in isolated pure form, undergoes rather specific calcium-dependent binding to both primary and secondary amyloid fibrils. In view of the substantial amounts of calcium which are present in amyloid-containing tissues it is possible that this may be the counterpart of a mechanism in vitro for deposition of AP.

Schneider & Loos (Vircrovs Archiz B: Cell Pathology, 1978, 29, 225) reported that fluorescent anti-(human AP) stained basement membrane in sections of normal tissues. We have confirmed this finding using both rabbit and sheep anti-(human SAP) reagents in direct and indirect immunofluorescence and immunoperoxidase techniques. There was linear staining in renal glomeruli and arterioles in normal and pathological kidneys and also in cardiac and smooth muscle and in lung. Immunological specificity of the staining was established by absorption experiments with isolated pure SAP. The pattern of staining was different from that produced by a rabbit anti-(human type IV collagen); for example, anti-(human SAP) did not stain renal tubular basement membrane. At the ultrastructural level, anti-(human SAP) staining of the glomerulus was confined to the internal layer of the basement membrane.

These findings, together with our studies of SAP in experimental animal models of secondary amyloidosis, suggest that amyloid F-component may be of greater significance in the pathogenesis of amyloidosis than has previously been suspected.

59. SIALIDOSIS TYPE II (ACID NEURAMINIDASE DEFICIENCY): CLINICAL AND BIOCHEMICAL FEATURES OF A FURTHER CASE

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Primary deficiency of acid neuraminidase (N-acetylmuraminic acid hydrolase, EC 3.2.1.18) has been described in a number of patients previously classified as having the cherry-red spot-myoclonus syndrome, mucolipidosis I, or primary β-galactosidase deficiency. All of these patients excrete abnormal amounts of sialyloligosaccharides in the urine and have recently been re-classified under a new general term, sialidosis (Lowden & O'Brien, 1979, American Journal of Human Genetics, 31, 1-18). We describe a further patient who falls into the group of patients who have progressive mental deterioration with dysmorphic features and have been classified as having sialidosis type II.

The patient, a white male now aged 22 years, is the second child of unaffected first-cousin parents. Development was normal up to the age of 9 months, when kyphosis and scoliosis of the spine developed and became progressively worse and was associated with developmental delay. During adolescence myoclonic jerks became apparent and there were three or four episodes of major convulsions. From late childhood there has been a significant coarsening of the facial features. Examination revealed a young adult with mild hirueroid facial features, marked kypho-scoliosis and joint contractures and moderate mental retardation. Both postural and action myoclonus was present. There was defective abduction of both eyes and fundoscopy revealed a cherry-red spot at the macula bilaterally; there were no corneal opacities. There was severe global weakness with brisk tendon reflexes and extensor plantar responses. Sensation, as far as could be tested, was normal. There was no hepatosplenomegaly.

Results of quantitative and qualitative tests for mucopolysaccharides in the urine were normal; however, abnormal amounts of oligosaccharides containing mannose, galactose, glucosamine and sialic acid were found. X-rays showed some of the changes of dysostosis multiplex. Foam cells were found on bone marrow aspiraion. Activities of lysosomal enzymes (β-galactosidase, hexosaminidases A and B, arylsulphatase A and sphingomyelinase) in fibroblast cultures were normal. Neuraminidase activity in fibroblasts was 1.3 nmoI of methylumbelliferone released min⁻¹ mg⁻¹ of protein (range in normals 82-116).

This patient is a case of sialidosis type II who demonstrates further heterogeneity within this group of disorders. The parental consanguinity makes it likely that he is homozygous at the neuraminidase locus and is not a compound heterozygote. The relationship of the clinical and biochemical findings to other patients with sialidosis type II will be discussed.

