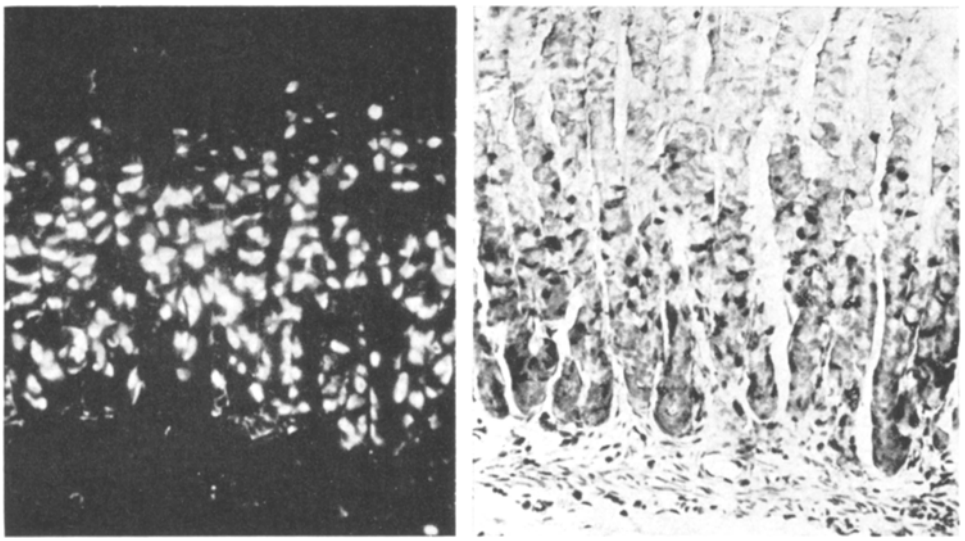

Fig. 2. Section from the same region of the stomach wall as in Figure 1, rat pretreated with L-DOPA. Formaldehyde-induced green fluorescence (left) in the enterochromaffin-like cells which have been induced to store dopamine. The argyrophil cells appearing after silver-staining are identical with dopamine-containing enterochromaffin-like cells. × 140.

affin-like) cells of the murine gastric mucosa are 2 different cell systems can be summarized as follows: (1) There is no evidence that argyrophil cells in this part of the gastrointestinal tract are ever transformed into argentaffin ones; the latter cell type is extremely rare in the oxyntic gland area, which on the other hand is very rich in argyrophil cells. (2) Argyrophil, non-argentaffin cells contain histamine and histidine decarboxylase; argentaffin cells do not. (3) Argentaffin cells are rich in DOPA decarboxylase; this enzyme is present also in the argyrophil, non-argentaffin cells but in considerably lower concentrations.

Nevertheless, the 2 systems of enterochromaffin and enterochromaffin-like cells have several properties in common – general morphology, all the characteristic features of protein-secreting cells, the capacity to produce and store amines – which may imply a similar function of these cells. It has been suggested that both the argentaffin and the argyrophil (non-argentaffin) cells of the digestive tract are endocrine in nature[14–18] and that they may be active in producing polypeptide hormones such as gastrin, secretin, pancreozymin-cholecystokinin and possibly also glucagon.

It should be pointed out that although the 2 endocrine cell systems of gastric mucosa are cytochemically different, the argyrophil but non-argentaffin cells observed elsewhere in the digestive tract may perhaps still be referred to as pre-argentaffin or as argentaffin cells temporarily devoid of their reducing cytoplasmic material[19].

Zusammenfassung. Durch fluoreszenzmikroskopische Untersuchung und nachfolgende Silberfärbung an ein und demselben Schnitt konnte gezeigt werden, dass das System argyrophiler, nicht argentaffiner Zellen in der Magenschleimhaut der Ratte mit demjenigen histaminspeichernder Epithelzellen identisch ist. Die histaminspeichernden Zellen, die argyrophil (aber nicht argentaffin) sind, bilden ein Zellsystem, das von dem der enterochromaffinen, serotoninhaltigen Zellen getrennt ist. Diese enterochromaffinen Epithelzellen sind im Gegensatz zu den histaminhaltigen Zellen sowohl argyrophil als auch argentaffin.

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Departments of Pharmacology and Anatomy, University of Lund (Sweden), 16 January 1969.

