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ABSTRACTS

Meeting of The American Pediatric Society and The Society for Pediatric Research

Atlantic City, New Jersey, May 4, 1968

SPECIAL SECTIONS
(APS) Paper submitted to The American Pediatric Society
(SPR) Paper submitted to The Society for Pediatric Research

1 *The Effects on Fluid and Electrolyte Balance of Angiocardiography.* AARON R. LEVIN*, HERMAN GROSSMAN*, EDWARD T. SCHUBERT* and ANGELA C. GILLADOGA*, N.Y. Hosp., Cornell Univ. Medical College, New York, N.Y. (introduced by Mary Allen Engle).

Forty-seven studies in 27 patients aged 1 month to 18 years were performed to determine the acute effects of angiocardiography on fluid and electrolyte balance in infants and children with congenital heart disease. Studies were carried out during diagnostic cardiac catheterization. Twelve patients had cyanotic congenital heart disease. Seventy-five per cent Hypaque® (1 ml/kg body weight) was utilized, the bolus being delivered within one second. Serum control values for sodium, chloride, potassium, BUN, bicarbonate, osmolality, pH, hemoglobin and hematocrit were obtained immediately prior to the first angiocardiogram and then within 5 minutes and 15, 30 and 180 minutes thereafter. No fixed pattern was noted in sodium or chloride values in the initial period. Osmolality showed a rise at 5 minutes, returning to normal by 30 minutes. BUN, serum potassium, pH, bicarbonate, hematocrit and hemoglobin all tended to fall dramatically immediately after injection; however, these parameters returned to near normal by 15 or 30 minutes. Patients having repeat angiocardiograms prior to return of all parameters to normal showed more pronounced and progressive changes. Analysis of data suggests a marked dilutional and acidotic effect occurring in the first 5 minutes after injection of 75 % Hypaque® with normality often being established by 15 minutes and usually present by 30 minutes after angiocardiography. Hence, repeat angiocardiography at shorter intervals than these is to be avoided, especially in infants in cardiac failure, on digitalis, and in cyanotic patients, to prevent the possible effects of severe acidosis, hemodilution and hypokalemia and to allow recovery from the acute biochemical insult produced by Hypaque® on fluid and electrolyte balance. (SPR)

2 *Abnormal Insulin Release in Cyanotic Heart Disease.* GERSHON HAIT*, MARINA A. CORPUS*, and FRANÇOIS R. LAMARRE*, Dept. of Pediatrics, Albert Einstein College of Medicine, Bronx, N.Y. (introduced by Henry L. Barnett).

Abnormal hemodynamics in congenital heart disease may be responsible for growth retardation. Cellular hypoxia, metabolic alterations and dietary factors are some important factors which have been incriminated in the pathogenesis of this syndrome. We recently demonstrated that infants in congestive heart failure had significantly higher plasma glucose levels at ½, 1 and 2 hours than normals following an oral glucose load. Accompanying this impaired glucose tolerance was a significant suppression of insulin release. In the present study, we investigated glucose metabolism and insulin release in children with cyanotic heart disease associated with pulmonary stenosis. Oral glucose tolerance tests were performed on 34 infants and children ranging from 1 month to 16 years of age. Three patient groups were studied: 11 normal children, 7 with cyanotic heart disease, and 8 with noncyanotic heart disease. The glucose tolerance tests in the three groups were normal; however, insulin levels of the cyanotic children were significantly higher ($p<0.01$) at 1 and 2 hours than normals and higher at ½, 1 and 2 hours than the noncyanotic children.

Since pancreatic secretory activities were reported to be directly related to blood flow, the increased insulin release in cyanotic cnildren with pulmonary stenosis may be due to hyperactivity of the Islets of Langerhans resulting from an increase in systemic blood flow in these children. Abnormal insulin release in children with cyanotic heart disease may also explain the hypoglycemic episodes observed occasionally following complete surgical correction. (SPR)

3 *Relationship Between pH, P_{CO_2} and P_{O_2} in the Pulmonary Vascular Bed of the Cat.* PETER H. VILES*, JOHN T. SHEPHERD* and WILLIAM H. WEIDMAN, Mayo Clinic and Mayo Foundation, Rochester, Minn.