60. AN EXTRINSIC FACTOR CONTROLLING NEUTROPHIL ALKALINE PHOSPHATASE IN CHRONIC GRANULOCYTIC LEUKAEMIA

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The mechanism underlying the reduced alkaline phosphatase (NAP) activity in chronic granulocytic leukaemia (CGL) was investigated by in vivo manipulation of CGL neutrophils. Between 2 and 12 x 10⁹ leucocytes collected from CGL patients undergoing leucopheresis on the continuous-flow blood cell separator were irradiated in vitro (1500 rad) and transfused to selected febrile neutropenic patients with leukaemia or aplastic anaemia. In the three previously neutropenic recipients the peripheral blood leucocyte counts were greatly elevated 14-17 h after transfusion and NAP scores were increased by factors of 36, 105 and 130 respectively. In one of these patients (a female receiving cells from a male CGL donor) Y-chromatin studies provided confirmatory evidence of the donor origin of the circulating neutrophils after transfusion. We conclude that NAP synthesis in mature neutrophils is controlled by an extrinsic factor and that the level of this factor is reduced in patients with CGL and perhaps increased in other patients with pyogenic infection.

61. BIOTIN-RESPONSIVE 3-METHYLCROTONYLGLYCINURIA WITH NORMAL CARBOXYLASE ACTIVITIES IN VITRO: DEMONSTRATION OF A METABOLIC DEFECT IN THE METABOLISM OF ISOVALERATE WITH A NEW ASSAY

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We have studied cultured skin fibroblasts from a patient with reported biotin-responsive 'floppy-infant syndrome' and an abnormal urinary excretion of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid and 3-methylcrotonic acid (Keeton & Moosa, 1976, Archives of Diseases in Childhood, 51, 636), in which we were unable to demonstrate deficient activity of 3-methylcrotonyl CoA carboxylase.

Activities, expressed as nmol of CO₂ incorporated h⁻¹ mg⁻¹ of protein, of 3-methylcrotonyl-CoA carboxylase and of propionyl-CoA carboxylase (assayed as control) in fibroblasts grown to confluence and disrupted for assay were 3.15 and 3.23, and 72.2 and 69.5, compared with activities in control fibroblasts (n = 8) of 3.83 ± 1.57 and 79.0 ± 13.2 respectively. Fibroblasts from two patients with proven propionic acidemia assayed simultaneously gave propionyl-CoA carboxylase activities of 6-59 ± 0.7 and 2-79 ± 0.3. Recently we reported a new assay for the detection of disorders of isovaleryl-CoA metabolism in the leucine metabolic pathway that measures incorporation of isovalerate into protein in dividing, sub-confluent, intact fibroblasts and amniotic cells (Chalmers & Spellacy, 1979, Clinical Science, 57, 25P). Use of this assay with fibroblasts from the present patient gave activities, expressed as pmol of isovalerate incorporated h⁻¹.
mg·1 of protein, of 21 and 23, compared with activities in control fibroblasts of 195 ± 41 (μ mol/h·mg of protein), and activity in fibroblasts from a patient with proven isovaleric acidaemia (Chalmers & Spellacy, 1979, Clinical Science, 57, 259) of 13 ± 3. Incorporation of acetyl-CoA into protein by the patient’s fibroblasts, assayed similarly, was normal at 102 and 107, compared with incorporation by control fibroblasts of 142 ± 57 pmol of acetyl-CoA incorporated h·1·mg·1 of protein.

These results demonstrate the presence of a metabolic disorder in intact cells from the patient, consistent with the clinical presentation and abnormal organic aciduria, in contrast to the normal 3-methylcrotonyl-CoA carboxylase activity in disrupted cells. The results may indicate defective biotin transport into the intact mitochondria or defective binding of the biotin to the apocarboxylase in the presence of normal holocarboxylase synthetase and this warrants further investigation in this and other, similar, patients.

