Hemoglobins of Two Urodeles: Changes with Metamorphosis (34251)

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Changes in hemoglobin patterns during anuran metamorphosis are well documented (1, 2), but there is no information on changes during urodele metamorphosis. This report deals with the electrophoretic patterns of the hemoglobins from two closely related species of urodeles, the Mexican axolotl, *Ambystoma mexicanum* and the spotted salamander, *Ambystoma maculatum*. Spontaneous metamorphosis is extremely rare in the laboratory reared Mexican axolotl which remains neotenous due to a functional inadequacy of the pituitary–thyroid axis (3, 4). This inadequacy can be overcome by exogenous thyroxine (5) or by hybridization of the axolotl with a metamorphosing ambystomid salamander, such as *Ambystoma tigrinum* (6). The spotted salamander normally undergoes metamorphosis in nature.

Methods. Metamorphosis was induced in adult axolotls from our inbred colony by a single intramuscular injection of 50 μg of sodium levothyroxine (Synthroid, Flint Laboratories) in 0.5 ml of 10% Steinberg's solution (5, 7). Metamorphosis occurred approximately 45 days after injection. Spotted salamander larvae and adults were collected from a pond south of Buffalo, New York. The adults were killed soon after collection to obtain blood for electrophoresis. The larvae were reared in the laboratory until they reached sufficient size to collect the blood easily, but before any indication of metamorphosis was apparent.

Blood was collected by cardiac puncture or decapitation using heparinized saline as anticoagulant. The red cells were washed with normal saline and then hemolyzed by the addition of distilled water. The resulting hemolyzate was shaken with carbon tetrachloride and centrifuged to removed stroma. Vertical starch gel electrophoresis of the fresh hemolyzate was carried out in a Tris–EDTA buffer, pH 8.6, as described by Weatherall (8). The gels were stained for protein using amido black or stained for hemoglobin using benzidine. The gels shown in Fig. 1a and 1c were stained with amido black but the gel shown in Fig. 1b was stained with benzidine since the applied hemolyzate in this case was rather dilute as a result of difficulty in obtaining sufficient blood from the unmetamorphosed spotted salamander. A hemolyzate from a normal human was included in each gel as a reference as it was not possible to run all test hemolyzates fresh on the same date.

Results and Discussion. Figure 1a demonstrates the electrophoretic pattern of the hemolyzates from the axolotl before and after induced metamorphosis. As shown, no change has occurred in the hemoglobin pattern, there being two major bands, one migrating cathodal to and the other anodal to human hemoglobin A. Figure 1b shows the electrophoretic pattern of the hemolyzate from the spotted salamander before metamorphoris and Fig. 1c the pattern after metamorphosis. Also included for comparison in Fig. 1c is the hemolyzate from the metamorphosed axolotl. The hemoglobin pattern of the spotted salamander changes. Before metamorphosis there is a rapidly migrating band anodal to human hemoglobin A which disappears after metamorphosis. Comparing the hemoglobin pattern of the axolotl and the spotted salamander prior to metamorphosis it is apparent that the patterns are different. In the axolotl the major band is anodal to human hemoglobin A whereas the major band in the

