169 URINARY OXIPURINOL-1-RIBOSIDE EXCRETION AND ALLOPURINOL-INDUCED OROTICACIDURIA
 Sebastian Reiter, Werner Löffler, Wolfgang Gröbner and Nepomuk Zöllner. Medizinische Poliklinik der Universität München, W-Germany.

Allopurinol-induced oroticaciduria which is due to inhibition of OMP-decarboxylase by oxipurinol-1- and -7-ribotide is markedly reduced by dietary purines. The underlying mechanism possibly is an influence of dietary purines on the formation of oxipurinol-ribotides. The latter being excreted as their corresponding nucleosides we first measured the urinary excretion of oxipurinol-7-riboside which was found not to be altered by dietary purines. Therefore we searched for oxipurinol-1-riboside which had not yet been detected in human urine. By HPLC we were able to demonstrate the presence of this metabolite. In the absence of dietary purines its excretion was similar to that of oxipurinol-7-riboside, but was strongly reduced by purine supplement. Having shown the indirect formation of allopurinol-1-riboside via allopurinol-1-ribotide *in vivo* (cf. the accompanying paper) we assume that the excretion of oxipurinol-1-riboside parallels the formation of oxipurinol-1-ribotide as well. We conclude from our data that dietary purines reduce allopurinol-induced oroticaciduria by inhibition of oxipurinol-1-ribotide synthesis.

170 ALLOPURINOL-1-RIBOSIDE FORMATION AND HGPRT-DEFICIENCY
 Sebastian Reiter, Werner Löffler, Wolfgang Gröbner and Nepomuk Zöllner. Medizinische Poliklinik der Universität München, W-Germany.

Allopurinol-1-riboside has been thought to be synthesized *in vivo* rather directly by purine nucleoside phosphorylase (PNP) than indirectly via the ribotide by hypoxanthine guanine phosphoribosyl-transferase (HGPRT). Consequently the lack of allopurinol-1-riboside formation in HGPRT-deficiency has been attributed to competitive inhibition of PNP by increased hypoxanthine levels (Elion, *Handb. Exp. Pharm.* 51). Having demonstrated elevated excretion of allopurinol-1-riboside in PNP-deficiency we studied the influence of hypoxanthine on allopurinol-1-riboside formation in healthy volunteers and a patient with partial (5% residual activity) HGPRT-deficiency. Allopurinol was given as a single dose of 5 mmol, the volunteers received additionally 7.5 mmol of different purines. The volunteers showed a reciprocal relationship between allopurinol-1-riboside excretion (0.615-0.034 mmol) per 24 hrs and hypoxanthine excretion (0.082-1.22 mmol) during the first 5 hrs following allopurinol and purine intake - the period of allopurinol-1-riboside formation. The corresponding values of our patient were 0.014 mmol allopurinol-1-riboside and 0.24 mmol hypoxanthine. These data indicate that the very low excretion of allopurinol-1-riboside in HGPRT-deficiency must be attributed to the enzyme defect itself as it cannot be explained by the moderate elevation of hypoxanthine formation.

171 *IN VIVO* INHIBITION OF MOUSE LIVER METHYLTRANSFERASE (MT) ENZYMES FOLLOWING TREATMENT WITH 2'DEOXYCOFORMYCIN (dCf) AND 2'DEOXYADENOSINE (AdR).
 Jane Renshaw and Kenneth R. Harrap, Inst. Cancer Res., Dept. Biochem. Pharmacol., Sutton, Surrey, UK.

Treatment of BDF₁ mice with AdR/dCf combinations (daily x5) leads to acute hepatic dysfunction with accumulative dose related hepatocellular necrosis (Paine et al *Cancer Treat. Rep.* 65:281-266, 1971). *In vivo* inhibition of liver S-adenosylhomocysteine (SAH) hydrolase by AdR is implicated since liver SAH levels increase dramatically following treatment. S-adenosylmethionine (SAM) levels also increase and the SAM:SAH ratios drop from control values of 15 to <1 (Renshaw & Harrap, in *Purine Metabolism in Man*, Vol. 165B, Plenum Press, pp 363-366, 1984). Further investigations have revealed that while SAH and SAM levels and their ratios normalise following the first 2 days treatment, recovery does not occur thereafter. These data indicate a correlation between the duration of depression of SAM:SAH ratios and the manifestation of hepatocellular necrosis. In addition we have obtained evidence of *in vivo* inhibition of MT reactions as a result of reduced SAM:SAH ratios: both plasma and urinary creatinine levels are significantly reduced (eg, 23% control urinary creatinine levels by day 5) reflecting inhibition of liver guanidinioacetate MT. Plasma:urinary creatinine ratios do not alter indicating a lack of kidney toxicity in mice. Treated animals also excrete significantly less pseudouridine (<30% from day 2) which may reflect a biochemical lesion at the level of tRNA post-transcriptional modification.

