20 N.GITZELMANN-CUMARASAMY* and C.C. KUENZLE* (Intr. by G.Duc). Department of Biochemistry, Tierspital, CH-8057 Zürich, Switzerland.

Mapping of the High-Affinity Bilirubin-Binding Site of Human Serum Albumin.

The nature of bilirubin binding to serum albumin is of considerable interest in the understanding of neonatal hyperbilirubinemia. Each albumin molecule has been shown to contain 1 high-affinity and 2 low-affinity bilirubin binding sites. The structural analysis of the high-affinity binding site was undertaken using the technique of affinity labeling. The label was prepared by reacting bilirubin with Woodward's reagent K, which converted both carboxyl groups of bilirubin into reactive enol esters. Coupling of this bilirubin derivative to albumin was achieved under N_2 at pH 9.4. A yellow monomer of albumin was isolated by gel chromatography. Dialysis of this monomer under denaturing conditions showed covalent attachment of the pigment to the protein. The specificity of the labeling reaction was confirmed by 6 independent methods. Monomer albumin (584 amino acid residues), thus labeled with ^{14}C bilirubin, was fragmented into 7 peptides by cyanogen bromide cleavage, followed by reduction and carboxymethylation. The bilirubin label was found to be covalently bound to peptide 3 (residues 124-297) and peptide 6 (residues 447-547). Further structural analysis of this bilirubin-binding site is now in progress.

21 C.DACOU -VOUTETAKIS and D.ANAGNOSTAKIS*. 1st Dept.of Pediatrics,Athens University,Athens, Greece. Phototherapy and serum LH levels in newborns.

The effect of phototherapy on serum LH levels was studied in newborns aged 3-30 days. LH was determined by radioimmunoassay in 11 icteric but otherwise healthy newborns prior to, during,and for several days after phototherapy (Ph).Similar determinations were performed in 15 icteric newborns without phototherapy (C). The results are shown below (mean±1SD):

Days :	3	7	11	15	19	30
Ph	:2.05(A) ±1.0	1.40*(B) ±0.81	2.48 ±1.13	2.96 ±0.45	7.0(D) ±2.2	3.7 ±0.57
C	:1.75 ±0.59	1.90 ±0.52	2.10 ±0.77	2.03 ±0.86	2.65(E) ±0.5	2.03 ±0.55

A> B ($p < 0.001$), D> E ($p < 0.001$). * phototherapy.

The results show that LH values are suppressed during phototherapy with a rebound increase shortly afterwards. These changes may be related to alterations in melatonin synthesis

22 A.F.BAKKEN* (Intr. by S.O.Lie). Department of Pediatrics, Rikshospitalet, University of Oslo, Oslo, Norway. Temporary intestinal lactase deficiency as one etiological factor of the diarrhea of light-treated jaundiced infants.

The cause of the loose stools observed in many light treated jaundiced infants is still obscure. As it has been found that the concentration of unconjugated bilirubin in the intestine of these infants is increased during light-treatment, we thought that the brushborder intestinal lactase might be inhibited by the unconjugated bilirubin, and thereby contribute to the diarrhea.

When we gave lactose-free diet to light-treated jaundiced infants with diarrhea, their stools normalized, even if the light-treatment was prolonged. The normal stools could be reversed to diarrhea when reintroducing human breast milk or formulas containing cows milk. Intestinal biopsies from these jaundiced newborn infants showed neglible activity of the lactase enzyme. Lactose tolerance test which we performed in the same infants showed almost no uptake of lactose, in contrast to normal newborn infants, which were able to hydrolyze lactose to a much higher extent (for ethical reasons intestinal biopsies were not taken in the normal newborns).

We think therefore, that lactose-free diet should be given to jaundiced newborn infants if they have diarrhea during light-treatment.

23 K.E.v.MÜHLENDAHL+, J. PACHALY+, and B. WEBER Dept. of Pediat., Free Univ., Berlin, Germany.

Lack of influence of physiological cortisol concentrations upon growth hormone secretion.

