61 How can one define urate overproduction in man?

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Initial studies suggested that a patient suffered from urate overproduction if the urine urate exceeded 600 mg/24 hours on a low purine diet or if glycine incorporation into urinary urate exceeded 0.3% in seven days (Seegmiller, Wyngaarden). Subsequent studies in normal subjects suggested that urate production relates to body size and that some relativity to body weight or surface area needs to be incorporated into any definition of overproduction (Sorensen). The variation in the percentage of produced urate which was excreted by the renal route also needs to be considered in assessments involving the 24 hour urinary urate excretion. More recent studies have shown a wider range of urine urate in healthy subjects on a low purine diet (Emmerson). In addition, lower values for urine urate excretion were obtained with synthetic purine free diets than with diets containing food low in purines (Loeffler & Zollner). Our recent studies in lymphoblasts from patients with urine urate exceeding normal values by standard criteria have not demonstrated any increase in de novo purine biosynthesis. Although severe degrees of urate overproduction can be defined from measurements of urine urate, it now seems very difficult to establish minor degrees of urate overproduction by such measurements.

62 USEFULNESS OF INTACT ERYTHROCYTE STUDIES IN THE DIAGNOSIS OF INHERITED PURINE AND PYRIMIDINE DEFECTS

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Diagnosis of the degree of severity of some inherited defects may be difficult where the normal enzyme is either very labile, or where the mutant enzyme is extremely unstable.Studies in intact red cells using physiological conditions and [8-^{14}C] labelled substrates (1mM P_i:10μM),or PP-ribose-P generating conditions (18mM P_i) have proved invaluable in such cases. Under these conditions control red cells at 1mM P_i convert less than 35% of adenine or hypoxanthine to the nucleotide,but more than 80% at 18mM P_i.

Studies in a variety of inherited disorders have shown: (1) that despite lack of detectable lysate activity,some partially HGPRT deficient patients have up to 25% of normal activity in intact cells; (2) raised endogenous PP-ribose-P levels are evident in the Lesch-Nyhan syndrome and PNP deficiency from the almost complete conversion of substrate even at 1mM P_i, but PP-ribose-P levels are normal in ADA, APRT and OPRT/ODC deficiency. These defects may also be detected using intact red cells and the appropriate substrate; (3)that despite normal lysate APRT and HGPRT activity, cells from a PP-ribose-P synthetase mutant failed to respond to phosphate activation using either substrate; (4) the presence of an unstable enzyme in some heterozygotes for ADA deficiency,where the same red cells showed no detectable lysate activity,but up to 60% of normal conversion of deoxyadenosine (10 μM) - at either 1 or 18mM P_i - using intact cells.

63 ZINC-MEDIATED URATE BINDING TO HUMAN ALBUMIN.

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Published data both support and dispute the existence of urate binding to serum proteins. The issue remains important since such binding could be relevant to both renal transport and tissue deposition of urate. Using equilibrium dialysis over 18 hours, we studied the effects of selected cations on ^{14}C-urate binding to human albumin (5 g/dl) in TRIS NaCl buffer at 300 mOsm, 4°C and pH 7.4. We calculated the binding ratio (BR) by dividing the ^{14}C (cpm/ml) inside the dialysis membrane by that in the bath. The mean coefficient of variation of the method was 4%. We observed minimal binding in the absence of added metal ions (BR 1.04). Added at 5mM, Zn^{++} resulted in strong binding (BR 9.53), Cd^{++} was less effective (BR 2.18),and Cu^{++} was least (BR 1.22). When added in the presence of Zn^{++}, Cu^{++} inhibited binding (BR 1.16), but Cd^{++} did not (BR 9.54). Ionic Zn led to an even higher degree of ^{14}C-hypoxanthine binding to albumin (BR 14.69) but did not affect the lack of albumin binding of ^{14}C-urea (BR 0.95) nor the avid binding of ^{14}C-salicylate (BR 10.98). Purine competition for the binding site(s) was suggested when saturation of the buffer with hypoxanthine, allopurinol, oxipurinol and adenine reduced the BR of ^{14}C-urate (1.50, 3.20, 4.40, 5.28, respectively). Histidine (5mM), a Zn^{++} chelator, strongly inhibited urate binding (BR 1.91), but salicylate (3.5mM) produced only a minimal change (BR 7.65). These findings indicate that zinc-purine complexes form readily in vitro and bind avidly to human serum albumin.

