

157 TOXICITY AND ANTITUMOR EFFECT OF 5-FLUOROURACIL (FU) AND ITS RESCUE BY URIDINE (UR).

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The application of FU in the treatment of colon cancer is limited because of myeloid and gastrointestinal toxicity. It has been demonstrated that UR is able to rescue mice from the toxic effects of FU, which precludes the use of higher FU doses. Antitumor effect and toxicity were studied in two murine colon carcinomas, both sensitive and resistant, which also show marked differences in FU metabolism (e.g. uridine phosphorylase is 10 times higher in Colon 26 than in Colon 38). Mice received FU weekly. Colon 38, grown in C57Bl mice, was sensitive to FU; at 100 mg/FU kg 70% of the tumors disappeared. UR administration (3500 mg/kg) after 2 and 20 hr, decreased the number of complete responders. Colon 26, grown in Balb/c mice, was not sensitive to 100 mg FU/kg. FU was lethal at 300 mg/kg, but UR increased the life-span, while a significant tumor growth delay was observed. 100 mg FU/kg caused a severe leucopenia which reached a nadir of 10% after the second dose. UR administration prevented this leucopenia and a faster recovery was observed. UR was not lethal, although, a sharp fall in body temperature was observed after 1 hr (+ 10°C). However, this hypothermia was not observed with patients in a Phase I trial with UR. In conclusion: hematological toxicity caused by FU can be prevented by delayed administration of UR; the antitumor activity seems to be affected, but this was not significant; the side-effects of UR seem to be acceptable to proceed Phase I trials.

158 URIDINE MONOPHOSPHATE KINASE: A NEW GENETIC MARKER OF SUSCEPTIBILITY TO HAEMOPHILUS INFLUENZAE TYPE B DISEASE.

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Alaskan Eskimos have the highest known incidence of invasive *Haemophilus influenzae* type b (Hib) disease, primarily meningitis, affecting 1-5% of all children in the first two years of life. We examined potential genetic explanations for this unique susceptibility by studying 93 Eskimo Hib cases and an equal number of healthy Eskimo controls matched for age and exposure (village). In this analysis, a polymorphic genetic variant of the pyrimidine pathway enzyme, uridine monophosphate kinase-3 (UMPK-3), was positively associated with invasive Hib disease, with a relative risk of 3.3 (McNemar matched pair, $P < .01$). All UMPK 3-3 homozygotes in this study were Hib cases. We also found that in cases and controls, there was no difference between UMPK phenotype and serum levels of total Hib antibody as measured by radioimmunoassay. This suggests that UMPK-3 may play a role in mediating the non-humoral immunity to Hib. However, unlike other enzyme variants in the nucleoside synthesis pathways which result in syndromes of severe immunodeficiency, this gene appears to confer a more subtle disease susceptibility. Thus, the UMPK-3 allele, although rare in many human populations, is associated with an increased risk of invasive Hib infection in Alaskan Eskimos.

159 ADENOSINE TRANSPORT BY ISOLATED RAT LIVER MITOCHONDRIA. Keijo J. Peuhkurinen, Albert R. Collinson, and John M. Lowenstein, Dept. of Biochemistry, Brandeis University, Waltham, MA 02254

Rat liver mitochondria were incubated with ^3H -adenosine (ado) and then separated from the medium and quenched by centrifugation through silicone oil into perchloric acid. The radioactive compounds in the mitochondria were analyzed by thin-layer chromatography. In the presence of 2 μM ado, 6 mM pyruvate and 2 mM malate, ado was incorporated into mitochondrial nucleotides at a rate of 0.79 pmol/mg mitochondrial protein/min. 23 μM Carboxyatractyloside, 1 mM ADP, 5 mM ATP, and 0.1 mM P_1P_5 -di(adenosine-5')-pentaphosphate decreased the incorporation by 78%, 37%, 65%, and 67%, respectively. Conversion of adenosine to AMP + ADP + ATP in the incubation medium (analyzed without separation of mitochondria from the medium) occurred at 3.8 pmol/mg protein/min. Addition of 5 mM ATP increased this to 4.8 pmol/mg protein/min in spite of the decreased incorporation seen in the mitochondria, suggesting that externally added ATP stimulates the phosphorylation of adenosine and release of nucleotides into the medium. The amount of adenosine associated with the mitochondria was constant from 0 to 10 min of incubation under all experimental conditions. It was higher than predicted from sucrose-accessible and mitochondrial matrix volumes determined under identical conditions, suggesting binding of adenosine to mitochondrial proteins. Our results suggest that adenosine is converted to ADP and ATP in the intermembrane space and that ADP or ATP or both are then transported into the matrix by the ADP/ATP translocase located in the inner mitochondrial membrane.