62. FAECAl PORPHYRIN ABNORMALITIES IN ROTOR’S SYNDROME

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An Iranian male presented with a 10 year history of chronic conjugated hyperbilirubinaemia but without any stigmata of chronic liver disease. All other liver-function tests were normal including fasting bile acids and oral cholecystogram. His markedly delayed plasma clearance of BSP and ICG and normal non-pigmented liver biopsy on light microscopy indicated a diagnosis of Rotor’s syndrome (Berthelot & Spellacy, 1979, Gut, 19, 474). The urinary coproporphyrin excretion was within the normal range (300–600 μg/day; 40% coproporphyrin I). The faecal porphyrins were, however, significantly increased (406 μg/g of dry stool). High-pressure liquid chromatography of the faeces revealed a porphyrin pattern similar to that of hepatic porphyrin metabolism. Total porphyrin excretion was 21 μmol in 24 h and was found primarily in the form of coproporphyrin (23 μmol). The activity in fibroblasts from the patient, assayed similarly, was normal at 102 and 107, compared with activities in control fibroblasts of 142 ± 57 pmol of acetyl-CoA incorporated h·1·mg·1 of protein.

We have investigated the effect of administering sodium 3-hydroxybutyrate to obese women more than 150% of ideal body weight. All subjects received a diet of 600 kcal/day (2.5 MJ) providing 34 g of protein, alternating with a fasting day. 24 hour urine collections were made daily. After a control 5 day period, each subject received a 3 day intravenous infusion of the hydroxybutyrate (18 g/day). After another 5 day control period, a 3 day intravenous infusion of glucose (18 g/day) was followed by a final 5 day control period. In a further six obese subjects the experiment was repeated, the hydroxybutyrate being given orally. In three of these subjects the order of hydroxybutyrate and glucose administration was reversed. Both intravenous and oral 3-hydroxybutyrate produced a significant reduction in urinary nitrogen output. The mean daily negative nitrogen balance decreased from 2·2 ± 0·4 g/day of urinary creatinine on the control days to a mean of 1·0 ± 0·3 g/day of urinary creatinine on the hydroxybutyrate days. The hydroxybutyrate did not significantly affect the mean rate of weight loss. Our results indicate that 3-hydroxybutyrate administration may increase the ratio of fat:lean tissue loss in obese subjects on semi-starvation diets.

64. ACTIVITY OF AMIDOPHOSPHORIBOSYLTRANSFERASE AND PURINE PHOSPHORIBOSYLTRANSFERASES IN DEVELOPING RAT BRAIN AND POSSIBLE IMPLICATIONS FOR THE LESCH–NYHAN SYNDROME

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Gross deficiency of hypoxanthine phosphoribosyltransferase causes the Lesch–Nyhan syndrome, in which there are highly characteristic behavioural and neurological manifestations. Previous indirect evidence suggested that the brain is particularly susceptible to the metabolic lesion because of limited ability to synthesize purines de novo during the critical phases of rapid cell replication (McKeran & Watts, 1976, Journal of Medical Genetics, 13, 91–95).

We have measured the specific activities of the purine salvage enzymes (hypoxanthine and adenine phosphoribosyltransferases (HPRT, EC 2.4.2.8, and APRT, EC 2.4.2.7, respectively)) and of the enzyme which catalyses the rate-limiting reaction on the purine synthesis de novo pathway (amidophosphoribosyltransferase (PRP-amidotransferase, EC 2.4.2.14)) in whole rat brain from day 18 of gestation until the postnatal age of 8 weeks, and in different parts of the brain between day 7 of postnatal life and age 8 weeks. The activity ratio [HPRT]/[PRP-amidotransferase] is similar in the different parts of the brain. It is lowest in the prenatal period and shows little tendency to rise until after day 14 of postnatal life.

Neuroblast and neoglia proliferate most rapidly during the period when activity HPRT is highest, which suggests that this activity is important for brain growth. The normal brain morphology with absence HPRT but active PRP-amidotransferase in all of the large number of areas of a Lesch–Nyhan syndrome patient’s brain which we recently studied (Watts, Slavin, Spellacy & McKeran, unpublished work) supports this concept.