ELECTROPHORETIC ASSAY OF PROTEIN 53K IN NEONATAL BLOOD LEUKOCYTES

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In a series of publications Paponov and co-workers [4-6] reported the discovery of a protein with mol. wt. 53 kilodaltons (53K) in blood leukocytes of patients with Down's syndrome, which could not be detected visually in leukocytes from healthy individuals either visually on electrophoretic gels or as a peak on densitograms. This protein was found in leukocytes of patients with different versions of the karyotype corresponding to Down's syndrome, namely trisomy 21, mosaicism, and translocation. Further investigations showed, however, that protein 53K cannot serve as a marker of Down's syndrome, but is only a factor associated with this syndrome, for it is found also in a certain number of patients with normal karyotype, but having children with Down's syndrome [7]. It could be suggested that protein 53K is a marker of some more widely spread pathology than Down's syndrome. Since the frequency of Down's syndrome as a congenital condition does not increase with age, which is unknown in the case of any hypothetical pathology, we attempted to determine the minimal frequency of its occurrence, in the absence of loading by diseases acquired in postnatal development, by studying the content of protein 53K in umbilical blood leukocytes. Meanwhile, as additional evidence that protein 53K can serve as a marker of pathology; we studied correlation between the prevalence of this protein in neonatal leukocytes and the state of health and course of pregnancy of their mothers.

EXPERIMENTAL METHOD

Umbilical blood (3-4 ml) was mixed with heparin (Richter, Hungary, 25 IU in 80 μl of 0.9% NaCl solution to 1 ml blood). Next, one part of 3% gelatin solution in 0.15 M NaCl was added to three parts of blood and the mixture was incubated for 30 min at 37°C in a conical centrifuge tube with a capacity of 10 ml. Hemolysis of erythrocytes remaining in the leukocyte suspension was carried out for 15 sec in ice in a 1:8 dilution with distilled water. Leukocytes were used for analysis of their proteins by PAG electrophoresis with SDS by Laemmli's method [8]. Gel disks were stained with Coomassie R-250, washed, and subjected to densitometry on a "Gilford" spectrophotometer (USA) at 570 nm. The presence and content of protein 53K in the cord leukocytes were determined as the ratio of the amplitude of peak corresponding to this protein on the densitogram, or the amplitude of the background line at the possible site of protein 53K, on the one hand, to the amplitude of the histone H2A peak, on the other hand.

EXPERIMENTAL RESULTS

The histogram of distribution of 90 neonates for the presence of protein 53K in cord blood leukocytes is shown in Fig. 1. Neonates for whom the values of 53K/H2A < 0.35 (i.e., not above the upper limit of the modal class

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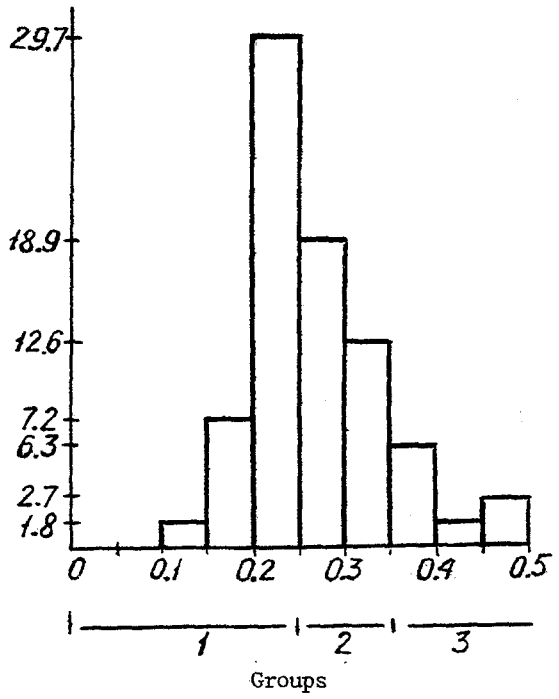

