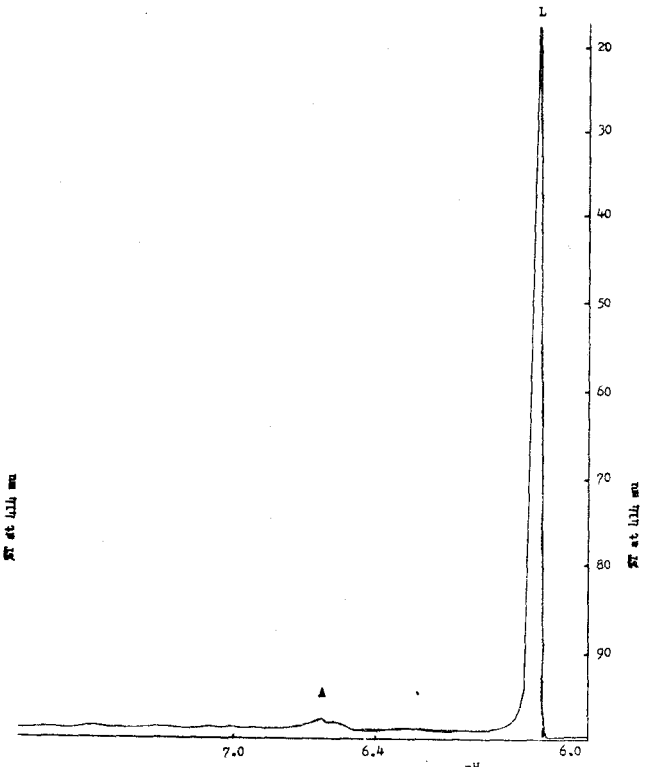

Fig. 14. Tadpole salamander hemoglobin elution pattern.

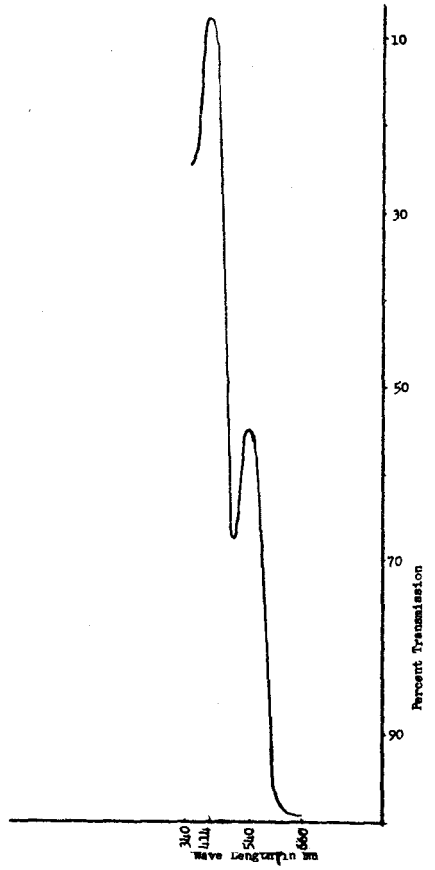

Fig. 15. Typical hemoglobin spectra.

Fig. 16. Adult salamander hemoglobin electropherogram.

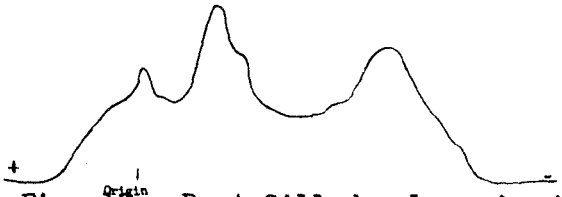

Fig. 17. Post Gilled salamander hemoglobin electropher- ogram.

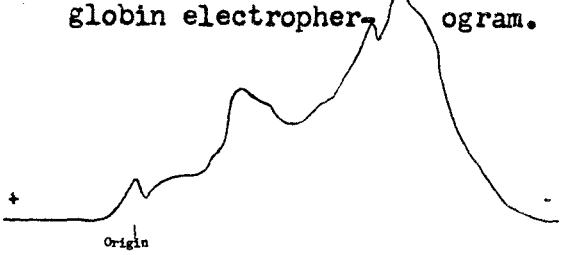

Fig. 18. Gilled salamander hemoglobin electropherogram.

