175 EFFECT OF DIETARY NUCLEOTIDES ON LYMPHOCYTE MATURATION, Frederick B. Rudolph, William C. Fanslow, Anil D. Kulkarni, and Charles T. Van Buren, Rice Univ. Dept. of Biochemistry and Univ. of Tex. Med. School, Dept. of Surgery.

We have shown previously that removal of purines and pyrimidines from the diet (nucleotide free diet-NFD) decreases immune responses both *in vivo* and *in vitro*. Allograft rejection and mixed lymphocyte responses and resistance to infectious organisms were all significantly influenced by NFD as compared to chow or NFD with RNA or isolated bases added. NFD affects T-helper cell number and functions as well as suppressor cell activity. In addition, NFD significantly decreases graft-versus-host disease mortality in allogeneic bone marrow irradiation chimeras suggesting that NFD impairs the functional T-cell maturation. To determine if this immunologic depression in our various diet fed mice is due to impaired maturation of T-lymphocytes, we assessed lymphoid tissues for the presence of terminal deoxynucleotidyl transferase (TDT). This enzyme is a well characterized marker for immature T-lymphocytes. A statistically higher number of cells positive for TDT activity were found in the thymus and bone marrow of mice on NFD ($p < 0.02$) as compared to cells from animals on chow or NFD with added RNA, uracil, or adenine. These results are consistent with a requirement for either a pyrimidine or purine or both for development of T-helper cell populations. These effects are likely due to a greater dependence of these cells on nucleotide salvage in G1 phase than other lymphocytes. (Supported by Grants 14030 and 35492 from the NCI.)

176 Changes in lymphocyte adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) levels in lung cancer after radical surgery. Michele Russo, Teresa Pizzella, Gaetano Liguori, Salvatore Nardiello and Bruno Galanti Clinic of Infectious Diseases, 1st Medical School, University of Naples, Naples, Italy.

Patients with solid tumors have low lymphocyte ADA levels, while operated patients without recurrence show normal levels(1). To elucidate whether ADA levels are restored by the surgical removal of tumor, we determined ADA and PNP activities in peripheral mononuclear cells (PMC), in T-enriched and T-depleted subpopulations from 13 healthy controls and from 6 patients with lung cancer before and 1 month after complete removal of tumor. Lymphocyte subpopulations were prepared by rosetting with SRBC. Before surgery the mean PMC ADA value (17.2 ± 4.8 SD, $\mu\text{M}/10^7$ cells) was significantly lower than the control value (24.8 ± 4.9 , $P < 0.01$). ADA levels were lower than controls in both T (18.1 $\pm$ 5.1 vs. 20.4 $\pm$ 3.9) and non-T cells (24.4 $\pm$ 5.1 vs. 26.7 $\pm$ 4.0). After surgery ADA levels, compared to pre-surgery values, were increased in PMC (24.2 $\pm$ 2.2, $P < 0.025$) and in T-enriched (25.7 $\pm$ 7.9, $P < 0.05$), but decreased in T-depleted subpopulations (19.9 $\pm$ 6.7). PNP pre-surgery levels did not differ from control values in PMC, T-enriched or T-depleted subpopulations; after surgery, they showed a trend similar to ADA. In conclusion 1 month after radical surgery ADA levels are apparently restored in unseparated PMC, however an ADA increase is found in T-cells, while low levels persist in non-T cells. 1) Russo M. et al.: Br. J. Cancer 43 (1981), 196-200.

177 KINETIC PROPERTIES OF HUMAN ERYTHROCYTE ADENOSYLHOMOCYSTEINASE. C. Salerno, A. Bozzi, C. Crifò, R. Strom Institute of Biological Chemistry and Department of Human Biopathology, University of Rome, and C.N.R. Centre for Molecular Biology, Rome, Italy.

Adenosylhomocysteinase (EC 3.3.1.1)-catalyzed hydrolysis of adenosylhomocysteine was measured in 0.1M HEPES, pH 7.0, at 37°C by continuous monitoring of the reaction product at 265 nm in the presence of adenosine deaminase. The initial reaction rate was a hyperbolic function of substrate concentration. The apparent K_m (60 μM) decreased upon addition of phosphate ions (P_i), reaching an asymptotic value of 20 μM in the presence of an excess of P_i (half saturation value = 8 mM P_i). The apparent V_{max} seemed to be unaffected by P_i . Pyrophosphate, arsenate, and sulfate exerted similar effects. Upon addition of 20-50 mM mercaptoethanol (but not ethylene glycol, ethanol, or glycerol), not only was the initial reaction rate lowered, but the hydrolysis process came to a stop without even reaching completion. Upon addition of new enzyme to the reaction mixture, there was further hydrolysis of the residual adenosylhomocysteine; no activity was instead restored if new adenosylhomocysteine was added to the inactivated enzyme. In the same concentration range, mercaptoethanol *per se* was totally ineffective on the enzyme in the absence of substrate.