Fig. 1

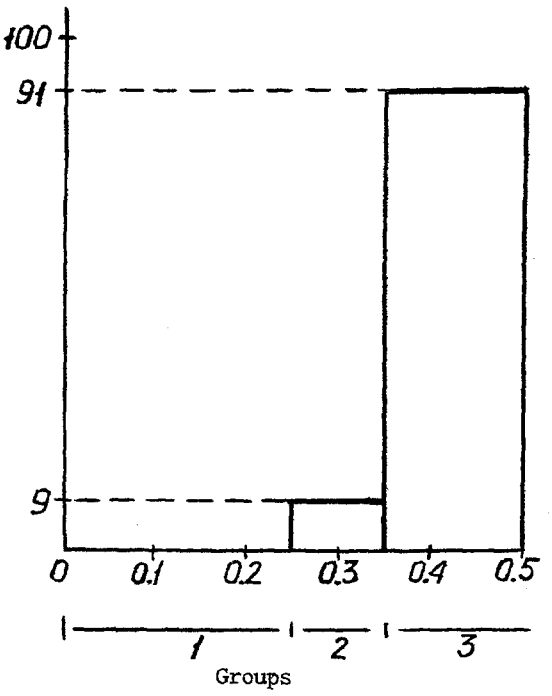

Fig. 2

Fig. 1. Distribution of neonates by relative presence of proteins 53K/H2A in cord blood leukocytes. Abscissa, ratio of amplitudes of peaks corresponding to proteins 53K and H2A on densitograms of electrophoretic gels (53K/H2A); ordinate, percentage of neonates with characteristics falling into individual classes of values of the ratios 53K/H2A.

Fig. 2. Distribution of patients with Down's syndrome by relative presence of proteins 53K/H2A in venous blood leukocytes. Abscissa, ratio of amplitudes of peaks corresponding to proteins 53K and H2A on densitograms of electrophoretic gels (53K/H2A); ordinate, percentage of patients with Down's syndrome with characteristics falling into individual classes of values of the ratio 53K/H2A.

of the distribution – the class with the highest frequency) are placed in group 1. The histogram of distribution of 35 patients with karyotypically confirmed diagnosis of Down's syndrome for the presence of protein 53K in venous blood leukocytes is given in Fig. 2. It is interesting to note that not a single patient with Down's syndrome was included in the group with 53K/H2A < 0.25, but almost half of the neonates (conventionally, a group of normal children), were placed in this group. The persons with the 53K/H2A < 0.25 parameter tested included most patients with Down's syndrome (91%), but only 13% of the neonates. It can therefore be concluded that neonates placed in this group are characterized by a pathology that is associated with Down's syndrome. A second intermediate group, including 9% of patients with Down's syndrome and 39% of neonates, can be regarded as a group with the initial manifestation of a pathological state, for which protein 53K serves as marker. These individuals have the parameter 0.25 < 53K/H2A < 0.35. Let us examine how the size of the normal (48%) and pathological (13%) groups, identified on the basis of the presence of protein 53K, corresponds to the analogous classification with respect to clinical features. The pathological group among the neonates, as regards parameters such as prematurity, malnutrition, or asphyxia, amounted to 12.7% [3]. The size of the healthy neonatal group can be judged indirectly by the fact that course of pregnancy in the mothers of the neonates whom we tested was physiological in 31% of cases (Table 1). In [1] this parameter amounted to 38.5%, based on the results of testing of 600 women in Moscow.

TABLE 1. Distribution of Models of Neonates Studied, Depending on Character of Course of Pregnancy

Course of pregnancy	Number of mothers	Percentage of total number of mothers (90)
Physiological	28	31
Threatened abortion	15	16,6
Edema	11	12
Anemia of pregnancy	4	4,5
Nephropathy	6	6,5
Early toxemia	17	19
infectious diseases ing pregnancy	11	12
Pyelonephritis	7	8
Edema of the limbs	4	4.5

TABLE 2. Distribution of Mothers of 3 Separate Groups of Neonates by Their State of Health and Course of Pregnancy

Groups of neonates	1	2	3
Numbers of mothers	43	35	12
Average age, years	30	26	28
Presence of chronic diseases	9 (21 %)	9 (26 %)	7 (58 %)
Presence of abortions on medial grounds	13 (30 %)	19 (54 %)	6 (50 %)
Presence of chronic diseases and/or complications of pregnancy	21 (49 %)	33 (94 %)	11 (92 %)