To study relationships between pH, P_{CO_2} and P_{O_2} on pulmonary vessels, isolated cat lungs were perfused with blood at constant flow and ventilated with 20, 10, 5 and 2.5 % oxygen. Airway and left atrial pressures remained constant. Lactic acid (0.3*N*) or sodium bicarbonate (0.9*M*) were infused to alter pH. P_{CO_2} was

* By invitation

zero or 60 mm Hg (0 or 10 % CO_2 in ventilating gases). In six lung preparations with P_{CO_2} of 0 and pH of 7.6, pressor responses to hypoxia were attenuated or absent. Lowering pH to 7.0 with oxygen tension normal caused a mean increase in perfusion pressure (P_p) from 17 to 25 mm Hg and augmented pressor responses to hypoxia. In six lung preparations with P_{CO_2} of 60 mm Hg and pH of 7.6, P_p increased from 14 to 26 mm Hg with severe hypoxia (P_{O_2} = 20 mm Hg); lowering pH to 7.0 with oxygen tension normal caused P_p to increase from 14 to 16 mm Hg, and near maximal increases in P_p (11 mm Hg) occurred with P_{O_2} of 40 mm Hg. Assuming that an element of pulmonary vasoconstriction, which may be caused in part by acidosis and hypoxia, exists in infants with respiratory distress syndrome, this study supports the rationale of treatment directed toward repair of acidosis, hypercapnia and hypoxia.

(Supported in part by USPHS Grants HE-5883 and HE-5515.) (SPR)

4 *Abnormal Cardiac Rhythms Associated with Congenital Anomalies of the Venae Cavae.* KAZUO MOMMA* and LEONARD M. LINDE, UCLA School of Medicine, Department of Pediatrics, Division of Cardiology, Los Angeles, Cal.

Electrocardiograms of 43 patients with congenital heart diseases (CHD) and anomalies of the venae cavae were studied. In 26 patients with persistent left superior vena cava draining into the coronary sinus, left axis of frontal P waves between +15 and —30 degrees was found in 35 %. Isorhythmic A-V dissociation with interference was observed in two patients. In nine patients with persistent left superior vena cava draining into the left atrium, there were eight instances of abnormal atrial activation. These included a vertical P wave axis between +75 and +90 degrees in three, extreme left P wave axis between —40 and —80 degrees in three, and left atrial rhythm in two patients. In electrocardiograms of patients with absence of the inferior vena cava and azygos continuation, eight of eleven showed left axis of the P wave (between —10 and —80 degrees). Wandering or shifting atrial pacemaker was observed in six cases. In five control groups of cardiac patients with presumably normal caval drainage, frontal P wave axis usually fell between +20 and +70 degrees. Therefore, abnormal left or vertical axis of the frontal P wave, left atrial rhythm, wandering or shifting pacemaker and A-V dissociation in patients with CHD suggests the presence of either persistent left superior vena cava or absence of the inferior vena cava. (SPR)

5 *Chronic Obstructive Lung Disease Following Viral Bronchopneumonia.* P. K. ADHIKARI*, N. V. RAO*, C. A. FERGUSON*, Department of Paediatrics, University of Manitoba and Children's Hospital, Winnipeg, Canada (introduced by H. Medovy).

Pathological changes in adenoviral bronchopneumonia is well documented. Histological study of material from our hospital led us to the study of survivors of severe bronchiolitis.

Thirty-four patients with proven virus bronchiolitis were studied between 1959 and 1967. Twenty-seven were American Indians. Fourteen out of the 34 came from the city. The median age at diagnosis was 10 months (range 4–70). Twenty-six survived the acute disease. Nineteen of them are being followed regularly. Their present median age is 34 months (range 12–100).

The diagnosis of viral infection was based on: (1) positive cultures for adenovirus in 7 patients (type 3–6, type 5–1); (2) positive culture plus significant complement fixing (C.F.) antibodies in 4 patients (type 3–2; type 1 and 5–1); (3) significant change in titre of C.F. antibody in acute and convalescent sera in 16; (4) significant change in C.F. antibody for both adenovirus and respiratory syncytial virus (RSV) in 2; (5) significant change in C.F. antibody for RSV in 5. Thus, 29 had adenoviral disease while 5 had RSV infection.

The acute stage was a typical picture of bronchiolitis with radiological changes in lungs.

Nineteen out of 26 survivors of acute bronchiolitis have recurrent episodes of coughing and wheezing requiring 94 hospital admissions and 437 out-patient visits. Seven have been placed in foster homes and one is in hospital 12 months after admission because of chronic respiratory failure. Nearly all have barrel-shaped chests and abnormal physical findings in the lungs. Only one has clubbing. Chest x-rays have shown hyperinflation in 14 and stable, but chronic changes in all 19. Three have partial atelectasis of lobe and 4 have proven bronchiactesis.

It is suggested that severe bronchiolitis caused by either adenovirus or RSV can lead to chronic disease of the lung. (APS)

6 *Hypoxic Constriction of Pulmonary Artery and Vein in Intact Dogs.* BEVERLY C. MORGAN and WARREN G. GUNTHEROTH, Univ. of Washington School of Medicine, Seattle, Wash.

