Chromatin Structure in Down's Syndrome

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Summary. In the chromatin of patients with Down's syndrome, changes are shown to occur in a short-term lymphocyte culture of the human peripheral blood. Some of them are induced by the patient's blood serum and are reversible when this is replaced by normal serum. A 100-fold dilution of the blood serum taken in subjects with Down's syndrome does not produce any changes in the structure of the lymphocyte chromatin of the patients. A similar procedure with the blood serum of healthy donors resulted in a drastic activation of their lymphocyte chromatin. These experiments, and investigations on the effect produced by the blood serum on the model desoxyribonucleoprotein systems, support the suggestion that the changed state of the chromatin in subjects with Down's syndrome is caused by a complex set of components contained in the blood serum, whose degree of dissociation deviates from the normal.

In recent years the present authors (Fedorova, 1971) and a number of other authors (Mittwoch, 1967; Pathak, 1972; Ridler, 1971) have obtained some data suggesting that the chromatin in humans with a changed karyotype (47,XY+21, XX+21) differs both in its structure and function from that of normal subjects. In principle, the karyotype changes can be caused by a number of intracellular events and may also be induced by some compounds of the cellular environment. The last proposition is supported by the data (Miller et al., 1971; Oster et al., 1964; Stiem et al., 1966) on the existence of substantial quantitative differences between the blood serum constituents in patients with Down's syndrome and those of healthy individuals. On the other hand, there are indications (Kroll, 1973) to the effect that the chromatin incorporates some proteins from the medium surrounding the cell.

The purpose of the present investigation is to ascertain the possibility of any structural changes occurring in the peripheral blood lymphocytes of individuals with the karyotype 47,XY+21;47,XX+21, which are dependent upon derangements in the blood serum composition.

Materials and Methods

The nuclear chromatin from the peripheral blood lymphocytes of 91 patients with Down's syndrome (mongolism), 8 to 18 years old, and of 106 healthy donors aged 18 to 30 years was studied. Furthermore, the nuclear chromatin of lymphocytes from 10 normal children 6 to 18 years old was also examined. The characteristics described below of the chromatin taken from donors of diverse age categories showed no significant differences and, therefore, all of them were included in a single control group.
Changes in the structure of the chromatin occurring directly in the human lymphocytes were tested against the capacity of the chromatin DNA of these cells to bind a fluorescent acridine orange tag (AO). An analysis of any possible effect that might be directly produced on the chromatin by a blood serum component of the ill and healthy individuals was made on model supramolecular DNP-systems using the thermomechanical method (Spitkovsky, 1969).

Making Ready the Preparations and the Method of Cytospectrofluorometry. 5 ml of blood were taken from the ulnar vein and placed into sterile test tubes containing 0.5 cm³ of Ringer’s solution with heparin (200 IU). The blood was allowed to settle in a thermostat for 60 min. The lymphocytes carrying plasma were then aspirated (drawn off), diluted 10-fold with Eagle’s medium and poured out into Petri dishes (50 mm diameter) each of which had 3 cover glasses at the bottom. All the operations were performed inside a thermostat at 37°C. 5 to 60 min after the diluted plasma had been poured into the Petri dishes the cover glasses with the cells deposited on them were rinsed with saline and placed in a fixative (acetone/ethanol, 1:1). In experiments involving replacement of the medium the cover glasses carrying the cells were, prior to fixing, washed with saline and transferred into a new medium and only then fixed afresh. Phytohemagglutinin (PHA) was introduced before incubation to bring its final concentration in some of the Petri dishes to 40 γ/ml.

The acridine-orange staining and subsequent treatment of the preparation were performed after Rigler (1966). The fluorescence intensity of AO bound with DNA of the lymphocytes chromatin was measured by means of an “Opten” scanning microscope-photometer, model MPS = 0.5.

Excitation was achieved by a luminous flux with a wave length of λ = 365 nm, the fluorescence intensity being measured at λ = 530 nm.

The Thermomechanical Method. In essence the method comes to determining, depending upon the temperature, the degree of elongation of an oriented deoxynucleoprotein (DNP) fiber in the study medium with corresponding blood sera (150-fold dilution with saline). The method of obtaining the DNP fibers and their rheological characteristics have been described earlier (Spitkovsky, 1969). DNP was isolated from a calf’s thymus following the standard method (Mirsky and Pollister, 1946), but replacing an IM solution of NaCl with a 0.75 solution. The separated DNP preparations had the following characteristics: 50% of protein, 40% of DNA and 10% of RNA. The mean viscous molecular weight was —2·10⁷ Dalton. Hyperchromism comprised 32 to 34%. The results were processed statistically with the Student’s t test.

Results and Discussion

Fig. 1a presents data on the intensity of the AO fluorescence in a non-stimulated and stimulated PHA, incubated for 5 and 60 min in the cells of healthy donors and patients with Down’s syndrome. The chosen incubation time was short enough to not allow RNA synthesis to start in the activated cells (Rigler, 1966). Therefore, the quantitative RNA changes should have only an insignificant effect on the binding of AO. From Fig. 1b 2 it follows that in mongolism a 60-minute incubation period of the lymphocytes tends to raise the fluorescence intensity by as much as 30 to 95% (P < 0.01). We term this effect “spontaneous activation”. At the same time, an incubation of the lymphocytes from healthy subjects performed under analogous conditions does not result in such an effect (Fig. 1a 2). However, a 60-minute incubation period of the lymphocytes under the same conditions, but with added PHA, produces a 40 to 60% rise of the AO-fluorescence intensity in the lymphocytes of healthy donors (Fig. 1a 3) (P < 0.01), but does not significantly change the fluorescence intensity of the stain in the cells of subjects with Down’s syndrome (Fig. 1b 3). Bearing in mind the fact that in staining after Rigler we used of surplus dye (10⁻⁴ μ) considerable differences in