Large amounts of glucocorticosteroids (CS) inhibit growth hormone (GH) secretion. The effect of physiological concentrations has not extensively been investigated. Therefore, we have correlated spontaneous CS and GH secretion during the night in 27 children. We have compared the integrated F values of three 4-hr-periods with GH secretion measured at half hour intervals. When F was high, GH was low, and vice versa. This correlation was statistically significant ($p = 0.028$). We think, however, that this correlation is not the expression of a causal relationship, but rather the reflection of the fact that GH secretion is highest during the first part of the night (due to onset of sleep) when F still is low (due to the circadian rhythm of F secretion). In the period from 4 - 8 hr. a.m. GH is low and F is high. A more detailed statistical analysis which eliminates those 2 factors influencing secretion indicates that there is no correlation between F and GH. Evaluation of GH after insulin induced hypoglycaemia in 77 children equally shows a lack of influence of endogenous F upon GH secretion. These results fit with the observation (summarized from all available published data) that the inhibiting effect of pharmacological doses of CS is clearly dose dependent. Endogenous F would be, under physiological conditions, too low to inhibit GH secretion.

24 Z. NEVO*, D. NOF*, M. BIR-ADLER*, M. MIMOUNI*, A. PERTZELAN., Z. LARON (++).

Dept. Chemical Pathology, Sackler School of Medicine, Tel Aviv University, (Z.N. & D.N.) and Institute of Pediatric and Adolescent Endocrinology, Beilinson Medical Center, Petah Tikva, Israel.

Serum Sulfate Levels and Plasma hGH, in Patients with Low and Excessive hGH Secretion

Serum sulfate determination by the bariumchloranilate reagent (Bertolacini et al, Analytical Chemistry, 30, 202, 1958; was measured in 60 children with isolated GH deficiency (IGHD), panhypopituitarism, high IR-hGH dwarfism and adults with active acromegaly. In acromegaly serum sulfate levels were subnormally low, where in the syndrome of high plasma IR-hGH the levels were markedly high. In untreated IGHD the basal levels of serum sulfate were variable, within the normal range. Upon institution of hGH therapy the serum sulfate levels of the IGHD patients decreased. We conclude that serum sulfate determinations might be a relatively simple indicator of serum hGH and/or somatomedin activity. Changes in serum sulfate levels seem to reflect the process initiated by hGH, via somatomedin generation, resulting in a higher consumption of sulfate by the target cells, and lowering its serum level.

(++) Established Investigator Chief Scientist's Bureau, Ministry of Health.

25 M.GOURMELEN*, M.T. PHAM-HUU-TRUNG,* F. GIRARD. Laboratoire d'Explorations Fonctionnelles. INSERM U 142 Hôpital TROUSSEAU, 75012 PARIS, FRANCE.

TEMPORARY PARTIAL HYPOPITUITARISM AS A POSSIBLE CAUSE FOR CERTAIN PUBERTAL DECREASES IN GROWTH.

The somatotrophic function of 88 subjects, aged 11 to 18 years, with pronounced short stature (-4σ in 48 cases) without apparent cause, was studied with the aid of the ornithine test. 61 responded with a normal hGH rise (mean of the peaks 28.7 ± 0.025), 6 proved to be suffering from complete hypopituitarism, and 21 responded with increases between 3 and 8 ng/ml (mean = 6.5 ng/ml).All these 21 subjects had reduced growth rates, 16 showed no signs of puberty and in 5, puberty had just begun. Insulin-provoked hypoglycemia carried out shortly after the ornithine test in 8 cases, confirmed partial hGH deficiency in every case. Similar tests applied to 12 of these patients after the onset of puberty and the re-establishment of normal growth resulted in positive reaction (mean of the peaks = 20.3 ± 0.057 ng/ml, range 11 to 45). In 2 of these who had been under growth hormone treatment for over a year, the distinctly enhanced growth rate was manifest well in advance of the first signs of puberty. The results are compared with those of measurements of testosterone and somatomedin levels, and it is suggested that certain decreases in growth accompanying puberty are related to reduced hGH secretion. This deficiency appears to be temporary, and is no longer evident after the establishment of the pubertal secretion of sexual hormones.