172 RESIDUAL PURINE NUCLEOSIDE PHOSPHORYLASE ACTIVITY IN A NEW DUTCH PATIENT WITH PNP-DEFICIENCY AND CELLULAR IMMUNODEFICIENCY

G.Rijksen¹, B.J.M.Zegers², L.J.M.Spaapen², W.Kuis², M.Duran², B.S.Voorbrood³, G.E.J.Staal¹ and S.K.Wadman¹.

¹ Dept. Hematology, Div. Med. Enzymology, University Hospital, Utrecht, The Netherlands. ² University Children's Hospital "Het Wilhelmina Kinderziekenhuis", Utrecht, The Netherlands. ³ Juliana Ziekenhuis, Dept. of Pediatrics, Rhenen, The Netherlands.

A deficiency of purine nucleoside phosphorylase (PNP) was detected in a three-year-old boy who was admitted for the investigation of behaviour disorders and spastic diplegia. The urinary excretion profile of oxypurines, analyzed by liquid chromatography, showed the presence of large amounts of (deoxy)inosine and (deoxy)guanosine together with a low uric acid. The PNP activity in red blood cells and peripheral blood lymphocytes was not detectable by standard methods. However partial purification of the red cell enzyme demonstrated some residual PNP activity equivalent to 0.5% of a control haemolysate. This residual activity enabled the characterization of the enzyme. Immune precipitation studies with anti-PNP antiserum (gift of Dr. W.R.A. Osborne, Seattle) showed that the amount of the residual protein was approximately 0.5% of the control value as well. The other enzyme properties studied including substrate affinity (inosine), substrate specificity, electrophoretic mobility and heat stability, were all completely normal. Erythrocyte PNP activities in both parents were in the heterozygote range (35 and 50%) and the enzyme properties were normal as well.

173 Identification of uridylylate nucleotidase as a potential target for 5-fluoro-5'-deoxyuridine in L1210 leukemia. C. ROOBOL and J.L. BERNHEIM

The modes of action of 5-fluoro-2'-deoxyuridine (FdUrd), 5-fluorouracil (FUra) and 5-fluoro-5'-deoxyuridine (dFUrd) were studied in L1210 leukemia, using precursor incorporation, salvage experiments and multireactant enzyme kinetics. It was shown that FdUrd has thymidylate synthetase as the decisive target for its cytotoxic action. FUra is converted to 5-fluoro-5'-uridinetriphosphate (FUTP) via a series of subsequent phosphorylation reactions and is incorporated into nascent RNA, leading to the formation of defective RNA species. FUra-induced inhibition of thymidylate synthetase does occur, but was shown to be unrelated to its cytotoxic action. dFUrd has since long been assumed to act as a pro-drug for FUra. However, in contrast to FUra, no effect of dFUrd on thymidylate synthetase could be demonstrated, which raises doubts about the prodrug function of dFUrd. Therefore, the effect of dFUrd on the *de novo* route of pyrimidine nucleotide synthesis via orotic acid was studied. It was shown that dFUrd does not interfere with the formation of uridine-5'-monophosphate (UMP), but strongly inhibits the degradation of UMP to uridine (Urd). An implication of this finding is that dFUrd treatment leads to accumulation of UMP, a phenomenon which presumably results in toxic death. More important, dFUrd is able to inhibit uridylylate nucleotidase without intracellular activation and does in this respect not act as a prodrug for FUra. Dpt. of Hematology, Immunology and Oncology, Laboratory for Experimental Chemotherapy, Medical School of the Free University of Brussels, Laarbeeklaan 103/E - B-1090 Brussels - Belgium.

174 PURINE NUCLEOTIDE SYNTHESIS IN CULTURED RAT EMBRYOS UNDERGOING ORGANOGENESIS
 Peter B. Rowe, Sandra E. McEwen and Annette

Kalaizis
 Children's Medical Research Foundation, University of Sydney, Camperdown, N.S.W., Australia.

The cultured rat embryo undergoing organogenesis (9.5-11.5 days of gestation) together with its associated yolk sac synthesizes purine nucleotides via the *de novo* synthetic pathway. Although both the embryo and its yolk sac contain significant levels of the purine base salvage enzymes adenine and hypoxanthine phosphoribosyltransferase, the culture medium that consists largely of rat serum contains high activity levels of purine catabolic enzymes. Short-term pulse-chase experiments with adenine and guanine, carried out under virtually serum-free conditions, confirmed that purine base salvage mechanisms were active and that there was no significant net transfer of purines between the embryo and its yolk sac. The 3-carbon atom of serine is the major source of one-carbon units for purine ring synthesis with a significant contribution from the 2-ring carbon atom of tryptophan. No one-carbon units are derived from glycine, histidine or choline. Most of the embryonic amino acid requirements are supplied by yolk sac mediated proteolysis of the culture medium protein. A high level of embryonic GTP is reflected in a very low ATP/GTP ratio and is presumably related to the relatively undifferentiated state of many of the cells. The reason for the high level of GTP is however purely conjectural.