There is general agreement that hypoxia produces pulmonary vasoconstriction. From pressure-flow data, it is not clear whether the vasoconstriction is pre- or postcapillary. The purpose of this study was to establish, by direct measurement of pulmonary vein diameter, whether pulmonary venous constriction occurs in intact dogs breathing an hypoxic gas mixture. Eighteen animals were studied following recovery from implantation of dimension transducers (miniature mutual inductance coils) on a pulmonary artery and pulmonary vein, ultrasonic flowmeters on aorta and pulmonary vein, and pressure cannulae in pulmonary artery, pulmonary vein, pleural space and femoral artery. Breathing hypoxic mixtures for 10 minutes produced an increase in depth and rate of respiration, respiratory alkalosis, a rise in cardiac output, and a rise in pulmonary and systemic arterial pressure. Pulmonary vein pressure and mean intrapleural pressure decreased, but distending pressure (intraluminal minus intrapleural) in the pulmonary vein rose during hypoxia due to a greater fall in intrapleural pressure. Vein diameter either decreased or was unchanged in the presence of increased distending pressure; thus pulmonary venous constriction was demonstrated in 15 of 18 experiments. Pulmonary artery diameter and pressure increased simultaneously in most animals; however, in one-third, pulmonary artery diameter remained unchanged despite significant increase in pulmonary artery pressure, demonstrating arterial constriction. These data support the hypothesis that both pre- and postcapillary pulmonary vessels constrict during hypoxia. For the first time in an intact animal, active constriction of the pulmonary vein is documented by direct measurement. (SPR)

7 *Pulmonary Valvular Dysplasia: A New Form of Pulmonary Stenosis.* JAMES H. MOLLER*, MICHAEL E. KORNS*, COLIN H. SCHWARTZ* and JESSE E.

EDWARDS*, Univ. of Minnesota Med. School., Dept. of Ped. and Path., Minneapolis, Minn. (introduced by Russel V. Lucas, Jr.).

We have observed an unusual and previously undescribed type of pulmonary valvular stenosis in six children. The pulmonary valve has three cusps, a normal sized pulmonary annulus and no commissural fusion. The valve leaflets, however, are thickened, redundant and immobile. Histologic examination of the leaflets revealed complete cellular disorganization and excessive amounts of myxomatous connective tissue. The right ventricular outflow tract obstruction present is related not to commissural fusion but to the immobility of the leaflets.

At operation, the absence of commissural fusion and the normal sized annulus implied normal valve function; infundibular resection was therefore carried out. Each patient died in the postoperative period.

Preoperative recognition of this anomaly is possible. Auscultatory findings are those of pulmonary valvular stenosis, except a systolic ejection click is absent. All six children were of small stature. Two had peculiar facies (hyperteleorism, low set ears and ptosis). The electrocardiogram revealed a marked right axis deviation (+150° to +270°) and right ventricular hypertrophy. Right ventricular pressures varied from 97/0 to 190/0 mm Hg. Right ventriculography is diagnostic. The thickened leaflets are clearly visualized. The leaflets are immobile; thus the valve does not 'dome' in systole. The 'jet' of radio-opaque dye seen in classic pulmonary valvular stenosis is absent.

We have no experience in successful operative therapy. Pulmonary valvular replacement appears to be the ideal operative approach. The surgeon recognizing this lesion at operation might try radical resection of one or more leaflets. (SPR)

8 *The Effect of Long-Term Propranolol Administration on Patients with Cyanotic Congenital Heart Disease.* RICHARD A. GREENE*, EMMANUEL MESEL*, and NORMAN J. SISSMAN*, Stanford University Medical School, Palo Alto, Cal. (introduced by Herbert C. Schwartz).

The efficacy of long-term administration of propranolol in ameliorating the clinical course of patients with congenital heart disease of the Tetrad of Fallot type was evaluated. Propranolol has been shown to improve the manifestations of 'hypoxic spells' when given intravenously but has not been studied when given orally chronically. Patients were selected on the basis of clinical criteria of a young age (when surgery is attended by a relatively high risk) and comparative well-being except for the occurrence of 'hypoxic spells'. Five patients were studied. Four had Tetrad of Fallot and one had a single ventricle with transposition and subvalvular pulmonic stenosis. Ages at onset of treatment varied from 6 to 30 months. Initial dose, given in divided doses 3 times daily, was 2 mg/kg/day but this was increased in all up to 5 mg/kg/day. Results showed that one patient had no improvement and required a systemic-pulmonary anastomosis 3 weeks after onset of treatment. The other four had marked decrease in the frequency and severity of their hypoxic spells. Two patients developed increasing dyspnea on exertion and polycythemia despite improvement in spells; they underwent successful anastomoses 8 and 9 months after starting propranolol. The other two patients are doing well 8 and 10 months after initiation of treatment. Ear oximetry showed little change in resting arterial saturation but a smaller decrease in saturation on crying while on the drug compared with pre-drug control in three of the five patients. No undesirable side effects were observed. It is concluded that propranolol may be useful as adjunctive treatment in selected patients with cyanotic congenital heart disease. (APS)

9 *Beta Receptor Activity in the Fetal Lamb.* CYNTHIA T. BARRETT*, MICHAEL A. HEYMANN* and ABRAHAM M. RUDOLPH. Cardiovascular Research Institute and Department of Pediatrics, University of California Medical Center, San Francisco, Cal.

