Gel preparation: The tray took about 40 ml of gel prepared with 1200 mg hydrolyzed starch (Sigma Chemical Co., Missouri, USA), 300 mg bacto agar (Difco Laboratories, Michigan, USA) and 40 mg saponin (Merck) to 45 ml Tris-edta-boric acid pH 8.6. This solution was transfered to a 500-ml pyrex flask, mixed well, and its contents heated over a flame. After complete dissolution of the starch agar, the air bubbles were removed by vacuum pump. The starch agar gel was poured into the horizontal tray fitted with the upper frame. The gelling was allowed to proceed at room temperature for 10 min.

Application of whole blood: The applications were made using blades 0.15 cm wide. The blades were wet with about 0.3 µl of blood, and inserted directly into the starch agar gel 2 cm away from the side that was in contact with the cathode. The distance between samples was 0.2 cm.

Electrophoresis procedure and staining of haemoglobins. Contact between the gel and the inner electrolyte was achieved by means of a piece of Whatman 3 MM chromatography paper on either side. The electrophoresis was carried out at 4 °C using 300 V for 45 min. The tray was then removed from the refrigerator and stained with 2% benzidine in 0.5% acetic acid.

*Results and discussion.* All abnormal haemoglobins tested, with different amino acid substitutions, showed their characteristic position in the electrophoresis systems described here (figure), when compared with starch gel and cellulose acetate. The Hb M Boston, whose substitution does not alter the electrophoretic mobility at alkaline pH, was recognized by the gray appearance of the band during the electrophoresis procedure. The separation of Hb F increased from Hb A and the identification of Hb $A_2$, were possible after staining with benzidine. Routine use of this method in our laboratory has shown that it is a simple and reliable method for identifying the presence of normal and abnormal haemoglobins. It requires only 0.3 µl of sample and it provides highly specific confirmation of the presence of haemoglobins $A_1$, $A_2$, F, S, C, M Boston, D Punjab and I. Furthermore, it has been sensitive enough to detect reproducibly cases of beta thalassaemia major and minor, revealing the increase of Hb F and Hb $A_2$. The combination of agar and starch produced an ideal gel for electrophoretic separation of human haemoglobin. Furthermore this method is more rapid than electrophoresis performed on starch gel or cellulose acetate, and equally sensitive.

Undoubtedly, the use of whole bloof for the electrophoresis procedure, without previous preparation of a haemolysate, which is made possible by the inclusion of saponin in the starch agar gel, makes screening for haemoglobinopathies easier. The results demonstrate that saponin does not alter the consistency of the gel or alter the electrophoretic behaviour of normal and abnormal haemoglobins.

The analysis of about 100 samples by each electrophoretic procedure showed this to be a suitable and rapid method for study of human haemoglobins in a large population.

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## Chromatin organization within nuclear blebs in leukocytes of *Xenopus laevis*

N. Chegini and N. Maclean[1]

*Department of Biology, Medical and Biological Sciences Building, University of Southampton, Bassett Crescent East, Southampton (England), 19 September 1979*

*Summary.* The nuclei from leukocytes of peripheral blood, liver and spleen of an individual anaemic *Xenopus laevis* have been found to possess numerous nuclear blebs or projections. These structures were found to be very variable in size and shape as viewed in electron micrographs, but commonly included an enclosed mass of cytoplasm bound on one side by a very thin section of nuclear material. Such sections are membrane bounded on each side and frequently display an interesting ordered array of chromatin.

Nuclear blebs or projections have been previously recorded and described, both in cells associated with certain pathologies[2-5] and in apparently normal cells[6-8]. All these observations have been made on mammalian cells. We here describe nuclear blebs found in many cells of liver, spleen and peripheral blood of a single specimen of *Xenopus laevis*, previously rendered anaemic by phenylhydrazine injection. The chromatin organization within these blebs is particularly noteworthy.

*Material and methods.* Mature *Xenopus laevis* were obtained from Harris Biological supplies (Weston-Super-Mare) and maintained as previously described[9]. Animals were made anaemic by phenylhydrazine injection using the method of Thomas and Maclean[10]. Samples of liver, spleen and buffy coat[11] were taken from anaemic *Xenopus* 11 days after the last injection, following anaesthetizing with MS222 (Sandoz Ltd, London). The tissue pieces and buffy coat were fixed, embedded and stained for Electron microscopy as previously described[12].

*Results and discussion.* Only 1 anaemic animal was found to have cell nuclei affected by blebs, and this phenomenon was found in all tissues examined though each has been separately prepared. We think it is unlikely that phenylhydrazine treatment initiated the appearance of nuclear blebbing, both because it is not apparent in other anaemic animals so treated, and because there is probably insufficient time since induction of anaemia for all of these cells to arise from a monoclonal origin. About 5% of all leukocytes were found to have at least 1 bleb per EM section. The form of the blebs is highly variable, but each consists of a very thin layer of nuclear material, bound on

