second antibody precipitation method we used thus gave reliable and reproducible results. Our results on cross-reactivity studies (figure 2) showed that cross-reactions of 142% and 3.8% were obtained, respectively, for digoxigenin and digotoxin. These also suggested that the EIA is specific for digoxin and the digoxigenin was succinylated at 3 positions during the succinylation reaction.

Maleimidobenzoyl derivatives have been used as cross-linking agents for protein-enzyme conjugation[14-16]. More recently, we reported m-maleimidobenzoyl derivatives of haptens as convenient derivatives for the preparation of enzyme-hapten conjugates[9,17].

In conventional enzyme-hapten conjugations, haptens were conjugated primarily to γ-amino groups of lysine residues of the β-galactosidase molecule, resulting in a substantial reduction of both enzyme activity and water solubility[12,18,19]. In digoxin-enzyme conjugation procedures, a γ-amino group of a lysine residue in an enzyme has been covalently linked to a terminal sugar moiety of digoxin through a diazo linkage. This procedure is rather tedious and difficult to replicate[11,20].

Our method of hapten conjugation to β-galactosidase is highly efficient and simple, and is easily replicated because sulfhydryl groups of the enzyme are involved in the hapten conjugation reaction. The present report also demonstrates that digoxigenin can be employed successfully for enzyme-hapten conjugate preparation for the development of EIA in digoxin quantification. The clinical application of this method is currently under study and will be reported elsewhere.

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Characteristic changes of cerebellar proteins associated with cerebellar hypoplasia in jaundiced Gunn rats and the prevention of these by phototherapy

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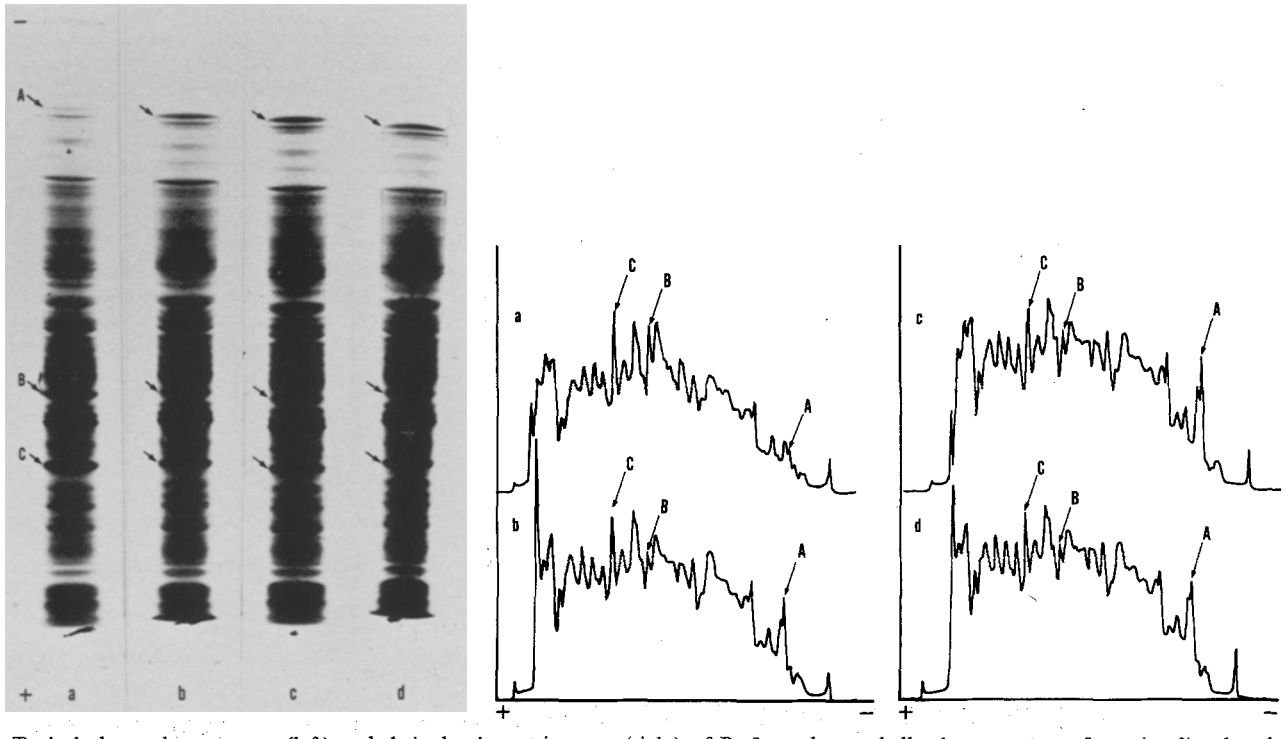
Summary. In the cerebellar particulate fractions from Gunn rat homozygotes 3 protein bands with apparent mol.wts of 250,000, 50,000 and 33,000 in SDS-polyacrylamide gel disc electrophoresis underwent major changes, and phototherapy of the newborns could effectively prevent the changes.