160 THE SYNTHESIS OF PHOSPHORIBOSYLPYROPHOSPHATE FROM GLUCOSE DECREASES DURING AMINO ACID STARVATION OF HUMAN LYMPHOBLASTS.

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When cultured human lymphoblasts are starved for an essential amino acid for 3 h, rates of purine nucleotide synthesis decrease markedly because of a decrease in the intracellular phosphoribosylpyrophosphate concentration (J. Biol. Chem. 257:4242-4247, 1982 and J. Biol. Chem. 259:2936-2941, 1984). In amino acid-starved cells, glucose transport was not changed whereas total glucose consumption and lactate production decreased by approximately 25% and 10%, respectively. Carbon flow through the oxidative pentose phosphate pathway decreased by 18% during amino acid starvation. Kinetic studies of ribulose 5-phosphate 3-epimerase and phosphoriboisomerase suggested that the ribulose 5-phosphate produced by this pathway is converted mostly to xylulose 5-phosphate instead of to ribose 5-phosphate, and thus that this pathway produces little phosphoribosylpyrophosphate. The activity of the nonoxidative pentose phosphate pathway, measured by HPLC following the incorporation of [^{14}C]glucose into phosphoribosylpyrophosphate and ATP and GTP, decreased by approximately 60% during amino acid starvation. None of the enzymes of either pathway changed in specific activity during amino acid starvation. We conclude that the nonoxidative pentose phosphate pathway is the major source of phosphoribosylpyrophosphate for purine nucleotide synthesis and that this pathway is regulated by a metabolite which changes in concentration during amino acid starvation.

161 METABOLISM OF PURINE NUCLEOTIDE IN THE LIVER AND IN THE KIDNEY OF CASTRATED RATS.

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The metabolism of purine nucleotides has been studied in the liver and in kidney of adult male Wistar rats before and after castration. The overall rate of purine biosynthesis was determined by following the incorporation of formate- ^{14}C into acid-soluble cellular purines as well as those incorporated into RNA (1). After castration, the level of acid-soluble purines remains the same both in the liver and in the kidney. The specific activity of the bases is only slightly enhanced in the kidney and significantly increased ($p < 0.05$) in the hypoxanthine and in the guanine of the liver, indicating an accelerated turnover. The G/A labeling ratio (counts per min in guanine/counts per min in adenine), which is an index of the "IMP branch point regulation", is slightly augmented in both tissues. The RNA content is unchanged, both in the liver and in the kidney, but the formate- ^{14}C incorporation is enhanced in liver RNA and decreases in kidney RNA. Results indicate that the testosterone deficiency plays different and discordant roles in purine nucleotide metabolism in the kidney and in the liver.

1) M.M. Welch, F.B. Rudolph J. Biol. Chem. 257, 22, 13253, 1982.

162 THE HORMONAL REGULATION OF PURINE BIOSYNTHESIS. CONTROL OF THE INOSINIC ACID BRANCH POINT.

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Purine biosynthesis "de novo" in levator ani muscle (LAM) of adult male Wistar rats has been studied before and after castration and after testosterone administration. The overall rate has been investigated determining the total pool sizes of free acid-soluble nucleotides and by following the incorporation of formate- ^{14}C into the acid-soluble purines (1), which were purified by ion exchange chromatography and analyzed by HPLC. After castration, the levels of bases decrease, specially the adenine, the specific activity (dpm/ μmole) decreases, while the guanine/adenine labeling ratio (counts per min in guanine/counts per min in adenine) remains unchanged. After testosterone administration (2.5 mg/100 g b.w.) the normal level of the bases is restored and a slight increase of guanine concentration is evident. The specific activity of the bases increases, the G/A labeling ratio also increases. We may conclude that testosterone plays a role in the regulation of the "inosinic acid branch point".

1) M.S. Hershfield, J.E. Seegmiller. J. Biol. Chem. 251, 23, 7348, 1976