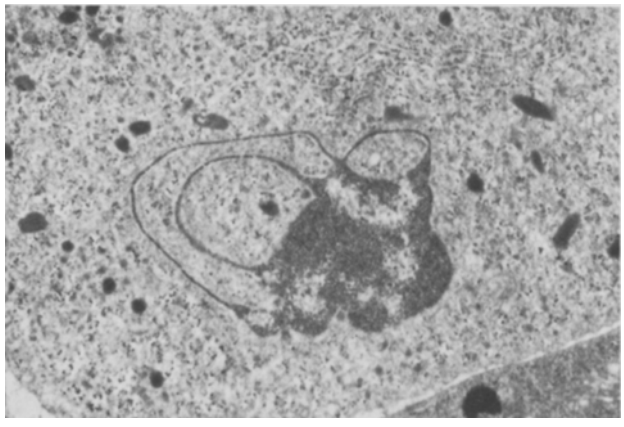

Fig. 1. Electron micrographs of a leukocyte from liver fixed first in glutaraldehyde and then with osmium tetroxide. Double stained with uranyl acetate and lead citrate. × 11,000.

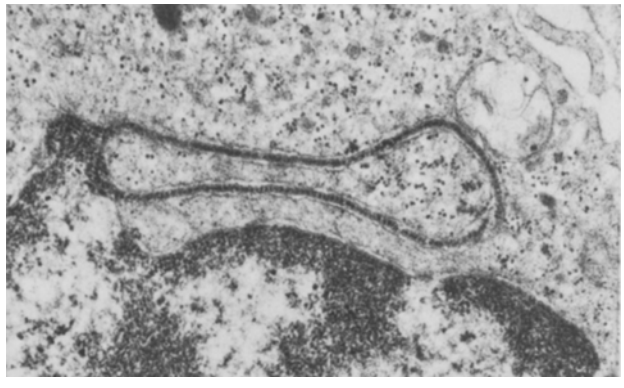

Fig. 2. Electronmicrograph of part of leukocyte from liver fixed first in glutaraldehyde and then with osmium tetroxide. Double stained with uranyl acetate and lead citrate. × 21,950.

each side by the nuclear envelope. In some sections blebs are found to be hollow spheres or tunnels of nuclear material, filled with cytoplasm.

We would like to draw attention to the chromatin organization within the extruded sheet of nucleus in the blebs which we have found. The chromatin is visualized in the sections as a row of discrete granules, each somewhat brick-shaped, and of dimensions of 20–40 nm across. Such a size suggests that they may represent a group of nucleosomes cut in sections. Such a stacking arrangement of chromatin granules has been previously visualized in the EM in the nuclear material immediately underlying the nuclear envelope in chicken erythrocytes[13] and in fish cells[3].

It has been proposed[14] that blebs of nuclear membrane may arise in nuclei which, for reasons unknown, produce an excess of nuclear envelope over and above what is needed to neatly enclose the chromatin. We are attracted by this view and cosider that this may occur in a low percentage of otherwise normal individual animals. The fact that in such nuclei chromatin is often extended between the sheets to reveal its beaded organization seems to offer a useful situation in which to study the organization of chromatin within the nucleus.


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## Estradiol treatment reduces a cytosol androgen binding protein in male rat liver[1]


N. Sato, M. Ota and S. Takahashi

*Department of Biochemistry, Iwate Medical University School of Medicine, Morioka, Iwate 020 (Japan), 17 October 1979*



*Summary.* The in vivo treatment of male rats with estradiol-17$\beta$ resulted in a significant decrease in liver cytosol of the protein which binds specifically androstenedione and testosterone.


There is a sex difference in androgen metabolism in the livers of rats. We have observed that an injection of estradiol in male rats induces feminization of the hepatic metabolism of testosterone[2] and that the treatment of female rats with testosterone results in the induction of enzymes which are involved in the androgen metabolism[3,4]. We have recently demonstrated the presence of a protein which binds androstenedione and testosterone with moderate affinity in the cytosol of male rat liver[5]. These findings led to an attempt to clarify the effect of estrogen on the androgen binding protein in male rat liver. We report here that administration of estradiol reduces the androgen-binding protein in the liver cytosol of male rats.

*Materials and methods.* Estradiol-17$\beta$ benzoate, dissolved in a small volume of ethanol and diluted with corn oil, was given s.c. to male adult Wistar rats weighing 200–250 g for 2, 4 or 7 days. The control animals received the vehicle only. The animals were castrated 15 h before sacrifice and were killed 24 h after the final injection. The liver was perfused with saline solution and quickly removed. The liver tissues were homogenized in 1 vol. of 0.01 M Tris-HCl buffer, pH 7.4, with 0.01 M KCl, 0.001 M EDTA and 0.001 M dithiothreitol (TKED buffer) in a glass-teflon homogenizer and then centrifuged for 10 min at 18,000 × g. Floating lipid was discarded and the supernatant was recentrifuged for 1 h at 105,000 × g. A clear supernatant (cytosol) was incubated with [1,2-$^3$H]androstenedione (sp. act. 60 Ci/mmole, New England Nuclear) or [1,2-$^3$H]testosterone (sp. act. 40 Ci/mmole, New England Nuclear). Separation of macromolecule-bound [$^3$H]steroids from free ones in the incubates was accomplished by a Sephadex G-100 column using 0.01 M TKED buffer and fractions of 5 ml were