control basal diet as well as tap water were given ad libitum. The mice were fed these diets for 14–56 days and killed at 14-day intervals to determine wet weights of the thymuses, spleens and adrenals.

Results and discussion. Body weights of the mice increased from the initial post-weaning mean value of 11–12 g to 25–26 g at the end of the experimental period. The weights of the thymuses and spleens in control groups follow the involution trend of the post-weaning period. No effects of aspartic acid treatment on body weight and on adrenals were noted. A statistically significant increase in thymus weight was observed after 2, 6 and 8 weeks of aspartic acid treatment; splenic weight increased significantly after 8 weeks (figure). In order to support these results, male mice of the same strain were treated at the age of 12 weeks with a 10-day administration of soluble K- and Mg-salts of aspartic acid in drinking water. Monokalium D,L asparagicum together with monomagnesium D,L asparagicum were dissolved in equal amounts in tap water at a level of 10 mg/ml (1%) and given ad libitum. Approximately similar daily intakes of aspartic acid as those used in the foregoing experiment were thus achieved. 4 experiments in various seasons of the year were performed and in all of them a significant increase in thymus weight was registered after aspartate treatment. No differences in the spleen weight were observed (table).

The results suggest that aspartic acid may play a role in the regulation of the size of the lymphatic tissue of the thymus, and that this function may be supported by dietary administration of this amino acid. The mechanism of the effect is not clear. K, Mg aspartate has been found to prevent the stress-induced disintegration of thymolymphatic tissue[5]. Ohnuma et al. emphasize the asparagine requirements of T-lymphocytes for cell growth[6]. In view of this evidence it could be hypothesized that, under the conditions of increased cellular level of aspartic acid, the enzymatically regulated balance between asparagine and aspartic acid is directed towards preservation of asparagine. Regardless of the mechanism involved, the importance of our findings for dietary attempts to modify cell-mediated immunity remains to be analyzed.

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An improved electrophoretic method for a screening program for haemoglobinopathies[1]

P.C. Naoum, M.C.R. Moura Campos, M.F. Parenti and A.M. Szymanski

Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, UNESP, São José do Rio Preto, SP (Brasil), 6 August 1979

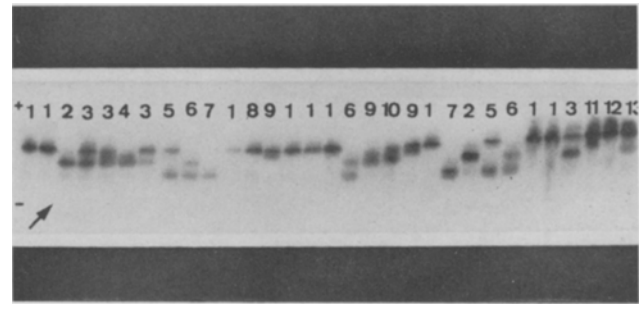
Summary. A method for a screening program for haemoglobinopathies in a starch agar gel mixed with saponin is presented. Normal and abnormal blood containing haemoglobins S, C, I, M Boston, D Punjab, beta thalassaemia major and beta thalassaemia minor, were applied, in a tray with the capacity for 100 samples. The electrophoresis was performed in 45 min using 300 V. This method offers special advantages for the examination of a large number of samples, using a small amount of whole blood and without the previous preparation of haemoglobin solution.

Millions of people throughout the world carry abnormal haemoglobins in various genetic combinations. Their identification is a public health problem of major importance[2,3]. It includes recognition of the relatively harmless mutants and of haemoglobins with known pathologic effects, particularly sickle cell anaemia and beta thalassaemia major[4]. This paper describes the application of an electrophoretic method for a screening program for haemoglobinopathies, and some of the advantages of this method are discussed.

Material and methods. Haemoglobin types used: In order to evaluate this procedure for human haemoglobins, whole blood samples were used, containing haemoglobins (Hb) A, S, C; fetal increased, from a patient with beta thalassaemia major; and Hb A_2 increased, from a patient with beta thalassaemia minor. All were identified by electrophoresis on cellulose acetate pH 8.6[5], starch gel pH 8.6, and agar gel pH 6.2[7]. Blood from patients with the rare abnormal haemoglobins M Boston. D Punjab and I, characterized by peptide maps and amino acid analysis[8], were also included in this study.

Electrolyte compartments and starch agar gel tray: The apparatus required for this study was made in our laboratory, and consisted of 2 electrolyte compartments separated by a support from the starch agar gel tray. Each electrolyte compartment measured $41 \times 5 \times 3$ cm in depth, and was constructed from acrylic plastic 0.3 cm thick. The tray, made from the same material, measured 38×8 cm. To its upper surface a frame of acrylic plastic 0.5 cm wide and 0.1 cm thick was fixed by adhesive.

Gel and electrode buffers: Discontinuous systems were used. For preparation of starch agar gel: 87 mM tris (hydroxy methyl) amino methane, 8.7 mM boric acid, 1 mM ethylene diamine tetracetic acid, pH 9.0[9], diluted 1:10 in water. For use in the electrolyte compartments: 300 mM boric acid and 60 mM sodium hydroxide pH 8.6[10], diluted 1:3 in water.



Electrophoresis of a part (1/3) of the agar gel tray. The arrow shows the line of application of whole blood. The number shows the haemoglobins types tested: 1 (AA), 2 (SS), 3 (AS), 4 (SF), 5 (AC), 6 (SC), 7 (CC), 8 (AA_2 increased), 9 (AF), 10 (mixture of ASF), 11 (mixture of AFI), 12 (AI) and 13 (mixture of ASI).

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