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The Investigation of *Xenopus laevis* Hemoglobins During Development by a Fluorescent Antibody

Analysis of hemoglobin solutions prepared from the blood of young tadpoles and mature adults of the South African Clawed Toad, *Xenopus laevis*, show that there are separate types of hemoglobin present in the tadpoles and adult animals respectively. Solutions of hemoglobin prepared from erythrocytes by osmotic shock were subjected to column chromatography on Whatman Chromedia CM52 carboxymethyl cellulose. The samples were eluted in a 0.01 *M* sodium phosphate pH gradient and eluent fractions were assayed for pH and optical absorption at 410 nm wavelength. Hemoglobin from *Xenopus* tadpoles emerges from the column in 2 elution peaks, at pH 6.50 and pH 6.82 respectively. The hemoglobin from adults emerges in 2 major and 1 minor peak at pH 7.41, pH 7.53, and pH 7.81 respectively. If an artificial mixture of hemoglobin solutions from adult and tadpole *Xenopus* is chromatographed, 5 elution peaks are seen, coincident with the 2 tadpole and the 3 adult peaks respectively.

Hemoglobin solutions from tadpole and adult *Xenopus* were also subjected to polyacrylamide gel disc electrophoresis in a *Tris*-Borate-EDTA buffer system at pH 7.8. The tadpole hemoglobin separated into 2 bands, one moving towards the anode more quickly than the other,

and hemoglobin from adult toads remained in one band, migrating at a speed intermediate between those of the 2 tadpole bands.

These observations indicate that separate hemoglobins exist in adult and tadpole *Xenopus* individuals. It is proposed to call the hemoglobin or hemoglobins found in adult *Xenopus* '*Xenopus*-HbA', and that which is found in tadpoles '*Xenopus*-HbF'. The presence of differing adult and tadpole hemoglobins has been described in the bullfrog, *Rana catesbiana*[1–10].

Hemoglobin solutions were also prepared from *Xenopus* individuals in various stages of metamorphosis. The solutions were each chromatographed separately on carboxymethyl cellulose as described above. In animals in early stages of metamorphosis only *Xenopus*-HbF was found to be present; in later stages *Xenopus*-HbA could be detected, and the amount of *Xenopus*-HbA progressively increased as metamorphosis proceeded until it comprised all the hemoglobin eluted. By summating the optical density values at wavelength 410 nm for each of the 2 tadpole and 3 adult elution peaks, a quantitative measurement of the relative amounts of *Xenopus*-HbF and *Xenopus*-HbA present at any one stage could be made. These results, expressed as percentages, are shown as crosses in Figure 1. It will be seen that during metamorphosis there is a progressive depletion in the amount of *Xenopus*-HbF present from 100% at the stage when the tail is finally lost to less than 2% at the 24 mm mouth-anus length. The relative proportion of *Xenopus*-HbA correspondingly increases during this period.

It would be of great interest to know if all, or almost all, the erythrocytes in circulation started to manufacture *Xenopus*-HbA during metamorphosis, or whether the *Xenopus*-HbA represented in the chromatographs is exclusively confined to a separate population of cells distinct from those which contain only *Xenopus*-HbF and which are presumably older. To investigate this problem a fluorescent antibody against *Xenopus*-HbA was prepared.

Fluorescent antibodies have been successfully prepared against human foetal hemoglobin[11–12], and against human adult and foetal hemoglobins[13]. The technique of DAN and HAGIWARA[13] has been adopted, with some modifications, by the present authors.

Erythrocytes from mature adult *Xenopus* were lysed by osmotic shock in distilled water. The resulting hemoglobin solution was used as the antigen, its concentration having previously been determined by comparison with cyanmethemoglobin standards. A solution containing 40 mg of this *Xenopus*-HbA in 2 ml of water was emulsified with an equal volume of Freund's adjuvant and was injected s.c. into a female New Zealand White rabbit. After 10 days 20 mg of *Xenopus*-HbA, precipitated with potassium aluminium sulphate after the method of PROOM[14], and resuspended in 1 ml 0.85% saline, was injected i.m. This dose of alum-precipitated hemoglobin was repeated 11 times at three-day intervals, followed by one 100 mg i.p. injection of alum-precipitated hemoglobin 14 days after the final i.m. injection. On each of the 2 succeeding days 10 mg of *Xenopus*-HbA in aqueous solution, concentration 20 mg/ml, was given i.v., and the rabbit was bled by superficial venesection of the ear 7 days later.