Beta receptor activity has not been well studied in the fetus, and it is not known when these receptors develop or whether fetal responses to beta stimuli differ from those occurring postnatally. We examined physiologic responses to both stimulation and blockade of beta receptors in lamb fetuses at varying gestational ages. Isoproterenol infusions produced a significant rise in heart rate in each of 15 animals studied (61–145 days' gestation) with return to control values upon cessation of the infusion. Umbilical blood flow was measured and cardiac output did not change, but redistribution of flow occurred with more of the cardiac output going to the placenta and heart and less to the carcass. Arterial pressure and blood gases did not change. Propranalol caused a significant decrease in heart rate in 3 fetuses and effectively blocked the effects of isoproterenol on heart rate in 5. No gestational differences were observed in response either to isoproterenol or to propranalol. We conclude from the increases in heart rate caused by isoproterenol that beta receptors are present in the heart and that they are active normally even at a very early gestational age since the heart rate fell after propranalol. Although postnatally beta stimulation alters myocardial contractility to produce an increase in cardiac output, our evidence suggests that although some redistribution of blood flow occurs in the fetus, cardiac output does not change.

(Supported by USPHS Grant HE-06285.) (SPR)

10 *Effect of Respiratory Rate and Airway Pressure on Arterial Oxygen Tension During Artificial Ventilation.* PENELOPE CAVE*, WILLIAM J. DAILY*, GRANT FLETCHER* and PHILIP SUNSHINE, Stanford Univ. School of Med., Palo Alto, Cal.

The purpose of this study was to investigate the effect of varying patterns of mechanical ventilation on arterial oxygen tension in infants with severe respiratory distress syndrome (RDS). Ventilation was begun only after treatment with O_2 and $NaHCO_3$ had failed and

Score	0	1	
PaO_2 (100 % O_2)	> 70	50–70	
pHa	> 7.3	7.20–7.30	
$PaCO_2$	< 60	60–70	
Score	2	3	4
PaO_2 (100 % O_2)	40–49	< 40	
pHa	7.0–7.19	< 7.0	Apnea
$PaCO_2$	71–80	> 80	

the infant scored 3 or more (see above). Twenty-seven studies were done with six infants. Each infant was studied 4–6 times in the first 120 hours of controlled ventilation. A study consisted of 4–8 randomized changes of respiratory rate and airway pressure selected to approximate similar levels of ventilation. Arter-

ial samples were drawn 25 minutes after each new pattern of ventilation was started. The studies were grouped according to age after initiation of ventilation. Correlations were made between change in rate and pressure and corresponding change in arterial pH and gas tensions. In every group of studies, PaO_2 varied directly with airway pressure and inversely with rate whether calculated as a change from the original settings or from the immediately preceding settings. No correlation was found between pH or $PaCO_2$ and rate, pressure or PaO_2, suggesting that the selected rate-pressure combinations approximated similar levels of alveolar ventilation. The data show that oxygenation of infants with severe RDS can be significantly influenced by variation of specific parameters of mechanical ventilation independent of changes of arterial carbon dioxide tension. (SPR)

11 *A Pediatric Reporting System Generated from Computer-Stored Narrative Medical Documents.* MARGARET LYMAN*, JULIUS KOREIN* and LEO J. TICK*, New York University, New York, N.Y. (introduced by Saul Krugman).

Quarterly reporting of progress in delivery of comprehensive health services under recent Children and Youth grants by the Children's Bureau is a requirement for continued support. The Bellevue Pediatric (C and Y) Project will be greatly assisted in its delivery of comprehensive health care to a large population by a computerized medical record. Such a system will be used to assist in assembling records of the several health facilities commonly used by indigent populations into an orderly, complete and readily available compilation. In effect, the various health facilities used by the population served will be 'subcontractors' for comprehensive care—each contributing casefinding, treatment or supervision to the child's health with full knowledge of the medical information accumulated by the others. The reporting system includes demographic data, acute episodic visits, diagnostic categories and the stages of comprehensive care reached at any given time. For the Bellevue Project, data for the reports are derived exclusively from those documents which serve as the patient's medical record: i.e., there are no additional or separate notations made just for the reporting system. The documents are formatted, and narrative content is entered into computer storage. Medical criteria have been established which classify categories of patients in accord with those definitions given for the reporting system, and appropriate computer programs written to effect analysis of the documents for automatic production of the required report. Examples of the documents used and results of this approach to analysis of medical records will be presented. (APS)

12 *Assaying the Process of Reaching a Clinical Diagnosis.* RAY E. HELFER* and CARL H. SLATER*, Univ. of Colorado, School of Medicine, Denver, Colo. (introduced by Arthur McElfresh).