PLATE V

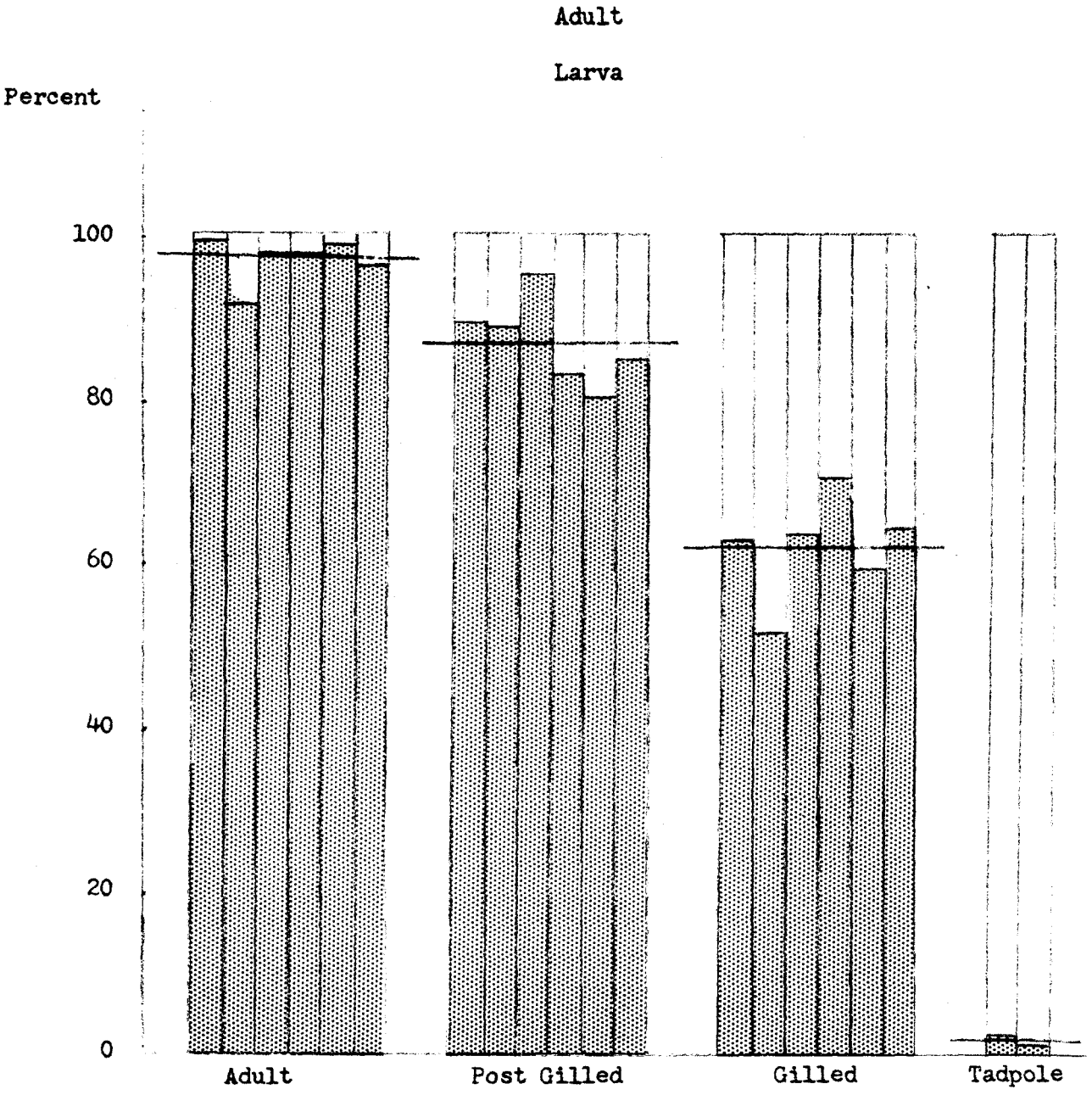

Fig. 19. Salamander hemoglobin. Percent adult vervus percent larva.

REFERENCES

Allen, D.W.; Schroeder, W.A.; Balog, J. 1958. Observations on the chromatographic heterogeneity of normal adult and fetal human hemoglobin: A study of the effects of crystallization and chromatography on the heterogeneity and isoleucine content. J. Am. Chem. Soc. 80:1628.

Allison, A.C. 1955. Notation for hemoglobin types and genes controlling their synthesis. Science 122:640-41.

Baglioni, C.; Sparks, C.E. 1963. A study of hemoglobin differentiation in Rana catesbiana. Dev. Biol. 8:272-285.

Bangham, A.D., and Lehmann, H. 1958. "Multiple" haemoglobins in the horse. Nature 181:267-68.

Barron, D.H. and Bataglia, F. G. 1955. The oxygen concentration gradient between the plasmas in the maternal and fetal capillaries of the placenta of rabbits. Yale J. Biol. Med. 28:197-207.

Chernoff, A.I.; Pettit. 1964. Some notes on starch-gell electrophoresis of hemoglobins. J. Lab. Clin. Med. 63(2):290.

Chernoff, A.I. 1965. The amino acid composition of hemoglobin IV. The preparation of pure polypeptide chain of human hemoglobin. J. Chromatog. 17:140-148.

Dessauer, H.C.; Fox, W.; and Ramirez, F.R. 1957. Preliminary attempt to correlate paper-electrophoresis migration of hemoglobins with phylogeny in amphibia and reptilia. Arch. Biochem. Biophys. 71:11-16.

Dunlap, J.S.; Johnson, V.L.; and Farmer, D.S. 1956. Multiple hemoglobins in birds. Experientia. 12:352-53.