64 CORRECTED CLEARANCE IDENTIFIES UNDEREXCRETION OF URIC ACID IN A GOUTY KINDRED.

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Both the rate and the efficiency of uric acid excretion were evaluated in a 28 year old gouty woman and in 16 additional members of her family. Mid-morning, spot blood and urine specimens were obtained from three generations of the kindred, living in Washington, Montana, British Columbia and Alaska. The excretion rate of uric acid, calculated per deciliter glomerular filtrate (GF), was normal in 15 family members. One person fell slightly above and one below two standard deviations from normal mean values of 0.37 ± 0.12 mg/dl GF in women and 0.39 ± 0.10 mg/dl GF in men.

Uric acid clearance was determined as an index of excretion efficiency. Since normal clearance is an exponential function of the serum urate, all values were "corrected" to a serum level of 5 mg/dl. Values from the kindred were compared to control groups of healthy, young men and women in whom the relative uricosuria of women was confirmed and quantified: the mean corrected uric acid clearance in women (9.6 ± 2.7 ml/min) being 133% of that in men(7.2 ± 1.9 ml/min). Corrected clearance of uric acid was significantly depressed in four of six family members with gout and in two additional subjects one of whom was hyperuricemic but still asymptomatic. Using these simple methods, we identified and quantified inefficient uric acid excretion which appears to obey autosomal dominant transmission.

65 DUCHENNE MUSCULAR DYSTROPHY: NORMAL ATP TURNOVER IN CULTURED CELLS.

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A reduction of ATP content has been observed in muscle from patients with Duchenne muscular dystrophy. We tested the hypothesis that there is an accelerated rate of ATP turnover in the cultured muscle cells and fibroblasts from patients with Duchenne muscular dystrophy. ATP and ADP levels were similar in both muscle cells and fibroblasts from normal subjects and Duchenne patients. The rates of ATP and ADP formed from radioactive adenine and the enrichment of these pools was the same. ATP degradation was evaluated during a 10-minute incubation in 5.5 mM 2-deoxyglucose or during a 72-hour period of growth. There was significantly less radioactively-labelled ATP after incubation with deoxyglucose in Duchenne cells than in normal muscle cells, but there was no difference in total ATP concentration or ADP concentration or radioactive labelling. There was no difference in ATP or ADP degradation in fibroblasts. In longterm study of fibroblasts, radioactively labelled ATP decreased to 50% of control values at 24 hours and about 30% of control values at 72 hours, but there was no difference between normal and Duchenne cells. The data suggest that ATP turnover is normal in cells from Duchenne muscular dystrophy under the study conditions.

66 BIOCHEMICAL AND GENETIC PROPERTIES OF $HPRT_{Cape\ Town}$

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$HPRT_{Cape\ Town}$ possesses the unique kinetic property in erythrocyte extracts of substrate inhibition by its purine substrates (1). In order to determine whether these properties reflect a mutation in the HPRT gene or in a gene for a putative regulatory polypeptide, the kinetic properties of HPRT from the proband's daughter, as well as growth properties of transformed lymphoblasts, were studied. Lymphoblasts from the daughter showed similar ^{14}C hypoxanthine uptake (relative to ^{3}H thymidine) in the proband's cells, and similar growth properties in selective media. However, the kinetics of HPRT in her erythrocyte extracts were intermediate between normal and proband erythrocytes indicating that she was heterozygous for the defect and therefore that the defect in $HPRT_{Cape\ Town}$ is in the gene for HPRT itself. DNA from the proband's and his daughter's cells were analysed by restriction endonuclease analysis and mRNA by S1 nuclease analysis using a cloned human HPRT probe.

References :

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