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INCORPORATION OF 5-FLUOROURACIL INTO NUCLEOTIDE SUGARS AND THE EFFECT ON GLYCOCONJUGATES IN RAT HEPATOMA CELLS AND HEPATOCYTES.

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Liver is the main organ in the elimination of 5-fluorouracil (5FU). Rat hepatocytes and hepatoma cells (H35) were incubated in monolayer in the presence of 0, 17, 50 and 100 μM 5FU for 4 hrs. The metabolism of 5FU was monitored with [3H]5FU, the protein synthesis was determined by the incorporation of [^{14}C]leucine and glycoconjugate synthesis by incorporation of [^{3}H]GlcNH$_2$ and [^{3}H]fucose. In rat hepatocytes the amount of soluble fluoronucleotides was dependent on the concentration of 5FU. With 50 μM 5FU the concentrations of FUDP-HexNAc, FUDP-hexose and FUTP were 17, 5 and 9 pmol/10^6 hepatocytes. In the presence of 0.5 mM thymine to inhibit the catabolism of 5FU, these concentrations were 23, 29 and 49 pmol/10^6 hepatocytes, resp. The effect of thymine at 17 and 100 μM 5FU was comparable. The incorporation of 5FU into RNA was also concentration dependent and was 72 pmol/10^6 hepatocytes at 50 μM 5FU. In contrast to nucleotide synthesis thymine enhanced the incorporation of 5FU into RNA only less than 1.5 fold at all 5FU concentrations. In H35 cells both synthesis of soluble fluoronucleotides and the incorporation of 5FU into RNA was linear with the concentration of 5FU. At 50 μM 5FU the amounts of FUDP-HexNAC, FUDP-hexose and FUTP were 30, 68 and 106 pmol/10^6 cells, and 345 pmol 5FU/10^6 cells was incorporated into RNA. In both cell types incubation with 5FU had no effect on the incorporation of leucine into proteins and of sugars into glycoconjugates.

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URIC ACID AND PURINE COMPOUNDS IN AORTIC AND CORONARY SINUS BLOOD IN MAN

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In the isolated perfused rat heart the main released purine catabolite is uric acid. Furthermore purine nucleosides and bases added to the perfusion buffer are converted into uric acid at the passage through the coronary circulation. In the human heart a low xanthine oxidase activity has been observed, however few data are available on the release of uric acid. In this work we have determined the purine catabolites by HPLC in deproteinated blood samples collected from the aorta and coronary sinus in human patients submitted to routine cardiac catheterization. Whole blood concentration of uric acid increased from 105 $\pm$ 21 uM in aortic blood to 189 $\pm$ 25 uM in coronary sinus blood, while the concentrations of xanthine and hypoxanthine were slightly decreased or in a few patients were unchanged. Only trace amounts of inosine and adenosine could be detected. The amount of the released uric acid is consistent with the xanthine oxidase activity in human heart. From these results it appears that in the human heart under rest conditions uric acid is the main purine catabolite and that the purine output exceeds the reuptake. In basal conditions the net purine output (including uric acid) should correspond to myocardial de novo synthesis of purine nucleotides. The formation of uric acid must be taken into account in the study of purine metabolism also in human heart.

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PHOSPHOCREATINE AS A POSSIBLE MODULATOR OF THE ADENYLATE POOL

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The administration of purine nucleotide precursors results in a small increase of the adenylate pool because of feedback control mechanisms; nevertheless different tissues have different levels of the adenylate pool. This suggests that other factors may modulate the feedback regulation. We have studied a possible relation between the creatine-phosphocreatine (PC) pool and the adenylate pool and we have determined purine nucleotides, creatine, PC in rat tissues after injection of PC. A significant increase of the creatine-PC pool was observed in those tissues (e.g. myocardium) in which the creatine-PC pool may freely expand. Adenine and guanine nucleotides were also significantly increased. Even more evident increases were obtained with Raji cell cultures: the creatine-PC pool showed a 5-fold increase (from 1.74 to 20.91 nmoles/10^6 cells) and the adenylate pool doubled (from 2.91 to 5.25 nmoles/10^6 cells). Similar results were obtained with hybridoma ascites cells. Both in rat tissues and in cell cultures energy charge was also increased. In cells in which no variation in the creatine and PC levels was observed the adenylate pool was not modified. These results suggest that the creatine-PC pool positively modulate the purine nucleotide pool and explain why tissues with high creatine and PC levels such as heart, muscle, brain, also have a high adenylate pool. It is possible that the increase in the energy charge maintains the adenylate pool at a higher level or that PC decreases the feedback inhibition. Compounds such as exogenous PC that increase the creatine-PC pool seem to be more effective in raising the adenylate pool than nucleotide precursors.

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PURINE METABOLITES AS MEASURES OF BIRTH ASPHYXIA AND PREDICTORS OF BRAIN DAMAGE. Vineta J. Ruth and Kari O. Raivio, Children's Hospital, University of Helsinki, Helsinki, Finland

The classical criteria of birth asphyxia, low Apgar score and metabolic acidosis, correlate poorly with each other and with perinatal brain damage. We evaluated if hypoxanthine (HX) and uric acid (UA) can be used as measures of the severity of asphyxia and/or predictors of adverse neurological outcome.