178 REGULATORY ASPECTS OF HYPOXANTHINE UPTAKE BY HUMAN ERYTHROCYTES.

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The uptake of ($8\text{-}^{14}\text{C}$)hypoxanthine by human erythrocytes, suspended in a Tris glucose NaCl isotonic medium, has been studied at 37°C in pH 6.7-8.0 range. In the presence of inorganic phosphate (3-10 mM), hypoxanthine was taken up by the cells and incorporated into IMP. The rate of hypoxanthine uptake was markedly stimulated by decreasing the pH and/or the oxygen partial tension in the incubation medium. The effect exerted by oxygen was more evident in the acidic pH range and could be overcome by removing the gas. At pH higher than 7.5, hypoxanthine was not appreciably taken up by the cells even in anaerobic conditions. Carbon monoxide inhibited hypoxanthine uptake as well as pure oxygen atmosphere.

179 INFERENCES ON THE RAPID METABOLISM OF EXOGENOUSLY APPLIED ATP IN ISOLATED SMOOTH MUSCLE PREPARATIONS USING PHARMACOLOGICAL STUDIES

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ATP causes relaxation of various smooth muscle containing preparations. ATP and related analogs were applied to strips of trachea and taenia coli from guinea-pigs in organ baths for 60 sec. The formation of adenosine was signalled by potentiation of relaxations in the presence of the adenosine transport inhibitor dipyrindamole. The basis for this potentiation is that adenosine which is also a potent relaxant is rapidly taken up into muscle cells decreasing its effect. ATP caused relaxations of both taenia coli and tracheal strips and both responses were potentiated by dipyrindamole signifying that adenosine was being formed during the contact times. In the taenia both the α,β and the β,γ -methylene isosteres of ATP caused relaxations. Dipyrindamole potentiated responses to the latter, but failed to affect responses to the former. Since methylene substitution renders the analog stable at that site it is presumed that the β,γ -methylene isostere rapidly formed adenosine due to the action of a pyrophosphohydrolase. In tracheal strips the β,γ -methylene isostere of ATP caused relaxations which were potentiated by dipyrindamole, the α,β -methylene isostere was inactive either in the presence or absence of dipyrindamole. This suggested that in contrast to the taenia, ATP is inactive in the trachea *per se* and must first be degraded to adenosine which in turn causes the relaxation.

180 PHOSPHORYLATION OF 3-DEAZAGUANOSINE (3-DGR) BY CHINESE HAMSTER OVARY (CHO) CELLS. Priscilla P. Saunders.

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3-Deazaguanine is metabolized by CHO cells via HGPRT catalyzed phosphoribosylation. Cells having a deficiency in HGPRT are highly resistant to 3-deazaguanine but retain sensitivity to the nucleoside, β -ribosyl-3-deazaguanine (3-DGR). Commercial purine nucleoside phosphorylase (PNPase) was employed to prepare 3-DGR and β -deoxyribosyl-3-deazaguanine (3-DGdR) having ^{14}C in the ribose and deoxyribose moieties for use in studies of the phosphorylation and other metabolism of these compounds. Incubation of HGPRT deficient cells with 3-DGR [^{14}C] results in the appearance of apparent metabolites in the triphosphate region of HPLC-derived nucleotide profiles. 3-DGdR [^{14}C] appeared not to be metabolized by these cells; no labeled metabolites were observed in the nucleotide profile. Crude extracts of CHO cells catalyze an ATP dependent transformation of labeled 3-DGR to a product which elutes in the monophosphate region of the nucleotide profile. Fractionation of extracts by DEAE-cellulose chromatography has revealed a single peak of apparent 3-DGR phosphorylating activity which elutes at a region distinct from other known purine nucleoside kinase activities. Attempts at identification of this enzyme are in progress. This work was supported by Grant CH-283 from the American Cancer Society.