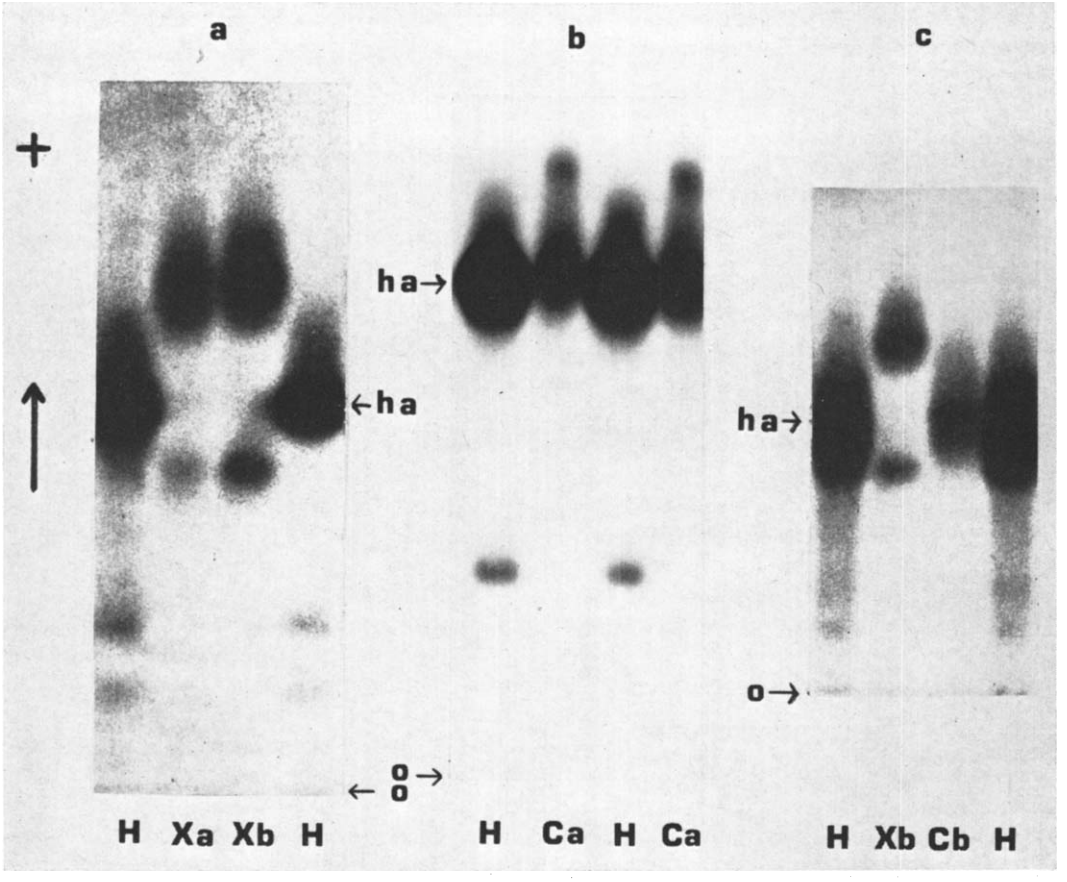

FIG. 1. Vertical starch gel electrophoresis of hemolyzates from normal human adult (H), the Mexican axolotl before (Xa) and after (Xb) metamorphosis, and the spotted salamander before (Ca) and after (Cb) metamorphosis. Sections (a) and (c) stained for protein with amido black; section (b) stained for hemoglobin with benzidine. O = origin; ha = human hemoglobin A.

spotted salamander has approximately the same mobility as human hemoglobin A.

The hemoglobin pattern of all amphibia previously studied changes during metamorphosis. Although information on other urodeles is not available we have demonstrated that the hemoglobin pattern of the spotted salamander also changes with natural metamorphosis, whereas that of the axolotl does not change with induced metamorphosis. This suggests that there is either a fundamental difference between natural and induced metamorphosis or that in the axolotl not only the genetic information to metamorphose spontaneously but also the genetic information to change its hemoglobin pattern during metamorphosis has been lost or has become inactive. As the transition from tadpole to adult hemoglobin in the frog occurs irrespective of whether the metamorphosis is natural or induced by thyroid hormone (2) the latter suggestion seems the more likely. Further work on the metamorphic changes in the hemoglobins of the Mexican axolotl and other urodeles may help to elucidate the control mechanisms governing the switch from embryonic to adult hemoglobin synthesis.

Summary. The hemoglobin pattern of the Mexican axolotl does not change with induced metamorphosis. In contrast, that of the related spotted salamander does change with natural metamorphosis. The genetic information required for metamorphic changes in hemoglobin pattern as well as that for

spontaneous metamorphosis may have been lost or become inactive in laboratory strains of the Mexican axolotl.

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