This paper describes a reliable and valid instrument for assaying the process by which a clinical diagnosis is reached. 'The Diagnostic Management Problem' (DMP) is made up of 96 consecutively numbered cards on which is recorded certain historical information, a physical finding, or a laboratory result which pertain to a specific clinical problem. An index which specifies the type of information on each card is provided to the student who is asked to formulate a diagnosis(es) for the problem represented by each card deck. The sequence in which the cards are selected is recorded by the student. Scoring is achieved by use of a computer which is programmed to compare the process used by the student in solving the clinical problem with that used by a group of 'experts'.

The reliability of this instrument was evaluated by comparing the scores of 19 students who solved two clinical problems ($r = 0.66$; $p < 0.05$). Concurrent validity was evaluated by comparing the DMP scores of 42 students with their grades in Pediatrics and of 19 students with their performance on a Patient Management Problem supplied by the University of Illinois ($r = 0.50$ and 0.60 respectively; $p < 0.05$).

Eighteen students who had completed an unstructured student-centered Pediatric clerkship solved the clinical problems presented to them in the DMP by a process which was more like that used by the 'experts' than did 18 students in a control group who concurrently participated in the common-place, highly structured clerkship. This difference was significant at the 0.05 level. Both groups were comparable by all other measures (National Board scores, Pediatric clerkship grades, and grade point average). These findings provide additional support for the validity (construct) of the instrument. (SPR)

13 *Factors Related to Patients' Failure to Follow Long-Term Medical Recommendations.* LEON GORDIS* and MILTON MARKOWITZ, Departments of Pediatrics, Johns Hopkins School of Medicine and Sinai Hospital, Baltimore, Md.

Quantitative methods have been applied to studying a factor important in health care evaluation—patient compliance with physicians' instructions. The extent to which patients fail to comply with medical recommendations and the reasons why they do not follow them are of particular importance in maintaining chronically ill children on long-term medical care. In order to study compliance in such a population, 136 children who had a history of rheumatic fever and who were on oral penicillin prophylaxis were selected for study. Compliance was determined using the *sarcinea lutea* method for detecting penicillin in urine, adapted for mail-in testing (Pediatrics *41:* 151 [1968]). Mothers were interviewed and medical records abstracted to identify medical, sociologic and cultural variables which might relate to noncompliance. Weekly random urine specimens were obtained at school during a 20-week period. Patients were classified as compliers (75 % or more urine tests positive) or noncompliers (25 % or less tests positive). Of the study group, 36 % were noncompliers and 32 % compliers. Compliance status could be predicted quite well using a 3-test sequence as a screening method. Compliance status on days of clinic visits did not differ significantly from that determined from random school specimens. Statements from child or mother about whether he had taken penicillin differed significantly from objective findings of urine tests. Relationships of compliance to age, sex, race, socioeconomic status and other variables were examined. The data indicate that the critical variable affecting compliance in this population is the degree to which mother and child sense he is personally vulnerable to effects of the disease. These results are important for long-term care of chronically ill children and have major methodological implications for health care research. (APS)

14 *Videotape Sampling of the Child's Day in the Hospital.* JEROME L. SCHULMAN and JOSEPH C. KASPAR*, Department of Pediatrics, Northwestern University Medical School, Chicago, Ill.

A videotape recorder was installed in a viewing corridor, separated from a patient room by a one-way mirror. This facility is located in the Clinical Research Center. The recorder was coupled to an automatic programming device which obtained records for twenty seconds of each five minutes during the daylight hours. Each segment was rated as to whether the child was out of bed, sleeping or awake. In addition, when awake, his mood was rated on a seven point scale. Each time someone was present in the room, the occupation and interaction were noted. Data was separately tabulated for the afternoon of admission, the morning before surgery, the postoperative afternoon and the day after surgery. The population consisted of nineteen children admitted for repair of inguinal hernias. The results should be assessed in view of the high nurse to patient ratio on this ward. Physicians were present only on the day of admission in relation to performing the admission physical examination. Nurses were present in the room 15 per cent of the time on the afternoon following surgery, but less at other times. The staff was significantly less likely to enter the room if the mother is present, although the reasons for this are not clear. The pattern of activity varied significantly during different periods of hospitalization, with, for example, only two children sleeping at all on the afternoon of admission. The child's mood did not vary in relation to who was present. (APS)

15 *A Project Progress Report of a Coordinated Pediatric Home Care Program in a Children's Hospital.* F. B. BECKER* and A. M. BONGIOVANNI, Children's Hosp. of Philadelphia and Univ. of Pennsylvania, Pa.