We measured plasma HX and UA, as well as pH and lactate in umbilical arterial blood of 62 infants born after severe pre-eclampsia of pregnancy (PP), 31 infants born acutely asphyxiated (AA) with 5-min Apgar <7 and/or pH $\leq$7.05, and 38 controls (C). Follow-up was at 2 yr: severe brain damage was found in 4 PP and 5 AA infants, mild in 12 and 6.

HX was higher in AA infants (geom mean; 95% conf: 24; 18-32 µmol/l), but not in PP (10;9-11), than in C (12; 10-16). UA was higher in PP infants (mean; 95%conf: 430;398-461 µmol/l), but not in AA (336;288-385) than in C (303;282-324). In PP group HX did not correlate with pH or lactate, whereas UA correlated with pH (r=-0.305, p=0.024) but not with lactate. In AA group HX correlated with pH (r=-0.647, p<0.001) and lactate (r=0.558, p=0.008), while UA correlated with lactate (r=0.734, p<0.001) but not with pH. No correlation was found between HX or UA and outcome in either patient group.

We conclude that cord HX is a measure of hypoxia in acute asphyxia, but not in prolonged intrauterine distress (ie. PP), whereas the reverse is true for UA. Neither HX nor UA is a predictor of perinatal brain damage.

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TRANSCRIPT-SWITCHING IS THE BASIS FOR CHANGE IN AMP DEAMINASE ISOFORMS DURING IN VIVO AND IN VITRO MYOCYTE DIFFERENTIATION, Richard L. Sabina and Edward W. Holmes, Duke University, Department of Medicine, Durham, NC, USA.

It is well established that many muscle-specific genes undergo switching of non-muscle to muscle isoforms during myogenesis, accompanied by a switch in RNA transcripts. Previously we had reported developmental isoforms of AMP deaminase (AMP-D) in skeletal muscle (Marquetant et al, PNAS 84: 2345, 1987). Subsequent cloning of the rat AMP-D cDNA (Sabina et al, JBC 262: 12397, 1987) has also enabled the analysis of transcript expression during myogenesis. The results of these experiments are as follows: 1) In the early embryo in situ or in proliferating myoblasts in culture, a 3.4 Kb transcript is expressed which encodes the embryonic AMP-D peptide. 2) The amount of this transcript increases during neonatal development in situ and during the transition to myotubes in culture. Concurrently, a 2.5 Kb adult muscle transcript begins to appear. During this time, however, all AMP-D activity is immunoprecipitated by anti-serum specific for the embryonic isoform 3) Late in neonatal development and in fully differentiated myotubes in culture, the embryonic transcript and the embryonic peptide disappear. During this interval there is an increase in the abundance of the adult muscle transcript and the adult muscle peptide appears. These results establish transcript switching as the basis for the change in AMP-D isoforms during myogenesis. These results also demonstrate that the AMP-D gene is controlled through transcriptional regulation presumably in response to tissue-specific factors. Additionally it appears that the adult muscle AMP-D transcript is subject to post-transcriptional regulation as well.

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CHARACTERIZATION OF THE AMP DEAMINASE AMPLICON IN COFORMYCIN-RESISTANT RAT MYOCYTES, Richard L. Sabina, Duke University, Medicine Department, Durham, NC, USA.

Coformycin resistance in Chinese hamster fibroblasts is associated with co-amplification of four unknown genes (i.e. W,X,Y_1,Y_2) presumably closely linked to the target gene, AMP deaminase (Debatisse et al, Mol. Cell Biol. 6: 1776, 1986). The recent cloning of rat AMP deaminase cDNA (Sabina et al, J. Biol. Chem. 262: 12397, 1987) has provided the opportunity to further analyze this presumed amplicon. In order to accomplish this goal a coformycin-resistant rat myocyte subline was established from the parental L6 cell line. One stably resistant clonal isolate (i.e. clone 17) exhibits a 7-fold increase in AMP deaminase gene sequences. Hybridization with the four hamster cDNA's for genes W,X,Y_1, and Y_2 indicates that all are co-amplified and over-expressed in clone 17. These results delineate the AMP deaminase amplicon in the rat and document its similarity to the hamster amplicon. Another clonal isolate (i.e. clone 18) exhibits increased abundance of AMP-D, W, X, and Y_2 transcript, yet none of these genes are amplified. This clone, therefore, represents an example of transcriptional activation without gene amplification and establishes that non-target (i.e. W, X, Y_2) as well as target (i.e. AMP-D) genes are subject to this phenomenon. Experiments are in progress to determine if transcriptional activation always precedes gene amplification and whether the pattern of transcriptional activation predicts those genes that will ultimately be amplified. Finally, developmental analysis of rat myocytes indicates regulated expression of some of these amplicon genes during myogenesis.