STATE OF THE NUCLEAR CHROMATIN IN CIRCULATING BLOOD LYMPHOCYTES OF PATIENTS WITH DOWN'S DISEASE AND PHENYLEKETONURIA AS REVEALED BY CYTOSPECTROFLUOROMETRY

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Incubation of blood lymphocytes from patients with phenylketonuria for 1h in Eagle's medium containing their serum causes an increase in the staining properties of the DNP-complexes of the lymphocytes, as reflected in an increased intensity of acridine orange fluorescence, i.e., spontaneous activation takes place, whereas the intensity of fluorescence of lymphocytes from healthy persons is not signifcantly changed. Phytohemagglutin does not increase the fluorescence of the acridine orange bound with the chromatin of the lymphocytes from patients with Down's disease, but causes a significant increase in luminescence of the dye in response of the action of phytohemagglutin in control donors.

An important problem in molecular genetics is the study of structural variations in chromatin at different stages of the mitotic cell cycle. The sequence of structural changes of the chromatin in this case is, of course, determined genetically. It would therefore be expected that the appearance of certain inherited disturbances must modify to some degree the course of this strikingly coordinated process. Disturbances of the normal structural transformations of chromatin in vivo can probably be assessed from changes in a number of the physicochemical parameters of the chromatin, with the result that the degree of blocking of the nuclear template by protein could be determined.

If this hypothesis is true, the structural changes in chromatin could be analyzed by Rigler's microfluorescence method [1, 2].

In the investigation described below an attempt was made to determine certain quantitative physicochemical characteristics of the nuclear chromatin of the lymphocytes from patients with Down's disease (trisomy), and from their parents and siblings.

EXPERIMENTAL METHOD

Blood (5 ml) was taken from the cubital vein into sterile tubes containing 0.5 cm^3 Ringer's solution with hepatin (200 i.u.). The blood was incubated for 40-60 min, after which the plasma containing leukocytes was decanted and diluted 1 : 10 with Eagle's medium and poured into Petri dishes containing cover slips. All manipulations were carried out in the incubator at 37°C. The cover slips were removed 5 and 60 min after addition of the diluted plasma (beginning of incubation), rinsed in physiological saline, fixed to a specially constructed teflon holder, and dipped in fixing solution (a 1 : 1 mixture of acetone and ethanol). Phytohemagglutin (PHA) was added to the incubation medium in a parallel series of tests before the beginning of incubation up to a final concentration of 40 μg/ml.

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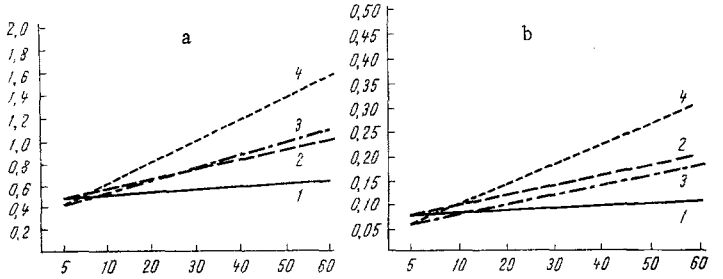

Fig. 1. Fluorescence of acridine orange bound with chromatin from circulating human blood lymphocytes (healthy donors and patients with phenylketonuria) as a function of incubation time at $\lambda = 530$ nm (a) and $\lambda = 640$ nm (b): 1) lymphocytes of healthy donors; 2) lymphocytes of healthy donors + PHA; 3) lymphocytes of patients with phenylketonuria; 4) lymphocytes of patients with phenylketonuria + PHA. Abscissa, time (in min); ordinate, intensity of fluorescence of acridine orange bound with DNP-complex of lymphocytes.

The methods of fixation, staining, and actual preparation of the specimens were carried out by Rigler's method [1, 2].

To measure the fluorescence, a single-beam microfluorometer designed on the basis of the Ortholux (leitz) luminescence microscope was used. Fluorescence was excited by light from a xenon lamp (λ = 360-405 nm). The appropriate interference filters were used to distinguish the spectral bands.

This paper gives the results obtained by investigation of the lymphocytes of healthy donors (35 observations), patients with phenylketonuria (20), the mothers (13), fathers (8), and sibs of these patients (7), and also the lymphocytes from patients with Down's disease (16), and the mothers (13), fathers (8), and sibs (8) of these patients.

EXPERIMENTAL RESULTS

Incubation of lymphocytes from healthy donors for 1 h caused no significant increase in the intensity of fluorescence, but the addition of PHA to the incubation medium caused a significant ($P < 0.001$) increase by 40-60% in the quantity of dye bound by the cells in both regions of the spectrum (Fig. 1a, b).

Incubation of the lymphocytes from patients with phenylketonuria for 60 min led to a significant ($P < 0.001$) increase in fluorescence (by 250-300%) in 90% of cases. This effect was called spontaneous activation of the nuclear chromatin of the lymphocytes. As Fig. 1 shows, it occurred only in the patients. An increase in fluroescence was observed in both the red and the green regions of the spectrum (Fig. 1). Addition of PHA to the incubation medium of the lymphocytes from patients with phenylketonuria led to a further moderate increase in the intensity of fluorescence. This increase corresponded to that in the control.