This report covers 27 months of a Coordinated Pediatric Home Care Program based in a children's hospital. It is current philosophy that hospital-based home care programs meet chronic illness needs, high costs of hospitalization, geriatric needs and shortage of professional personnel. A pilot study was begun into methods of extending and coordinating medical and paramedical facilities into homes of pediatric patients. Need was determined by surveys of in-patient and outpatient population. Approximately 30 % of in-patients on any given day and 85 out-patients per week were potential candidates strictly on medical grounds without social or economic factors. Two basic categories emerged. The home care category included 206 patients which represented 1650 hospital days saved; the community health category, 444 patients with no saving. Ancillary hospital services, nursing, social service, drugs, medications, medical supplies, equipment, oxygen, inhalation therapy, speech therapy and medically indicated transportation are available to all categories regardless of ability to pay, type of illness or services needed. Admission to the program is based on medical and nursing needs, the parents' attitude and home facilities. Our program has demonstrated that home care will work for children. Planning, evaluation and parent education in health supervision can be accomplished. Physician-directed nursing, social and related services at home can be achieved. Early discharge from hospital and/or prevention of hospitalization is safe and feasible. Continued care at home can be provided and coordinated for the pediatric patient, and the physician can save valuable time for other more acutely ill patients. (APS)

16 *Environmental Influences on Drug Effect.* CHARLOTTE S. CATZ* and SUMNER J. YAFFE. Dept. of Pediatrics, School of Med., State Univ. of New York at Buffalo, N.Y.

We reported previously that in mice, strain variations in hexobarbital response (reflected as sleeping time—ST) occur and that this response is under genetic control. A corresponding increase in hexobarbital oxidase activity was obtained *in vitro*. At this age (21 days) in the normal developing mouse, weaning occurs. Therefore, the influence of this environmental event on hexobarbital response was investigated. Littermates (weaned and nonweaned) were compared at three different weaning schedules: early (15 days), normal (21 days), and late (28 days). Half the litter remained with the nursing mother; the other half was separated and started on regular laboratory pellets. In the early weaned group, hexobarbital response was measured at 18 and 21 days of age (3 and 7 days after weaning respectively). At 18 days of age the weaned group had a significant decrease in ST when compared to their nonweaned littermates (31 min versus 62 min). This difference between groups was no longer present when tested 7 days postweaning. Animals were used only once experimentally. A significant difference in hexobarbital response was noted between littermates on the normal or late weaning schedules. ST was 29 min versus 69 min at 24 days and 17 min versus 45 min at 28 days. The concentration of hexobarbital in blood obtained upon awakening was similar, indicating that end-organ sensitivity is equivalent in all groups. The significance of the reported observations in the rodent for infants and children is unknown but merits investigation and consideration. (SPR)

17 *The Long-Term Effects of Embryonic and Fetal Irradiation.* ROBERT L. BRENT, Jefferson Med. Coll., Philadelphia, Pa.

Human epidemiology studies indicate that relatively high doses of irradiation can increase the incidence of certain malignancies in irradiated populations. In 1958, STEWART reported that the mothers of leukemic children had twice the frequency of diagnostic irradiation to the abdomen during the relevant pregnancy as did mothers of nonleukemic children. The studies from the Atomic Bomb Casualty Commission have not substantiated an association between preconception irradiation, *in utero* irradiation and malignancy in the offspring. This area of interest is unique in that although there are scores of clinical studies, few animal studies have been concerned with the long-term effects of *in utero* irradiation. Ten years ago our laboratory initiated such a study in mice. Over 6000 embryos have been irradiated and allowed to live out their normal life span. Preimplantation, differentiating and fetal stages were irradiated with 0, 30, 60 or 90R. The animals were weighed throughout their life and a complete gross and microscopic examination was performed at the time of death. The results dealing with the length of life and incidence of tumors indicate that low dosage irradiation to mouse embryos at the stages of gestation that were studied did not decrease life expectancy or increase the incidence of tumors. In fact, the embryo demonstrated no life-shortening effects following 90R, although this same dose of irradiation in the adult mouse produces a substantial life-shortening effect. These and other animal data do not support the con-

cept that diagnostic irradiation during pregnancy is an indication for therapeutic abortion. This controversy has become important since some physicians have utilized the occurrence of diagnostic x-ray exposure during pregnancy as an indication for therapeutic abortion. This latter approach is confusing and will eventually result in an increase in litigation.

(Supported by Grant AEC NYO 2071-41.) (APS)

18 *Effect of Early Underfeeding on the Growth of the White Carneau Pigeon.* JEROME LIEBMAN and AARON LEASH*, Case Western Reserve University School of Medicine. Department of Pediatrics and Animal Facilities, University Hospitals, Cleveland, Ohio.

The white carneau pigeon spontaneously develops severe aortic and coronary atherosclerosis grossly and microscopically similar to that of humans. Aortic fatty streaks develop after 12 weeks; 50 % have significant atherosclerosis by one year, and all have disease at three years. Long-term experiments are under way to determine whether underfeeding from birth to weaning (28 days) will be protective to the adult. MCCANCE and WIDDOWSON in various animals have shown that early underfeeding followed by ad lib feeding after weaning markedly suppresses eventual adult size. After attempting multiple methods, the most satisfactory type of underfeeding has been determined to be that of giving the parents (both of whom feed the squabs) one-third of their normal diet. There was marked undergrowth with a 30 % attrition rate compared to a 15 % death rate in the controls. All weights are in grams.