Gratzer, W.B.; Allison, A.C. 1960. Multiple hemoglobins. Biol. Rev. 35:495-506.

Halbrecht, I. and Klibanski, C. 1956. Identification of a new normal embryonic haemoglobin. Nature 178:794-95.

Halbrecht, I.; Klibanski, C.; Broqoqa, H. and Lahav, M. 1958. Further studies on the various hemoglobins and the serum protein fractions in early embryonic life. Am. J. Clin. Pthol. 29:340-344.

Halbrecht, I.; Klibanski, C.; and Barilan, F. 1959. Co-existence of the embryonic (third normal haemoglobin fraction with erythroblastosis in the blood of two full-term newborn babies with multiple malformations. Nature 183:327-28.

Hamada, K.; Sakai, Y.; Shukuya, R. and Kaziro, K. 1964. Biochemical metamorphosis of hemoglobin in Rana catesbiana I. Purification procedures and properties of hemoglobin from bullfrog and tadpole erythrocytes. J. Biochem. (Tokyo) 55(6)636-42.

Helm, J.J.; Van Der; Visser, H.H.A.; Vliet, G. Van and Huisman, T.H.J. 1958. Some investigations dealing with animal haemoglobins. Clin. Chem. Acta. 3:114-19.

Herner, A.E.; Frieden, E. 1961. Biochemical changes during anuran metamorphosis VIII. Changes in the nature of red cell proteins. Arch. Biochem. Biophys. 95:25-35.

Huisman, T.H.J.; Meyering, C.A. 1960. Studies on the heterogeneity of hemoglobin I. Heterogeneity of different human hemoglobin types in

carboxy-methyl-cellulose and in amberlite IRC-50. Chromatography Qualitative Aspects. Clin. Chim Acta. 5:103-123.

Hunt, J.A. 1959. Identity of the -chain of adult and foetal human haemoglobins. Nature 183:1373-75.

Itano, H.A. and Robinson, E. 1957. Hemoglobin Intermediates. Fed. Proc. 16(3):199.

Kollros, J.J. 1959. Thyroid gland function in developing cold-blooded vertebrates. In:Grobman, B Ed. Comp. Endocrinology N.Y., Wiley & Sons p. 340.

Kunkel, H.G.; Ceppellini, R.; Muller-Eberhard, U.; and Wold, J. 1957. Observations on the minor basic hemoglobin components in the blood of normal individuals and patients with thalassemia. J. Clin. Invest. 36:1615-25.

Kunkel, H.G. and Bearn, A.G. 1957. Minor hemoglobin components of normal human blood. Fed. Proc. 16:760-62.

Manwell, C. 1957. The respiratory pigments (Doctoral thesis, Stanford University, Stanford, California).

McCarthy, E.F. 1954. Hemoglobin and Iron in the fetal and newborn mammal. Cold Spring Harbor Symposia Quant. Biol. 19:133-40.

McCutcheon, F.H. 1936. Hemoglobin function during the life history of the bullfrog. J. Cellular Comp. Physiol. 8:63:81.

McCutcheon, F. H. 1938. The oxygen affinity of hemoglobin in splenectomized bullfrogs. J. Expt'l Biol. 15:431-36.

Prins, H.K. 1959. The separation of different types of human haemoglobin. J. Chromatography 2:445-486.

Riggs, A.J. 1951. The metamorphosis of hemoglobins in the bullfrog. J. Gen. Physiol. 36:1-16.

Rodnan, G.P. and Ebaugh, F.G. Jr. 1957. Paper electrophoresis of animal hemoglobins. Prod. Soc. Expt'l. Biol. Med. 95:397:401.

Schapira, G.; Drefus, J.C. and Kruh, J. 1958. Heterogeneity of hemoglobin and methods of isotope biosynthesis. Nat. Acad. Sci. Nat'l Research Council, Publ. No. 557:201-11.

Scheinberg, H. 1958. The structural Basis of differences in electrophoretic behavior of human hemoglobins. Conference on hemoglobin. Nat. Acad. Sci. Pub. 557:227.

Sydenstricker, V.P.; Oliver, R.; Chandler, B.M. and Sydenstricker, O. 1956. Electrophoretic behavior of some animal hemoglobins. Proc. Soc. Expt'l. Biol. Med. 93:396-97.

ABSTRACT

Analysis of hemoglobins were conducted on animals of the species Ambystoma tigrinum. Separation of hemoglobin types was performed with a Carboxy-methyl-cellulose column (6 X 50 mm.) and pH gradient (pH 6 thru 8) of .01 M phosphate buffer. The hemoglobins in the effluent were detected and analysed with a micro-flow cell in a Beckman DB-G spectrophotometer attached to a Photovolt Varicord 43 recorder.

Two major components of hemoglobin were observed: larval and adult. These two components varied in concentration with the stage of development of the individual. In addition, it was noted that minor peaks and shoulders appeared on each peak which were consistant and characteristic for each major hemoglobin type.