SPONTANEOUS DNA SYNTHESIS IN NORMAL ADULT AND NEONATAL HUMAN LYMPHOCYTES

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Recent reports have described the discovery of a phenomenon of DNA synthesis in peripheral blood lymphocytes, which incorporate thymidine label during culture for 2-4 h after taking the blood sample from persons with transplanted organs and patients with infectious diseases and chronic lymphatic leukemia [3-5].

The aim of this investigation was to determine the level of DNA synthesis in lymphocytes and individual variations in this parameter in normal adults and also to determine its values in cord blood of normal neonates.

EXPERIMENTAL METHOD

Altogether 156 healthy subjects aged from 20 to 30 years (91 men and 65 women) were investigated; blood samples were taken for testing twice from 25 subjects (men and women) and 3 or 4 times from seven women. Heparinized blood from the cubital vein or umbilical cord, in a volume of 5-7 ml, was fractionated in a Ficoll-Verografin density gradient. The isolated lymphocytes were washed in medium 199 during centrifugation for 10 min at 1000 rpm. Lymphocytes (1 million) were incubated at 37°C for 30 min, after which they were treated with 4 μCi of [^{3}H]thymidine (specific activity 25 Ci/mmole) and incubated for a further 2 h. The cells were then washed 3 times with cold physiological saline during centrifugation for 10 min at 1000 rpm, covered with cold 5% TCA solution for 20 h, and then transferred to millipore filters. The results were determined on a scintillation counter and expressed in cpm. Statistical analysis of the results was carried out by Student's t test.

EXPERIMENTAL RESULTS

The statistical data on spontaneous DNA synthesis in lymphocytes from normal women and men are given in Table 1.

TABLE 1. Individual Variations in Spontaneous DNA Synthesis by Lymphocytes of Normal Adults (M ± m)

Investigation	Number of tests	DNA synthesis, cpm
Once only		
women	53	74,06±5,78 (13,16—332,83)
men	69	73,37±4,08 (13,00—316,00)
Twice		
first blood sampling	25	68,71±4,47
2nd " "		70,10±3,67

Legend. Here and in Table 3: limits of variations shown in parentheses.

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TABLE 2. Incorporation of [^{3}H]-Thymidine (cpm) into Lymphocytes from Normal Women Tested 3 and 4 Times

No.	Number of blood samples taken			
	1	2	3	4
1	120,33	16,33	54,83	47,83
2	158,83	86,13	62,00	13,00
3	51,16	89,50	42,83	41,16
4	66,16	70,50	81,16	291,66
5	125,66	29,00	140,83	155,66
6	99,75	154,66	85,25	—
7	65,66	124,83	59,66	—

TABLE 3. Spontaneous DNA Synthesis in Lymphocytes from Normal Neonates (M ± m)

Group tested	Number tested	DNA synthesis, cpm
1) Normal neonates disregarding state of mother's health	64	2126,60±398,35 (64,1—16281,8)
2) Normal neonates from normal mothers	17	281,79±33,73 (64,1—617,16)

The results showed that there were no significant differences between men and women with respect to the mean values of these parameters. Concentration of the values was observed between limits of 50 and 90 cpm. On the 53 women tested, the parameters in 10 (about 18%) did not fall within the confidence interval (t = 95) but were on the high side. This percentage in the group of men reached 14.

Differences in the mean values between subjects tested once and twice were not significant (Table 1).

The absolute values of parameters of spontaneous DNA synthesis in lymphocytes of normal women when tested 3 and 4 times are given in Table 2.

In view of the possibility that spontaneous DNA synthesis in lymphocytes may vary during ontogeny, tests were carried out on a group of healthy neonates. The results are given in Table 3.

Altogether 64 infants with a normal course of the neonatal period were tested. The mothers of these infants were clinically healthy at birth. Analysis of values of spontaneous DNA synthesis in lymphocytes of this group of neonates revealed wide scatter of this parameter (Table 3, group 1).

Careful analysis of the history of the mothers of these infants revealed close correlation between high values of DNA synthesis in neonatal lymphocytes with ill health in the mothers and also enabled a group of healthy neonates (17 of the 64) from healthy mothers to be distinguished. Table 3 (group 2) shows that the mean values of DNA synthesis differed significantly in the two groups ($P < 0.001$); the two groups also differed significantly ($P < 0.001$) from normal adults.

These observations suggest that high values of DNA synthesis obtained by the test used indicate intrauterine stimulation of the fetal immune system.

There have been few studies of spontaneous DNA synthesis in lymphocytes. A study of patients with Hodgkin's disease and with acute and chronic bacterial and virus infections, and of immunization of healthy volunteers revealed elevation of DNA synthesis in lymphocytes compared with its level in normal subjects. The important conclusion was drawn that the value of the parameter depends on the phase of the disease and activity of the process (a tendency toward a decline during clinical improvement) [4]. The prognostic value of sponta-

neous incorporation of labeled thymidine by circulating lymphocytes has been demonstrated in chronic lymphatic leukemia [5]. The writers previously studied the dynamics of the rise in spontaneous incorporation of labeled thymidine during physiologically normal pregnancy [1]. These findings agree with those obtained by other workers [6]. The origin of the lymphocyte subpopulation spontaneously incorporating the thymidine label has been associated in different studies with stem cells, precursors of immunocompetent cells, and sensitized lymphoid cells [2-5].

It can be concluded from analysis of the results that the peripheral blood of normal adults contains a definite level of proliferating lymphocytes, on the basis of which a criterion of normal can be deduced for a given age group allowing for the technical conditions under which the test is performed. Reduction of the time of culture and manipulations *in vitro* to the minimum ensures that the test is more physiological than others.

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INTERACTION BETWEEN SYNTHETIC PEPTIDES AND INDIVIDUAL COMPONENTS OF THE BLOOD CLOTTING SYSTEM

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Intensive research is currently in progress into the effect of various peptides on the vascular system. These substances appear in large quantities in the blood stream as a result of the action of blood hydrolytic enzymes — trypsin, thrombin, and plasmin. Peptides formed during plasmin hydrolysis of fibrinogen and fibrin possess vasoconstrictor activity and increase vascular permeability [6]. Investigation of these substances and of their synthetic analogs has shown that the biological activity of these compounds depends on the presence of three amino-acid residues in them: proline, arginine, and lysine [6, 7].

Investigations have demonstrated the effect of peptides on blood coagulation. For instance, placental peptides lengthen the prothrombin clotting time [12]. Peptides obtained during fibrinogenolysis exhibit marked anticoagulant properties [11]. They lengthen the prothrombin and thromboplastin times of blood plasma, reversibly inhibit factors VIII, IX, XI, and XII of the blood clotting system, and control thromboplastogenesis. In the investigations which have been undertaken not only have natural peptides been studied, but compounds with high anticlotting potential have also been synthesized. Stereoisomeric analogs of the α(A)-chain site of fibrinogen in the region of the Arg-Gly bond [2] have been created, which exhibit anticoagulant activity by removing thrombin from the fibrinogen molecule. Synthetic peptides, constituting N-terminal regions of the fibrin α-chain can bind fibrinogen and frag-

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