Comparison of the results of the present investigation with those obtained previously shows that the chromosomes of A/He mice are characteristically more sensitive to thiotepa than those of C57BL/6 mice, whether the cells treated are in the g_0 phase of the cell cycle (the liver of adult animals) [3] or actively proliferating embryonic liver and bone marrow cells are treated in the g_1-S phase of the cell cycle. Calculation of the percentage of cells with damaged chromosomes (structural aberrations and deficiencies) per unit dose of the compound showed that embryonic liver cells are rather more sensitive to the mutagenic action of thioTEPA than bone marrow cells of adult animals. For instance, per milligram of thioTEPA, 22.6 and 11.5% of damaged cells were observed in the embryonic liver and 10.7 and 9.2% in bone marrow of A/He and C57BL/6 mice respectively.

A/He mice differ from C57BL/6 mice not only in the greater number of aberrant cells after thioTEPA treatment, but also in the fact that more cells with multiple chromosomal injuries are induced both in the embryonic liver and in the bone marrow of mice of this line. In the writers' view, the existence of a greater number of these cells among the total number of aberrations in A/He mice reflects the greater severity of the damage and, consequently, the higher sensitivity of mice of this line to the mutagen.

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BLOOD SERUM AS A FACTOR IN CHROMATIN CONDENSATION IN TRISOMY-21 (DOWN'S SYNDROME)

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The effect on the blood serum of patients with Down's syndrome and of healthy persons and also separate fractions of sera on structural parameters of model nucleohistone systems (DNP-systems) was studied. Unfractionated patients' sera were found to have a condensing effect on DNP-systems, unlike healthy human sera. Analysis of the action of the individual serum fractions showed that different degrees of condensation can be attributed to the influence of high-molecular weight, undialyzable, thermolabile components, the action of which disappears after gel-filtration of the serum proteins. The problem of possible humoral control over the structural organization of chromatin in vivo is discussed in the light of data showing similarity between blood serum proteins and certain nonhistone proteins of chromatin.

KEY WORDS: chromatin; condensation, serum.

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The writers showed previously [10] that an increased degree of condensation of interphase chromatin observed in lymphocytes in Down's syndrome is largely dependent on the blood serum of these patients. If autologous serum in the culture medium of cells from heathly donors is replaced by patients' serum the packing density of the chromatin is increased, whereas healthy human serum "normalizes" chromatin in the patients' lymphocytes. This fact provided the stimulus for a study of the mechanism of the action of active serum factors on the structural organization of the nuclear substrate of the cell and, in particular, on isolated chromatin. As a preliminary step [2] it was shown that blood sera from patients with schizophrenia and systemic lupus erythematosus differ in their action on model systems of chromatin in vitro from healthy human sera, for the former increases whereas the latter decrease the degree of condensation of chromatin.

The object of the present investigation was to study the character of the action of sera from patients with Down's syndrome on model chromatin systems in vitro and to investigate the action of separate serum fractions on these models with the aim of identifying the active factor.

EXPERIMENTAL METHOD

Blood sera from 55 healthy donors and 40 patients with Down's syndrome were used.

The sera were fractionated by gel-filtration on Sephadex G-200 in columns measuring 2.4 × 96 cm. The eluent was 0.14 M NaCl in 0.001 M Na-phosphate buffer, pH 7.0. The protein fractions thus obtained were analyzed by differential disk electrophoresis in polyacrylamide gel [13].

The effect of sera from healthy subjects and patients, and also of separate serum fractions were investigated by a thermomechanical method [5] on model systems (DNP fibers) obtained in a physiological medium from DNA preparations isolated from calf thymus in 0.7M NaCl. The magnitude and character of structural modifications to the model systems were judged from measurements of deformation of the DNP fibers detectable in response to the action of temperature. This deformation was expressed quantitatively by the following parameters: 1) the relative length of the fibers $E = [(l_t - l_0)/l_0] \cdot 100\%$, at each given temperature, and 2) the maximal change in length of the fiber $A = [(l_m - l_0)/l_0] \cdot 100\%$, measured at 80°C, where l_0, l_t, and l_m represent the length of the fiber before heating, during heating at each given temperature between 25 and 100°C, and before the beginning of melting of the fibers at 80°C [2].

The medium in which the model DNP systems were tested contained serum diluted 1 : 150 in order to balance the action of bivalent ions contained in the blood. The serum fractions were added to the medium in amounts corresponding to their content in whole serum. The significance of differences in the values of the structural parameters of the DNP fibers in each experiment was determined by Wilcoxon's criterion [1].

EXPERIMENTAL RESULTS

Unfractionated sera from healthy subjects and patients with Down's syndrome differed in their action on DNP fibers. In 90% of cases (35 of 40) the relative length of the fibers (E) decreased in the patients' sera within the temperature range 55-80°C (Fig. 1). The maximal change of length (A) under these circumstances was reduced on average by 40% ($P < 0.01$) (Table 1). In view of data showing that the parameters of the fibers depend on the degree of condensation of DNP [4, 6], it can be concluded that the decrease in the degree of deformation of the fibers in medium containing patients' serum is the result of the condensing action of the components of this serum by contrast with normal serum. It is important to emphasize that this result agrees with data to which reference has already been made [7] on the condensing action of the patients' sera on interphase chromatin in lymphocytes.

