activity of preparations and with the absence of such potentiation of toxic side effect. This may be the explanation of this same action of other derivatives of hydrazine, notably hydrazine sulfate.

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SPONTANEOUS AND INDUCED PRODUCTION OF TUMOR-NECROTIZING FACTOR BY NEONATAL BLOOD CELLS

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Tumor-necrotizing factor (α-TNF), a pleiotropic mediator with a broad spectrum of biological effects, not only possesses direct antitumor cytotoxicity, but it is also involved in the inflammatory and immune reactions of the body, interacting with other soluble factors and, in particular, with γ-interferon and interleukin 1 [5]. Participation of TNF both in protective reactions agains tumors or infections, and in reactions harmful to the body (hyperstimulation of macrophages under infectious or tumor conditions), is due mainly to its ability to increase expression of surface antigens during interaction with the specific receptor for TNF on a wide variety of cells [1, 3, 9]. An increase in the expression of HLA-antigens of the I and II classes may stimulate induction of a specific antitumor response as a result of increased ability to present an Ia-protein–antigen complex [3, 9]. Increased expression of the surface antigens on endothelial cells modulates their hemostatic properties, promoting adhesion of polymorphonuclear cells to endothelial cells, stimulating secretion of procoagulant factor, and reducing expression of thrombomodulin on endothelial cells [1].

The principal producers of TNF are activated macrophages [6, 8]. Increased sensitivity of newborn infants to infection have been linked with a defect of certain functions of the macrophages (ability to present antigens and to respond to lymphokines) [2, 7]. Accordingly the study of the ability of cells of newborn infants to produce TNF is of definite interest.

The aim of the work was to assess the level of TNF activity in the serum and to determine spontaneous and induced production of this factor by neonatal human blood cells.

EXPERIMENTAL METHOD

The test material consisted of mononuclear cells and blood serum from the umbilical cord of newborn infants. In some cases venous blood from infants aged 5-6 days also was investigated. The serum was frozen and kept at −20°C until required for testing. Mononuclear cells were isolated from heparinized blood with the aid of differential centrifugation on a Ficoll–Hypaque density gradient (α = 1.077) as described by Boyum [4]. Into four wells of a 24-well panel $1 \cdot 10^6$ mononuclear cells were introduced in 1 ml of medium RPMI-1640 with 10% calf embryonic serum, 1% glutamine, and antibiotics. After incubation for 1 h, cells not adherent to plastic were removed from two wells and the adherent cells were washed 3 times and treated

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TABLE 1. TNF Activity in Serum from Cord and Peripheral Blood of Newborn Infants (in %)

Neonates	Serum from cord blood	Serum from venous blood of infants aged 5-6 days (%)
R. A.*	41,7	56,9
P. N.*	52,3	—36,8
M. V.	—7,1	—31,0
K. B.	—21,0	—23,1
K. V.	—25,0	14,1
M. G.	—15,5	0,2
N. A.*	20,2	38,0
K. D.	—7,0	4,0
K. E.	—16,0	12,8
Zh. B.*	—6,1	—47,1
Kh. S.*	51,2	0,2

TABLE 2. TNF Production by Cord Blood Cells

Neonates	Serum	Supernatant of mononuclear cells		Supernatants of adherent cells	
		medium	LPS + GMDP	medium	LPS + GMDP
Yu. S.	—74,1	22,0	30,6	23,2	24,7
Sh. N.	—6,3	—7,5	36,1	30,3	78,1
K. B.	—21,2	23,4	49,5	—34,4	51,0
S. A.	—16,5	16,1	79,1	14,0	44,8
V. N.	—3,1	31,0	34,5	21,6	24,6
K. A.	—22,0	8,6	13,7	25,8	24,3
Sh. M.	—12,2	57,1	79,3	81,0	90,5
M. A.	—20,5	28,7	38,4	14,0	25,6
Zh. B.*	—6,1	—21,3	89,2	—52,3	79,5
B. S.*	54	32,6	90,1	33,4	94,5
N. A.*	20,2	3,2	76,3	35,7	57,3
B. M.*	16,3	47,1	76,4	43,2	81,5

Legend. Here and in Table 2, infants whose mothers showed signs of toxemia of threatened abortion.

with 1 ml of medium. To one of each pair of wells were added 10 μliters each of bacterial lipopolysaccharide (LPS, 1.0 μg) and the synthetic analog of the repetitive fragment of bacterial wall glycolipids, namely glucosamine-muramyl dipeptide (GMDP, 20 μg). The supernatants of the cultures were collected after 24 h and kept until testing at −20°C.

