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25. FURTHER STUDIES ON THE EFFECT OF SOYPROTEIN AND CASEIN ON THE VITAMIN B_6 STATUS IN RATS. S.C.Lu* and P.C.Huang. Department of Biochemistry, College of Medicine, National Taiwan University, Taipei, R.O.C.

Young adult rats of Long-Evans strain were depleted of vitamin B_6 without giving deoxypyridoxine, which was used in our previous experiments. The test diets contained soy protein isolate or casein at 40% protein level with(control groups) or without(deficient groups) pyridoxine. Diets were given by pair-feeding method for 5 weeks. At the end of each week, each rats was loaded with 20 mg of L-tryptophan and the 24-hour urine was collected. At the end of the experimental period, blood was collected by decapitation.

Time course of the change in urinary xanthurenic acid(X.A.) excretion was studied. The soyprotein-B_6 deficient group(S-B_6D) excreted significantly higher amount of X.A. than the casein-B_6 deficient group(C-B_6D) as early as the 7th day on the test diets, 1.75±0.66 vs 0.84±0.46 mg/d, while both of the pyridoxine supplemented control groups excreted negligible amount of X.A. The rats of both deficient groups excreted more and more X.A. during the following weeks, but the difference became less significant. At the end of the 5th week urinary X.A. were 3.21±0.61 and 2.70±1.35 mg/d for the S-B_6D and C-B_6D respectively. Activities of erythrocyte tranaminase(EGOT and EGPT), with or without pyridoxal phosphate addition, were lower in the S-B_6D than in the C-B_6D. Urinary excretion of another tryptophan metabolite, kynurenine, was also determined. All the data indicated that soyprotein, as compared with casein, would somehow increase B_6 requirement.

26. DETERMINATION OF GALACTOSE AND GALACTOSE-1-PHOSPHATE IN DRIED BLOOD SPOT BY ENZYMATIC METHOD. K.-J. Hsiao, S.-H. Chiang*, K.-S. Huang. Clinical Biochem. Research Lab., Dept. of Medical Research, Veterans General Hospital; Taipei, Taiwan 11217, R.O.C.

A quantitative method was developed for micro-determination of galactose and galactose-1-phosphate (Gal-1-P) in dried blood spots. Free galactose and galactose hydrolyzed from Gal-1-P by alkaline phosphatase (ALP) was measured by the fluorescence of NADH reduced from NAD in the presence of β-galactose dehydrogenase (GALDH). Two 3mm diameter discs were punched from dried blood speciman, and each one placed into one glass tube. Blood proteins were denatured by ether/methanol/acetone/water mixture and the galactose & Gal-1-P were eluted with buffer. Then a reaction mixture containing GALDH was added to the first tube, and a reaction mixture containing GALDH and ALP was added to the second one. After inclubated at 37°C for 90 min, the fluorescence was measured by a fluorometer (Auto FP-1). Concentrations of blood galactose and Gal-1-P were calculated against standard dried blood spots. The reference range of galactose and Gal-1-P were estimated to be 47-234uM (n=103), 0-197uM (n=103), respectively, in Chinese neonates. A galactosemic newborn, detected by neonatal screening with Paigen's phage-*E. Coli* method, was confirmed with the same screening specimen immediately by this method (galactose 3253 uM, Gal-1-P 2606 uM). This rapid and sensitive method could be used as a primary confirmatory test for galactosemia detected by neonatal screening. It could also be applied to monitor the lactose-free dietary therapy for patients with galactosemia.