Parallel investigations of fluorescence of the lymphocytes from the siblings and parents of the patients showed that the effects of incubation of the lymphocytes from the siblings and fathers for 1 h and their reaction to PHA were indistinguishable from the changes in dye-binding properties of the lymphocytes from healthy donors. However, the intensity of fluorescence of lymphocytes from the mothers of the patients with phenylketonuria after incubation for 60 min was a mean value between the corresponding parameters for the lymphocytes of the patients and the control group. No significant difference was found between the intensity of fluorescence of the lymphocytes from the patients and from their mothers.

Incubation of lymphocytes from patients with Down's disease for 60 min, just as the control tests, revealed no significant increase in the dye-binding properties of the chromatin in these cells.

Meanwhile, significant differences were found on analysis of the action of PHA on the nuclear chromatin of the lymphocytes from these patients. In this case, by contrast with the control experiments and the results obtained with lymphocytes from patients with phenylketonuria, the PHA had no activating effect, i.e., intensity of fluorescence of the dye was unchanged in response to the action of PHA in both regions of the spectrum (Fig. 2a, b).

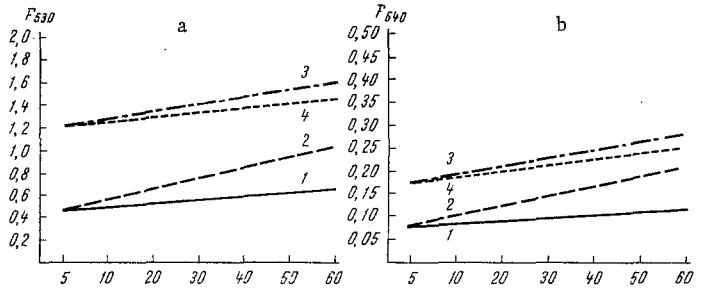

Fig. 2. Fluorescence of acridine orange bound with human circulating blood lymphocytes (healthy donors and patients with Down's disease) as a function of incubation time at λ = 530 nm (a) and λ = 640 nm (b): 1) lymphocytes of healthy donors; 2) lymphocytes of healthy donors + PHA; 3) lymphocytes of patients with Down's disease; 4) lymphocytes of patients with Down's disease + PHA. Legend to abscissa and ordinate the same as in Fig. 1.

It is interesting to note that PHA likewise did not activate the nuclear chromatin of lymphocytes from mothers of children with trisomy, but this was not the case with the sibs of the patients, who were indistinguishable from control donors.

The initial fluorescence, i.e., the intensity of fluorescence after incubation for 5 min, was 2.5-3 times higher, on the average, in the patients with Down's disease than in the control. The writer's previous investigations showed that thymocytes (functionally more active cells than lymphocytes) give a very high initial fluorescence, which does not react to PHA, probably because the number of liberated PO_4 groups bound with the dye is already at a maximum.

The chromatin of lymphocytes from patients with phenylketonuria thus differs from that of the lymphocytes of healthy donors by becoming spontaneously activated after 60 min. Chromatin of lymphocytes from patients with Down's disease, on the other hand, as the results of microspectrofluorometry show, is evidently in an activated state already without additional incubation, and is not modified on subsequent treatment with a powerful mitogen such as PHA.

The results of these experiments show that lymphocytes from patients with phenylketonuria are potentially readier for activation than healthy human lymphocytes. In patients with Down's disease, on the other hand, this potential preparedness is probably already accomplished in the isolated cells, i.e., changes had already taken place in their chromatin in vivo. Whether these changes were the cause or the result of the disease is a question which cannot be answered without further investigations. However, even at this stage it is clear that the nuclei of thymocytes (functionally more active cells than lymphocytes) behave to some extent like the lymphocytes in Down's disease, at least as reflected by results obtained by the microfluorescence method. Normal thymocytes, however, have a normal karyotype. Consequently, activation of chromatin is an essential but not an adequate condition for the development of numerical chromosomal anomalies, at least for trisomy. Of course, the possibility cannot be ruled out that activation of chromatin is the result of whatever it is which has caused the numerical chromosomal anomalies. If such a hypothesis is excluded, for it seem less probable to the writers even though it is more in accordance with the prevailing opinion, at least three events untypical of the normal situation and which could lead to activation of chromatin must be mentioned: a) the onset of a gene mutation and synthesis, as a result of this mutation, of an abnormal protein which participates in the formation of the supramolecular system of the chromatin, b) the onset of a gene mutation and a resulting disturbance of certain normal metabolic processes whose products lead by a feedback mechanism to changes in the normal structural conversions of chromatin, and finally, c) a primary disturbance of the regular three-dimensional structure or of the continuity of the supramolecular formations (chromosomes) without injury to DNA (the justification for this conclusion depends on the discovery of the true picture of chromosomal structure). The writers are inclined to believe that cases of trisomy are connected with chromatin modifications described in event c. However, this view requires further experimental verification.

LITERATURE CITED

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