	Number	Hatch	1 week	2 weeks	3 weeks
Controls	160	22.2	187.8	389.3	493.9
Underfed	103	21.9	134.2	212.8	271.0

	Number	4 weeks	5 weeks	6 weeks	6 mos.
Controls	160	520.2	528.1	526.6	579.1
Underfed	103	387.9	470.0	478.3	568.7

The weights are the same at hatch and at 6 months, but are significantly different in between, maximally so at 3 weeks, when the squabs begin to feed themselves. Thus, though there was tremendous undergrowth during the underfeeding period, the deprived squabs rapidly caught up to the controls after weaning and on ad lib feedings. Therefore, underfeeding and undergrowing during the preweaning period apparently do not affect adult size in the white carneau pigeon. (SPR)

19 *Life Table Analysis of the Changing Prognosis for Children with Cystic Fibrosis.* WARREN J. WARWICK and RICHARD G. POGUE*, Dept. of Ped. and Biomed. Data Proc. Unit, Medical School, Univ. of Minnesota, Minneapolis, Minn.

Life table analysis of age-specific mortality rates permits the formation of life expectancy curves. Such tables and curves were prepared for four groups of patients. Group I: patients diagnosed prior to 1940 who received no known effective treatment. Of this group, 72 % died by 1 year of age and 96 % died by 5 years. Group II: patients who received antibiotic and other medical treatments but did not receive pulmonary therapy. They were analyzed by data obtained from six sources. The best results from these showed 42 % survival to 5 years and 23 % to 10 years of age. Group III: 4040 patients seen at 43 of the 60 CF Centers. All of this group received some type of pulmonary therapy in addition to the medical measures used in Group II. Life expectancy for this group was 49 % to 10 years and 14 % to 25 years of age. Group III was divided into male and female cohorts. The life expectancy curve for males was better than for females after the age of 3 years. Group IV: 379 patients with 1801 patient years of observation who received prophylactic pulmonary therapy. Life expectancy for this group was 85 % to 10 years and 61 % to 21 years. *Conclusion:* (1) The earlier in life treatment is started and the more intensive the treatment, the better the prognosis. (2) Life table survival analysis may permit discovery of minor factors, other than sex, which have significant but minimal influences on survival. The summation of these factors, however, may provide further improvement in the outlook for children with cystic fibrosis. (SPR)

20 *A Rapid Screening Psychometric Testing for Pediatricians.* FERNANDO J. DE CASTRO*, KENNETH L. VAUGHN* and RALPH M. GIBSON*, University of Michigan, Department of Pediatrics, Ann Arbor, Mich. (introduced by James L. Wilson).

The Kent Emergency Scale (KES) is a screening test for mental age which can be performed by a physician in approximately ten minutes. To determine the validity of this test in a pediatric out-patient unit, a sample of forty patients (ages 6 to 17 years) requiring psychological testing was selected. These patients were tested (a) by one of two pediatricians using the KES, and (b) by one of four psychologists using a Weschler, a Stanford-Binet or a Leiter test. The mean IQ obtained by pediatricians was 82 and by the psychologists 83. The differences between pediatricians' results and psychologists' results were 0 to 4 IQ units in 42 % of the cases; 5 to 9 IQ units in 22 %; 10 to 14 IQ units in 25 %; 15 to 19 IQ units in 5 %; and 20 to 21 IQ units in 5 % of the patients. The standard error of the estimate was 11.4 IQ units and the correlation coefficiency was $+0.78$ (significant $\alpha < 0.01$). These results confirm the usefulness of the KES as a screening device for pediatricians confronted with children presenting behavioral and educational problems. (APS)

21 *Intestinal Manifestations in Immune Deficiencies.* R. S. DUBOIS*, C. C. ROY*, D. MERRILL*, V. FULGINITI* and K. DRUMMOND*. University of Colorado Medical Center, Denver, and Montreal Children's Hosp., Montreal, Canada (introduced by D. O'Brien).