The serum was separated from the blood cells by allowing it to stand overnight at 2 °C, and the γ_G-globulin component was separated by adsorbing unwanted plasma proteins onto Whatman DE52 diethyl-aminoethyl cellulose equilibrated at pH 7.5[15].

The γ_G-globulin was used for precipitin-ring tests with the *Xenopus*-HbA antigen: precipitates were formed within 2 min with the antigen at a dilution of $^1/_{256}$ its original concentration of 20 mg/ml. No precipitates were formed when *Xenopus*-HbF was used instead of *Xenopus*-HbA. Ouchterlony plates confirmed this specificity of the antibody. Two precipitin lines appeared between the γ_G-globulin and the *Xenopus*-HbA in all the plates made, but no lines could be detected between the globulin and the *Xenopus*-HbF.

The γ_G-globulin antibody protein was conjugated with fluorescein-isothiocyanate (FITC) after the method of NAIRN[16]. In an attempt to remove any non-specific fluorescent protein the preparation was treated with pig-liver powder[17]. Smears of blood cells from *Xenopus*

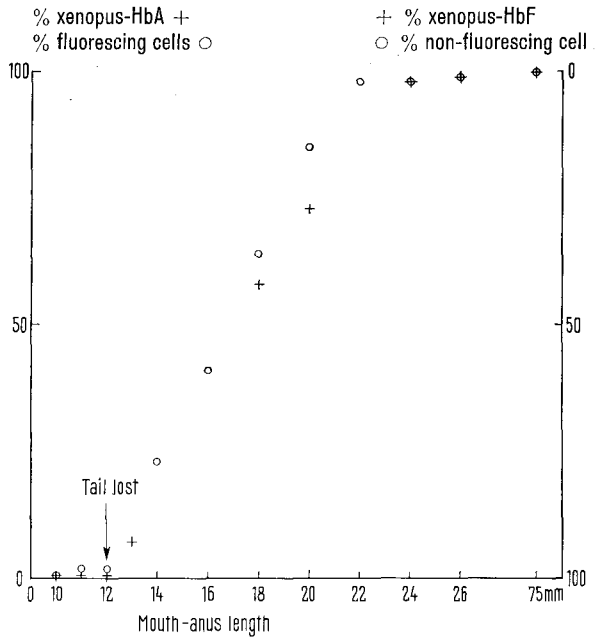

Fig. 1. Relative proportions of *Xenopus*-HbA and *Xenopus*-HbF in animals of various sizes as revealed by column chromatography. Relative numbers of cells fluorescing when blood smears taken from animals of various sizes are treated with anti-*Xenopus*-HbA-FITC.

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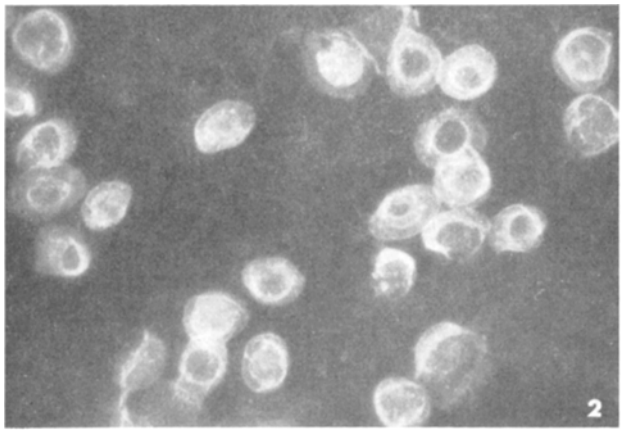

Fig. 2. Blood cells from a mature adult *Xenopus* treated with anti-*Xenopus*-HbA-FITC.

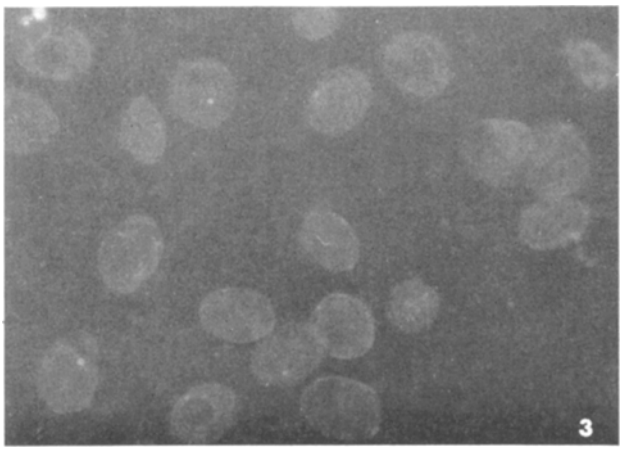

Fig. 3. Blood cells from a young *Xenopus* tadpole treated with anti-*Xenopus*-HbA-FITC.