Data on the distribution of mothers of the three separate groups of neonates by state of health and character of the course of pregnancy are given in Table 2. Clearly the mothers' age was found to have no effect on the inclusion of their children in the pathological group on the basis of the 53K marker. The presence of a history of abortion on medical grounds in the mothers correlates (by the chi-square test) with inclusion of the neonates in the first or second and third groups ($p > 0.95$). In the absence of chronic diseases in the past medical history of the mothers, the neonates were placed more often in group 1 than in the pathological group ($p > 0.95$ by the chi-square test). It will also be clear from Table 2 that in the presence of chronic diseases and/or complications of the course of pregnancy in their mothers, the neonates were placed significantly more often in groups 2 and 3 ($p > 0.99$). This is a weighty argument in support of the fact that the more frequent or more abundant presence of protein 53K is connected with pathology. This pathology could be a disturbance of the immune status, which may occur in neonates as a result of chronic maternal diseases or infection arising during abortion or unsuccessful pregnancy. This hypothesis is supported by the relatively high incidence of pathology in the newborn, and the high predisposition of patients with Down's syndrome to tumors and infectious diseases, which is associated with immunopathology [3, 9].

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THIOL-INDUCED FRAGMENTATION OF CHROMOSOMAL DNA

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The role of S–S groups of residual protein in the tandem organization of subunits of chromosomal DNA has been demonstrated in [6-8]. We have shown [3, 4] that in supramolecular DNA–residual protein complexes, of different complexity, isolated by a mild phenolic method [1], three types of specific S–S bonds are present (mercaptoethanol-dependent at acid and neutral pH, glutathione reductase-dependent), and that these can determine different levels of organization of the DNA not only of eukaryotes, but also of prokaryotes.

In the investigation described below the sedimentation method was used to assess the size of thiol-induced DNA subunits, and their secondary structure was studied by the thermal denaturation method.

EXPERIMENTAL METHOD

Supramolecular DNA complexes, isolated by methods in [1] from loach sperm and erythrocytes, and from hens' erythrocytes, contain 0.15, 0.2, and 0.5% of residual protein respectively; the composition of the protein includes cysteine and 40% of acid amino acids; it consists of 4-5 peptides with mol. wt. of 12-70 kD, of which the duplex of 50-70 kD proteins is constant for different objects [5]. As S–S-cleaving agents we used 2-mercaptoethanol (ME, from "Serva," Germany), dithiothreitol (DTH, from "Serva," Germany), and sodium borhydride (25 mM). The DNA preparations were incubated with the thiols under sterile conditions with the addition of 10 mg% of sodium azide, using the following solutions: 0.07 M NaCl, 0.07 M acetate buffer, pH 4.4-5.9; 0.07 M NaCl, 0.07 M phosphate buffer, pH 8.0; 0.14 M NaCl, 0.025 M EDTA, 0.003 M phosphate buffer, pH 8.0. After treatment with the thiols the DNA samples were dialyzed against 0.14 M NaCl-0.01 M SSC, pH 7.0. W-sedimentograms were recorded by means of a chromatographic densitometer (type KhD, USSR). The sedimentation constant and molecular weight of the DNA were estimated by the method in [10], with correction for the experimental conditions used. DNA from phage T4 and phage lambda, isolated by the method in [1], served as the standard. Floating the DNA (20 μg/ml in solution of 0.14 M NaCl, 0.01 M SSC, pH 7.0) was carried out on an SF-16 spectrophotometer at 260 nm, with exposure of 5 min at the points for reading optical density (interval 5°C).

EXPERIMENTAL RESULTS

As we showed previously by a viscosimetric method [3, 12], a characteristic feature of the thiols (ME and DTH) is fragmentation of supramolecular DNA complexes only at acid pH values (4.1-5.5); the intensity of the effect, moreover, was determined by the incubation time (2-24 h), the nature of the thiol, and the nature of the complex. In

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