To test activity of the TNF, cells of mouse fibroblast line L-929 sensitive to it were used. Into each well of a 96-well flat-bottomed panel were introduced $5 \cdot 10^4$ cells. After 24 h, actinomycin D (final dilution 1 μg/ml), followed by serum (1:10) or supernatant (1:1) were added to wells containing a cell monolayer. After culture for 18 h the culture fluid was withdrawn and the cells were stained with a 0.2% solution of crystal violet in 2% ethyl alcohol for 10 min. After careful washing to remove the dye, the panels were dried. The intensity of staining of the cell monolayer was determined by means of a Titertek Multiscan system, using a 540-nm filter. Cytotoxic activity (CTA) was expressed as a percentage:

$$\text{CTA} = \frac{a - b}{a} \cdot 100,$$

where a denotes the intensity of staining of cells in the control wells (addition only of medium with actinomycin D), and b denotes the intensity of staining of cells in wells after addition of serum or supernatant.

EXPERIMENTAL RESULTS

Table 1 gives the results of estimation of the cytotoxic action of cord blood serum of a group of healthy full-term newborn infants (8-10 points on the Apgar scale). In most cases, the serum had no cytotoxic activity whatever against target cells. Moreover, many sera had a "protective" action on target cells, as shown by the more intensive staining of the cell monolayer in the experimental wells than in the controls (negative values). In 36% of cases, however, a marked degree of CTA (over 15%) was detected. On reinvestigation at the age of 5 or 6 days, the character of activity of the serum of some infants changed, whereas that of others did not. Retrospective analysis showed that the mothers of those infants whose serum possessed CTA had exhibited signs of late toxemia or threatened abortion.

The results of the study of the ability of cord blood cells to produce the cytotoxic factor spontaneously or after induction by means of LPS and GMDP are given in Table 2. Nearly all the supernatants had a cytotoxic action of target cells. In most cases addition of the inducers led to an increase, to a varied degree, of production of the cytotoxic factor. No definite correlation was observed between its concentration in the supernatants and serum, although a definite tendency was noted toward an increase in cultures of cells from blood whose serum possessed cytotoxicity. It was shown [6] with the aid of antibodies to TNF that this factor alone is the lytic mechanism for target cells sensitive to it (L-929 and WEHI-164). The authors cited suggest that normally TNF acts in the membrane-bound form, mainly locally. In response to stimulation by LPS, macrophages secrete TNF if they have been activated beforehand [8]. The results of our own investigations indicate that TNF is

not present in detectable amounts in the blood serum of the majority of neonates, although mononuclear cells, including those adherent to plastic, are able to produce this factor and, to a certain degree, to respond to induction by bacterial products. Considerable TNF activity was found in the serum of some infants, evidently due to intrauterine activation of the TNF-producing cells. It is important to discover to what extent the presence of TNF in the serum reflects pathological conditions arising during hyperstimulation of macrophages. In these children, in the case of infection, there may perhaps be a high degree of disk of onset of systemic release of TNF, giving rise to physiological decomposition, tissue damage, and lethal shock.

The mononuclear cells of newborn infants can thus produce TNF, but TNF activity cannot be found in serum from the cord blood of the majority of healthy infants.

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ANALYSIS OF CORRELATION BETWEEN KINETICS OF BLOOD SERUM CHEMILUMINESCENCE AND EXPERIMENTAL TUMOR GROWTH

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One of the particular metabolic features of a growing tumor is its accumulation of bio-antioxidants [7], which stimulate tumor growth by inhibiting free-radical oxidation (FRO) [4]. One manifestation of interaction between host and tumor [9] is the correlation between free-radical oxidation and antioxidative activity in the body media during tumor growth, an appropriate indicator of which is the kinetics of spontaneous chemiluminescence (SCL) [1, 2, 14], and the most convenient object for investigation is blood serum (plasma). The view has developed in the literature that reduction of the intensity of luminescence, as a result of "pumping over" of antioxidants from the tissues into the developing tumor, is characteristic both of tumor tissue and of blood [6, 13].

Our experimental and clinical data indicate a different, and even opposite, effect of tumor processes of different nature on the kinetics of SCL of blood serum [2, 3, 12, 14]. However, there has been no attempt to analyze the correlation which we found between the kinetics of SCL and the kinetics of tumor growth. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

The intensity of SCL of the blood serum was determined on an original apparatus based on the FÉU-39a photoelectronic multiplier [14]. After rapid decapitation of the animals their blood was centrifuged at 1500 rpm for 10 min. Samples of serum, 1.5 ml in volume,

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