

217

**PROTEINS, SIALOGLYCOPROTEINS AND EXTERNAL SURFACE COMPONENTS OF HUMAN PLACENTAL MICROVILLOUS MEMBRANE.**

Carl H. Smith, Lucky K. Kelley, Washington University School of Medicine, St. Louis Children's Hospital, Department of Pediatrics, St. Louis, MO 63110.

The human placental microvillous membrane is known to possess a variety of enzymatic, transport and receptor activities. As an initial step in understanding the protein structure of this membrane and its relationship to these activities, we have analyzed an isolated membrane preparation by SDS-polyacrylamide gel electrophoresis. Microvillous membrane was prepared as we have previously described. The principal sialoglycoproteins were identified with periodate-<sup>3</sup>H-borohydride and the principal external surface components with lactoperoxidase-<sup>125</sup>I.

Ten major peptide bands and an approximately equal number of minor bands were seen with Coomassie blue staining. The most heavily stained component (45,000 molecular weight) was presumptively identified as actin. A band of apparent molecular weight 69,000 was consistently the most heavily labelled with both <sup>3</sup>H-borohydride and <sup>125</sup>I. Minor components of 100,000, 45,000 and 40,000 were labelled more faintly with both procedures. In addition 2-4 bands in the molecular weight range of 70,000 to 200,000 were labelled with each procedure. The human placental microvillous membrane has a relatively simple pattern of peptide components. There is one principal and several minor external surface proteins most of which are sialoglycoproteins. The identification of these components should make possible investigation of their role in a variety of placental functions.

218

**FETAL AND MATERNAL ARGININE VASOPRESSIN IN SPONTANEOUS OVINE PARTURITION**

Raymond I. Stark, Salha S. Daniel, Kazim M. Husain, Raymond L. Vande Wiele, L. Stanley James. Div. of Perinatal Med., College P. & S., Columbia Univ., N.Y.C., N.Y.

In order to assess the possible correlation between plasma arginine vasopressin (AVP) levels and the process leading to the initiation of parturition, catheters were placed in maternal and fetal vessels of 11 pregnant ewes. Samples of blood withdrawn during a 20 day period preceding delivery and during parturition were analyzed for AVP by radioimmunoassay, pH, PaCO<sub>2</sub>, and PaO<sub>2</sub>.

Detactable levels of hormone were found throughout the interval prior to labor, fetal AVP  $1.74 \pm 0.18$  pg/ml and maternal AVP  $1.47 \pm 0.10$  pg/ml (mean  $\pm$  S.E.). Daily fetal AVP levels taken during the 4 days prior to the onset of labor did not differ significantly from the levels during the preceding two weeks. Levels increased progressively during labor to reach peak values in cord blood (range 7.5 to 8000 pg/ml). There was no concomitant rise in maternal AVP. A persistent relationship between antepartum intrauterine asphyxia and increases in fetal AVP was noted. Antepartum and intra partum increases in fetal hormone correlated with decreases in fetal PaO<sub>2</sub> ( $r = -.790$  and  $-.981$ ).

It is concluded that the markedly elevated levels of AVP seen in both ovine and human cord blood are the result of intrapartum "stress" but are not related to the initiation of parturition.

219

**EOSINOPHILIA IN PREMATURE INFANTS.** Yvonne Vaucher and Emma L. Gibson (Spon. by J.J. Corrigan) Univ. of Arizona Health Sciences Center, Tucson, Arizona.

Absolute eosinophilia is common in premature infants. No clear correlation or etiologies have been proposed for eosinophilia in the premature. This study correlates the onset and peak of eosinophilia with a positive nitrogen balance. Serial eosinophil counts ( $\bar{x} = 8$ /pt) were determined in 38 hospitalized, appropriately grown premature infants whose gestational ages ranged from 27-35 weeks. Absolute eosinophilia ( $> 700/\text{mm}^3$ ) was documented in 76% (29/38). Eosinophilia was mild ( $700-999/\text{mm}^3$ ) in 9, moderate ( $1,000-2,999/\text{mm}^3$ ) in 17, and marked ( $> 3,000/\text{mm}^3$ ) in 3 patients. The average time of onset was day 21. Peak eosinophilia was usually seen within one week of onset and lasted an average of 16 days. A consistent relationship ( $r = 0.8$ ) was found between the day of peak eosinophilia ( $\bar{x} = \text{da } 24$ ) and the day at which birthweight was regained ( $\bar{x} = \text{da } 23$ ). Eosinophilia seldom occurred before steady weight gain began. No association was apparent between the occurrence or degree of eosinophilia and gestational age, birth stress, presence of umbilical catheters, hyperalimentation solution, time of beginning or type of oral feeds. The majority of infants had respiratory disease and received antibiotics and transfusion therapy. Since there is increasing evidence that eosinophilia is an immunologic response, it is possible that it represents the response of a maturing immune system to antigenic stimuli. The data suggest that eosinophilia does appear to be strongly associated with the establishment of an anabolic state.

