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INCREASED UNSATURATED TRANSCOBALAMIN II IN AUTOIMMUNE DISEASE; INFLUENCE OF IMMUNOSUPPRESSIVE THERAPY.

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Transcobalamin II (TC II) is a serumprotein responsible for transporting vitamin B 12 to the cells. Congenital deficiency of TC II leads to severe impairment of hematopoietic, gastrointestinal and immunologic functions. We have studied TC II levels in patients with acquired immunodeficiency states due to chemotherapy with prednisone, azathiorpine and/or chlorambucil. Two different assays to determine the B 12 binding capacity of unsaturated TC II were used.

Two groups of patients were investigated: 1. Autoimmune diseases (AID), lupus erythematodes 35, dermatomyositis 3, autoimmune hemolytic anemia 2; 2. Renal transplant patients 39 under immunosuppressive therapy.

Results: TC II was found normal or elevated 2-5 fold in untreated AID, normal in AID under therapy in remission, increased 1.5-3 fold in treated, but active AID and normal or elevated 1.5 fold in treated renal transplant patients. Thus, immunosuppressive treatment reduces high TC II serum concentrations in AID to normal but not to subnormal levels (group 1)

One other report in the literature found equally (2-6 fold) elevated TC II in acute leukemia and lymphoma, which decreased after therapy. These data and normal or elevated TC II found in group 2 indicate that synthesis of TC II is not restricted by immunosuppressive drugs.

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STUDIES ON TRANSCOBALAMIN (TC): QUANTITATION OF TC II; COMPARISON OF AN ELECTROPHORETIC WITH AN IMMUNOCHEMICAL ASSAY OF TC II IN HUMAN SERUM.

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Vitamin B12 in blood is transported by specific carrier proteins, the transcobalamins (TC I, II and III). TC II is of particular interest since it carries and delivers vitamin B12 to the cells. Polyacrylamid gel electrophoresis was used to separate TC I and III from TC II. This was achieved after saturation of TC I, II and III with 57 Co-labelled vitamin B12 and subsequent neuraminidase degradation of TC I and III. Unchanged TC II could then be separated electrophoretically from the other transcobalamin fractions. Autoradiographic evaluation showed that the TC II fraction is composed of several isoprotein bands (individual isoprotein patterns, probably genetically determined, were observed). Absolute radioactivity of the gel and relative intensities of the radioactive bands were used to quantitate the TC II fraction.

Unsaturated TC II levels were also determined using a new immunochemical method, based on the precipitation of TC II by insolubilised anti-TC II antiserum (radio immunosorbent technique = RIST). The results of TC II determinations with these two basically different assays correlate satisfactorily.

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ACID PHOSPHATASE (AcP) ISOENZYMES IN CHILDHOOD ALL

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In attempt to further differentiate cytochemically and immunologically defined subtypes of ALL AcP isoenzyme patterns of various cell extracts were investigated by isoelectric focusing in gels.

Isoenzyme patterns of T-ALL blasts were found to be identical to that of normal and fetal thymus cells. Also one case of BURKITT lymphoma, investigated so far, did not show any significant difference of AcP isoenzymes to T-ALL.

The so called Non T- Non B-ALL however turned out to be a heterogenous group with different cytochemical and isoenzymic AcP patterns: cytochemically undifferentiated cells either contained only one single band of AcP or no AcP was detectable. In cases of cytochemically weak and insignificant positive AcP reaction several AcP isoenzymes could be demonstrated by isoelectric focusing but fewer than in T-ALL.

Subgrouping of Non T- Non B-ALL seems to be possible by the determination of enzyme markers.

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HEXOKINASE IN ERYTHROCYTES OF NEWBORN INFANTS AND ADULTS: ISOLATION AND CHARACTERIZATION OF THE ISOENZYMES.

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The metabolism of erythrocytes of newborn infants (nRBC) is different from that of adult erythrocytes (aRBC). Since hexokinase plays an important role in the regulation of glycolysis we investigated the distribution and properties of the hexokinase isoenzymes (Hxi) in nRBC and aRBC.

By column chromatography with DEAE-Glycophase we isolated two Hxi in aRBC. Hxi I represented more than 90% of the total hexokinase activity in aRBC. In nRBC we found only one Hxi which was eluted with a slightly higher conductivity than Hxi I from aRBC.

Km values for glucose and ATP, inhibition by ADP, pH optimum and thermostability of aRBC Hxi were identical to rat liver Hxi I and III. The Hxi from nRBC, however, is different from all rat liver and aRBC Hxi. It shows a higher Km values for glucose and ATP (0.1 mM and 1.8 mM respectively) than Hxi I from aRBC (0.047 mM and 0.98 mM respectively). 2,3-DPG inhibited the Hxi from nRBC more than Hxi I from aRBC.

The low affinity for glucose and the increased inhibition by 2,3-DPG of the nRBC Hxi may explain the decreased glycolytic rate compared with aRBC of similar age distribution of nRBC.

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USE OF SPECIFIC ANTISERA AGAINST LEUKEMIA-ASSOCIATED ANTIGENS IN DIAGNOSIS AND TREATMENT OF CHILDHOOD ALL.

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Rabbit antisera against the common forms of childhood acute lymphoblastic leukemia (ALL) the 0- and T-ALL were produced. Extensive absorptions of the antisera removed the crossreactions with normal mature lymphocyte populations and bone marrow progenitor cells. 1) A characterization of the type of ALL by use of the highly purified antisera is possible by different test systems such as cytotoxicity, immunfluorescence and quantitative complement fixation. The results show that the former test systems such as rosette technique failed to identify some 0-ALL. Therefore by use of specific antisera the benefit of chemotherapeutic treatment for the different forms of ALL can be better calculated. 2) Treatment of a patient with 0-ALL during his 3rd bone marrow relapse by high doses of Anti-0-ALL globulin revealed a marked decrease in circulating blast cells but no effect on bone marrow blast cells. Our approach aims for an elimination of residual leucemic cells in bone marrow during haemopoietic remission using the cytotoxic effect of the antisera. The use of A-0-ALL-G for "antileukemic autotransplantation" is proposed.

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THE EARLY ANEMIA OF PREMATURITY. PLASMA LEVELS OF TRANSFERRIN, IRON, FERRITIN, AND CERULOPLASMIN.

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As indicative of the iron status during the early anemia of prematurity, transferrin, iron, and ferritin levels of plasma were followed in infants with birth weight less than 1500 g. Ceruloplasmin, thought to act as ferroxidase, was also studied. Oral iron therapy was started at the age of four weeks.

Transferrin concentration rose steadily from a mean value of 1.58 g/l at 1 week of age to 2.17 g/l at 8 weeks of age. Plasma iron concentration at 1 week of age was 15.6 ± 5.5 umol/l and did not change significantly during the 8 week period. Ferritin in cord blood of full term infants was within the range of normal adult males, while the levels in cord blood from 10 infants with birth weight less than 2000 g were lower. Ferritin levels during the course of the early anemia will be discussed. Ceruloplasmin showed a more variable course than transferrin, but after 4 weeks a progressive rise occured. Mean value at 1 week of age was 0.11 g/l, at 6 weeks 0.13 g/l, and 0.15 g/l at 8 weeks.

The plasma iron levels observed are within the normal range for full term infants. However, whether they provide suffient available iron for the accelerated erythropoiesis of this period is unclear. The progressive rise in transferrin suggests that in this period the level more reflects the increase in general protein synthesizing capacity than the iron status. Ferritin concentration, on the other hand, seems to reflect iron stores.