Hyperbilirubinemic homozygous Gunn rats[2] have been known to show a marked cerebellar hypoplasia[3-6]. In view of the inhibitory effect of bilirubin on the protein synthesizing machinery in Gunn rat cerebellum[7,8], the first attempts were made to characterize cerebellar protein patterns and to investigate the effect of phototherapy in Gunn rat homozygotes (jj), using SDS-polyacrylamide gel disc electrophoresis (SDS-PAGE) and comparing the patterns with those from control non-jaundiced heterozygotes (j+).

Materials and methods. Newborn jj and j+ rats from the same dam were allotted to irradiated and non-irradiated groups. The irradiated group was isolated from the dam and subjected to blue light irradiation (phototherapy) for 5 h/day (09.00-14.00 h) from the 4th-7th day of life at an energy level of about 2.4×10^4 erg/cm^2/sec near the animals.[6]. The non-irradiated group was treated in the same way without irradiation. The rats were sacrificed on the 31st day of life, and the cerebellar homogenates were prepared at 4 °C with 9 vol. of 0.32 M sucrose-10 mM phosphate buffer, pH 7.0. The subcellular fractions obtained by differential centrifugation at 4 °C were termed P_1 (1000 × g for 10 min; twice washed), P_2 (12,000 × g for 20 min; twice washed), P_3 (105,000 × g for 60 min) and S (final supernatant). SDS-PAGE in a linear gradient of 5-15% polyacrylamide was performed according to Ames[9], except that the gels were polymerized at 30 °C with 0.0125% ammonium persulfate in final concentration, and the electrophoresis was carried out at 4 °C at 1 mA/gel tube. Protein was determined by the method of Lowry et al.[10] with bovine serum albumin as a standard. The mol.wt estimation was made using the following protein subunits as internal and external standards: thyroglobulin (330,000) and ferritin (220,000 for half of the native protein and 18,500) from Pharmacia, RNA polymerase (165,000, 155,000 and 39,000)



Typical electrophoretograms (left) and their densitometric scans (right) pf P_3 from the cerebellar homogenates of non-irradiated and irradiated jj and j+ rats. a, non-irradiated jj (41 g and 57 mg in body weight and cerebellar wet weight, respectively, at the 31st day of life); b, irradiated jj (50 g and 181 mg); c, non-irradiated j+ (55 g and 211 mg); d, irradiated j+ (53 g and 221 mg). Sample load was 43 µg (20 µl) per gel tube. A, B and C with arrows indicate protein bands with respective apparent mol. wts of 250,000, 50,000 and 33,000.

from Boehringer-Mannheim, and rabbit muscle phosphorylase a (93,000).

Results. The figure shows typical electrophoretic protein patterns of P_3 from non-irradiated and irradiated jj and j+ rats. Major and highly reproducible changes in the protein profile of the non-irradiated jj rat (a) when compared with the j+ control (c) were observed in bands of A, B and C (arrows) with apparent mol. wts of 250,000 (hereafter GR-250), 50,000 (GR-50) and 33,000 (GR-33), respectively. GR-250 located mainly in P_3 and to a lesser extent in P_1 and P_2, but not in S. Staining of the gel by the PAS method[11] gave a positive reaction to this band. A protein band corresponding to GR-250 was lacking both in jj and j+ cerebra. Reduction of this protein was evident particularly in jj P_3 (a). GR-50 and 33 bands showed an apparent increase in the hypoplastic cerebellum. GR-50 was relatively rich in P_1, and also found in other fractions. GR-33 was distributed almost exclusively in P_3. Although protein bands corresponding to GR-50 and 33 were present in the cerebrum as well, there were no measurable differences between non-irradiated jj and j+ cerebra. Photo-irradiation of jj newborns could effectively prevent the decrease of GR-250 and the increase of GR-50 and 33 (b). The protein profiles of the subcellular fractions from the irradiated j+ cerebellum (d) were essentially the same as those from the non-irradiated (c). The present results were confirmed in many repeated experiments with littermates from different dams.

Discussion. The characteristics of GR-250 are in appearance similar to those of a protein specific for cerebellar Purkinje cells, P_{400}[12], the lack of which has been evidenced in homozygous staggerer and nervous mouse cerebella[12] with defects of the Purkinje cells[13,14]. The jj rat cerebellum with a marked hypoplasia is also reported to show an altered morphology and a reduced number of the Purkinje cells[3,5,15]. However, because of a large difference in apparent mol.wt between the 2 proteins (400,000 for P_{400}[12]) we cannot at present offer a definite conclusion as to whether they are identical. Although the physiological functions of GR-250, 50 and 33 in the development of cerebellum have not been established, it is clear that they are, directly or indirectly, involved in pathogenic mechanisms for bilirubin-induced cerebellar hypoplasia in hyperbilirubinemic Gunn rats.

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