220

**EFFECT OF THYROID HORMONE ON TISSUE NERVE GROWTH FACTOR (NGF) CONCENTRATIONS IN THE MOUSE.** Peter Walker, Morton E. Weichsel, Jr., Shirley M. Guo,

David A. Fisher, and Delbert A. Fisher. Fetal Maternal Research Laboratories, UCLA-Harbor General Hospital, Torrance, CA

NGF is a protein which has a wide tissue distribution in the mouse and is able to stimulate growth, maturation, and function of autonomic neurons. Because thyroid hormone also is known to affect nervous system growth and function, it is possible that some of its effects may be mediated through NGF. Using a newly developed, highly sensitive (10-20 pg) radioimmunoassay (RIA) for  $\beta$ -NGF, we studied the effects of thyroxine (T4) and propylthiouracil (PTU) on NGF concentrations in several tissues of the adult male mouse. T4 (25  $\mu\text{g}$  i.p. daily X 12 days) caused a significant increase in submaxillary gland and liver NGF concentrations (180 and 225% respectively) compared to controls ( $p < 0.001$ ), when expressed relative to wet weight or supernatant protein. There was no consistent effect on kidney NGF concentration. PTU (0.05% in drinking water X 21 days) led to a reduction in kidney NGF to 25% of control values ( $p < 0.001$ ) with no discernible effect in submaxillary gland and liver NGF concentrations. Conclusions: 1) A highly sensitive RIA for NGF has been developed. 2) T4 appears to have a significant effect on the concentration of NGF in submaxillary gland and liver of the adult male mouse, while PTU-induced hypothyroidism affects kidney NGF. These observations in adult mice suggest that thyroid hormone may play an important regulatory role in NGF metabolism.

221

**PRENATAL DEVELOPMENT OF ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN RAT LUNGS.** Kendall B. Wallace, Jerry B. Hook and Michael D. Bailie. Departments of Physio-

logy, Pharmacology and Human Development, Michigan State University, East Lansing, Michigan, 48824.

Angiotensin I (AI) is rapidly converted to angiotensin II (AII) during a single transpulmonary passage. The enzyme responsible for this hydrolysis, angiotensin-converting enzyme (ACE), is present in small amounts in lungs of newborn animals. Inasmuch as ACE is the final catalytic component of the renin-angiotensin system, and since fetal plasma renin activity increases with advancing gestational age, it was of interest to determine the prenatal development of converting enzyme activity in lungs of fetal rats. ACE activity was measured *in vitro* by virtue of its ability to generate hippuric acid by hydrolysis of the AI-homologue hippuryl-L-histidyl-L-leucine (HHL). Hippuric acid was extracted from the reaction mixture and quantitated spectrophotometrically. ACE activity was first detectable in fetal lungs at 18 days of gestation and increased thereafter until birth (day 21 of gestation). The *in utero* development of ACE activity was paralleled by increases in fetal lung weight and protein content. The affinity of converting enzyme from fetal lung for HHL ( $K_m = 2.0 \text{ mM}$ ) was similar to that of the adult enzyme, suggesting that the increase in ACE activity was due to increased enzyme content rather than further activation of pre-existing enzyme. This antenatal increase in ACE activity may play an important role in the maturation of the renin-angiotensin system which has been implicated in the regulation of body fluid homeostasis during the perinatal period.

222

**THE LOCALIZATION OF ADENYLATE CYCLASE AND INSULIN RECEPTORS IN THE HUMAN PLACENTA.** Jeff A. Whitsett, Carl Johnson, Kim Hawkins, (Spon. by Reginald Tsang)

University of Cincinnati, College of Medicine, Department of Pediatrics, and Cell Biophysics, Cincinnati, Ohio.

The interactions of hormones with plasma membranes in the human placenta have not been characterized for specific maternal and fetal components. The human placenta is exposed to maternal blood at the microvillus brush border membrane (MVBB) and to fetal blood at basal plasma membranes (BPM). In order to clarify possible hormone membrane interactions at these two sites, hormone sensitive adenylate cyclase (AC), insulin receptor and marker enzyme analyses were compared in MVBB and in BPM from 10 term human placentas. MVBB was 12 fold enriched in alkaline phosphatase, 5' nucleotidase, Mg-ATPase and in specific insulin binding. Adenylate cyclase could not be demonstrated in the MVBB. Basal plasma membranes, prepared after removal of the MVBB, were rich in AC; basal activity was  $46 \pm 5.6 \text{ pmoles mg}^{-1} \text{ min}^{-1} \pm \text{SE}$  and sensitivity was demonstrated for epinephrine, fluoride, prostaglandins E<sub>1</sub>, and F<sub>2</sub> $\alpha$ . Enzyme analysis of this fraction showed no enrichment with MVBB markers or insulin receptors. In the MVBB, insulin receptor concentration was  $242 \pm 42 \text{ nmoles } 10^{-6} \text{ mg}^{-1}$  compared to  $20.7 \pm 2.9$  in the BPM ( $p < .01$ ). Alkaline phosphatase was  $3.02 \pm .43$  in MVBB compared to  $.30 \pm .032$  in the BPM ( $p < .01$ ). Microvillous plasma membrane is rich in insulin receptors suggesting interaction with maternal insulin but does not contain adenylate cyclase. Basal membrane, presumably exposed to fetal hormonal influence, is rich in adenylate cyclase but not in insulin receptors.