adults and tadpoles were made, the cells having been first washed in RUGH's Ringer solution[18]. When the smears were dry they were soaked in 0.85% saline, buffered with 0.01 *M* sodium phosphate at pH 7.1, for 1 h. A drop of the fluorescent antibody was placed on the cells and allowed to interact with them and their contents for 30 min. The smears were then thoroughly washed in the buffered saline to remove surplus antibody before mounting in buffered glycerol.

The antibody-treated blood smears were viewed under UV-light. Cells taken from adult *Xenopus* were seen to fluoresce brightly: the dye was taken into the cytoplasm, and the nucleus was visible as a dark region surrounded by a very brightly fluorescing ring of cytoplasm (Figure 2). Cells taken from young tadpoles showed only a very dim fluorescence over their whole surfaces; it was almost impossible to distinguish any nuclei in these cells (Figure 3). Artificial mixtures were prepared containing known proportions of adult and tadpole erythrocytes. In smears made from such mixtures and treated with the antibody it was found that the proportion of the cells fluorescing corresponded with the known proportion of adult cells present.

A second rabbit was immunized as above against *Xenopus*-HbA, and the γ_G-globulin prepared from it was conjugated with the sulphonyl chloride of lissamine rhodamine B (RB200SC)[16]. Orange fluorescence was

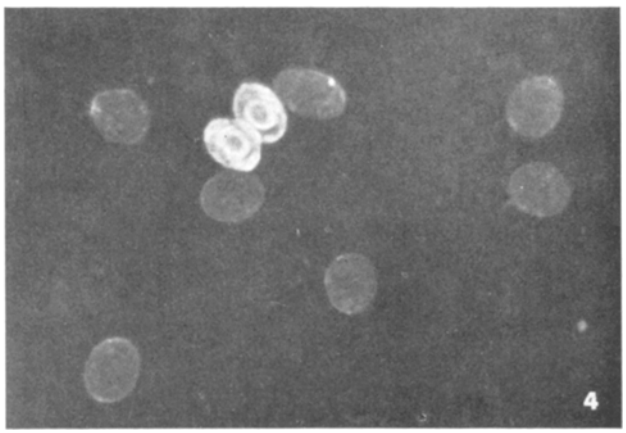

Fig. 4. Blood cells taken from a recently metamorphosed toad, 14 mm tail-anus, and treated with anti-*Xenopus*-HbA-FITC.

observed in adult *Xenopus* erythrocytes treated with this conjugate, but not in tadpole cells.

From these results it was concluded that an antibody specific against *Xenopus*-HbA had been prepared. This was called anti-*Xenopus*-HbA-FITC or anti-*Xenopus*-HbA-RB200SC according to whether it was conjugated with FITC or RB200SC respectively.

Smears from erythrocytes were made from the blood of toads in various stages of metamorphosis. These smears were treated with anti-*Xenopus*-HbA-FITC and the proportion of cells fluorescing was noted on each smear. One such smear of blood cells from a metamorphosing toad is shown in Figure 4. The circular points on the graph in Figure 1 show the proportion of blood cells which fluoresce in smears made from toads in a number of different stages of metamorphosis. It will be seen that the percentage of cells containing *Xenopus*-HbA as indicated by fluorescence is slightly greater than the percentage of *Xenopus*-HbA present at the same stage in metamorphosis as indicated by column chromatography. We hope to discover, by the preparation and use of a fluorescent antibody against *Xenopus*-HbF whether this discrepancy is caused by some of the cells containing both hemoglobins.

If smears from metamorphosing animals are treated with anti-*Xenopus*-HbA-RB200SC, the proportions of fluorescing cells are identical, $\pm 3\%$, with those obtained using anti-*Xenopus*-HbA-FITC[19].

Zusammenfassung. Bei *Xenopus laevis* findet man im Kaulquappenstadium 2 fötale Hämoglobine (HbF) und nach der Metamorphose 3 neue Hämoglobine (HbA) vom Erwachsenentypus. Immunofluoreszenz-Untersuchungen lassen erkennen, dass während der Metamorphose keine oder nur wenige Erythrozyten gleichzeitig HbF und HbA enthalten.

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