To discover the serum components responsible for changes in the degree of condensation of the model systems, the action of separate fractions obtained by several methods was investigated. The action of the total protein and nonprotein fractions obtained from patients' and healthy subjects' sera by dialysis against 0.14 M NaCl for 24 h at 4°C was analyzed. As Table 1 shows, a decrease in A, observed in the whole patients' sera, was characteristic of the undialyzable, high-molecular-weight fraction. Since the protein content in the low-molecular-weight fraction was extremely small (under 0.001%), it can be concluded that the factor under investigation is connected with the protein component of the sera.

This conclusion was tested in a series of experiments in which sera from patients and healthy subjects was heated to the temperature of protein denaturation. After heating to 97°C for 30 min, the denatured proteins were removed by centrifugation for 2 h at 40,000g. As Table 1 shows, this procedure led to disappearance of the differences in the value of parameter A. These results confirmed the suggestion that the active factor is linked with serum proteins.

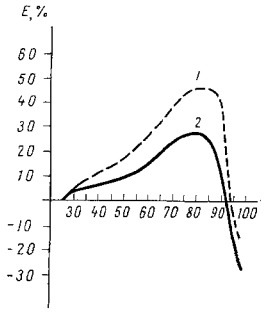

Fig. 1

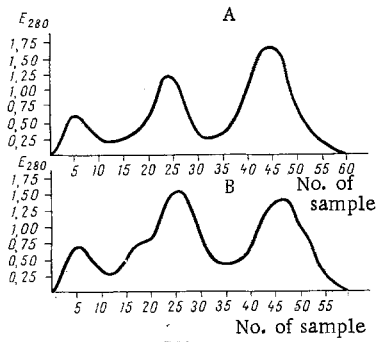

Fig. 2

Fig. 1. Relative length of DNP fibers (E) as a function of temperature in the presence of serum from healthy blood donors (1) and patients with Down's syndrome (2).

Fig. 2. Typical elution profiles of serum proteins on gel filtration on Sephadex G-200. Serum from: A) healthy donors, B) patients with Down's syndrome.

TABLE 1. Effect of Unfractionated and Fractionated Blood Sera of Healthy Subjects and Patients with Down's Syndrome on Parameters of DNP Fibers

Sera	$\frac{A_D}{A_N}$	P
Unfractionated	0,61±0,18	<0,001
After dialysis:		
undialyzable fractions	0,60±0,11	<0,01
dialyzable	0,99±0,07	≧0,05
After heating (97°C 30 min)	0,95±0,06	>0,05
After gel filtration		
fraction 1	1,07±0,06	≧0,05
fraction 2	0,99±0,14	>0,05
fraction 3	1,03±0,07	≧0,05
Reconstituted after fractionation	1,09±0,07	>0,05

Legend. A_D and A_N represent amplitudes of maximal change in length of DNP fibers in sera (or fractions) from patients and healthy donors respectively.

Fractionation of the serum proteins by gel-filtration on a column with Sephadex G-200 revealed basically a difference in the ratio between globulins and albumins in the sera of the healthy subjects and patients (Fig. 2). When this method is used for fractionation, three protein fractions are obtained, the first containing mainly macroglobulins, the second γ-globulins, and the third albumins. The results of fractionation showed that the globulin content in the patients' sera was on average 12% higher, and the albumin content 12% lower than in the healthy area.

Differences in the action of the high-molecular-weight fractions of sera from the patients and healthy donors could therefore be attributed to two factors: qualitative differences in the proteins or quantitative differences in the proportions of the main protein fractions. Comparative analysis of the effects of homonymous fractions of sera from patients and healthy subjects on DNP systems failed to detect in any of them differences found in the unfractionated sera (Table 1). On the other hand, the abnormal content of globulins and albumins in the patients' serum, which other workers also have found [11, 12], is also evidently not the cause of the condensing effect, for reconstruction of the composition of the sera of the healthy subjects and patients by the readdition of all the fractions did not restore the initial differences (Table 1). Probably the active serum components exist as a protein complex which, broken down on fractionation, is not restored during subsequent re-

constitution of the sera. Admittedly, the possibility of loss of the active factor as a result of its adsorption on the Sephadex during gel filtration cannot be ruled out (the total yield of protein was not more than 8% below its content in unfractionated serum).

The change in the degree of condensation of the model chromatin systems under the influence of blood sera observed normally and in the case of pathologically changed sera from patients with Down's syndrome, and also in patients with schizophrenia and systemic lupus erythematosus [2] suggest the existence of humoral control over the structural organization of the genome. This may perhaps be one method of regulation of gene expression: A change in the degree of condensation of chromatin is reflected in the accessibility of individual genes for specific regulator proteins [6, 8, 9].

The identical character of the structural modifications under the influence of the sera in chromatin in vitro and in vivo in the cell in Down's syndrome and in schizophrenia [2] indicates the possibility of direct contact between chromatin and the active serum protein components. This argument is supported by data showing similarity between blood serum proteins and certain nonhistone proteins of chromatin [14].

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