Five congenital hypogammaglobulinemics (Bruton type), three cases of isolated IgA deficiency, four of idiopathic acquired hypogammaglobulinemia (IAH), five with thymic dysplasia and one thymic alymphoplasia were studied and shown to have a high incidence of disaccharidase deficiency, a finding not previously reported. All these patients except one with isolated IgA deficiency had intermittent diarrhea; however, in only four could steatorrhea be documented, one Bruton type, one IgA deficiency and two IAH. Both duodenal juice and stool cultures have been negative for ova, parasites and pathogenic bacteria. Light microscopy studies of the jejunal mucosa have been uniformly normal except for one case of IgA deficiency and two with IAH. All three had steatorrhea and presented with various degrees of villous atrophy; in addition, one had features of lymphoid nodular hyperplasia. Lactose and sucrose tolerance tests were done in 14 and 13 patients respectively. Five of the LTT's were abnormal and yet

jejunal lactase was deficient in 12–14 while five STT's were flat with 8–14 showing sucrase deficiency. In all patients, serum levels of IgG agreed with duodenal juice measurements. There was a perfect correlation between salivary and duodenal juice. However, in one patient with IgA deficiency, a normal level of 11S IgA was found in both saliva and duodenal juice. The disaccharidase deficiencies found could not be correlated with histological changes or with the type of immune defect except in thymic dysplasia where all five cases had sucrase deficiency. (APS)

22 *Lithocholic Acid in Meconium.* HARVEY SHARP*, JAMES CAREY*, JANET PELLER* and WILLIAM KRIVIT, Univ. of Minnesota Medical School, Minneapolis, Minn.

Lithocholic acid increases the mitotic indices of bile duct epithelium and eventually causes cirrhosis in all the animals tested (J.Lab.clin.Med. *69:* 737 [1967]). Because of an observation of improved liver function concomitant with reduction of serum bile acids (J. Pediat. *71:* 723 [1967]) an investigation of the development of bile acid metabolism was undertaken in fetuses and newborns. One hundred grams of meconium pooled from normal newborns in 15 to 20 g batches were analyzed. Following separation by silicic acid columns, fatty acids comprise approximately 3 % g weight, cholesterol 0.1 % g weight and bile acids 0.5 % g weight. The bile acid fractions were then separated on a celite column. The predominant bile acids were chenodeoxycholic and cholic acid. Lithocholic and deoxycholic acids were isolated in half of the meconiums pecimens. These secondary bile acids were verified by both thin layer and gas chromatographic techniques. The meconium has been examined for bacteria and found to be sterile. Analysis of fetal gallbladder bile demonstrated predominantly chenodeoxycholic acid, with cholic acid the only other bile acid isolated. Limitations in sample size and strong alkaline hydrolysis might fail to detect lithocholic acid but not deoxycholic acid. Lithocholic acid has never been detected before adulthood and deoxycholic acid has not been isolated under one year of age. Serum lithocholic acid concentrations in healthy adults may reach 0.1 μ g/ml. These findings suggest that secondary bile acids traverse the placental barrier. Since lithocholic acid can be present in the gastrointestinal tract *in utero*, a possible relation to infantile obstructive jaundice must be considered. (SPR)

23 *Studies on the Mechanism of Sodium Transport Inhibition in Cystic Fibrosis of the Pancreas.* JOHN A. MANGOS* and NONA R. MCSHERRY*, Univ. of Wisconsin Medical School, Madison Wis. (introduced by C. C. Lobeck).

We have demonstrated that the sweat and saliva of patients with cystic fibrosis contain a macromolecular substance that inhibits sodium (Na) reabsorption in the rat parotid gland by 60–80 %. Similar inhibition of transductal Na transport was observed when the luminal side of the cells of the duct system of the rat parotid was exposed for 90 seconds to solutions containing one of several strongly positively charged macromolecular compounds (protamine, histones, polyethylene imine, poly-L-lysine, poly-ornithine) in concentrations as low as 10^{-7} M. Our results demonstrate that interaction of these compounds with the membrane of the transporting cells affects transport of Na. Addition of the negatively charged substances heparin or polyglutamic acid to the above solutions resulted in elimination of their transport inhibitory effect. Similarly, addition of heparin to the viscous and turbid saliva from patients with cystic fibrosis eliminated its Na transport inhibitory effect, decreased its turbidity, and made it qualitatively less viscous. We concluded that the Na transport inhibitory factor of cystic fibrosis may be a positively charged macromolecule that interacts with the membrane of transporting cells of exocrine glands causing defective sodium reabsorption. The same factor could be responsible for the abnormality of mucus in cystic fibrosis. (SPR)

24 *Studies on Infant Diarrhea. IV. Composition of Jejunal Fluid after a Single Feeding.* R. TORRES-PINEDO* and H. RODRIGUEZ*, Dept. of Pediatrics, Univ. of Puerto Rico School of Medicine, San Juan (introduced by A. Ortiz).

Studies of the change in the volume and composition of ingested milk-like mixtures containing glucose, lactose or maltose, during the digestive-absorptive process were performed in infants with acute diarrhea and shortly after recovery. The luminal fluid was sampled at two points of the jejunum, 25 cm apart, hourly during 5 hours. Since transmucosal sugar transfer is essentially unidirectional, maximal jejunal absorptive and hydrolytic abilities may be assessed by comparing original to sampled concentration ratios of sugar with those of polyethylene glycol (S out/in + PEG out/in) because variations in gastric emptying volumes apply to both terms and cancel out. The following average ratios were obtained at proximal collecting points (diarrhea *versus* recovery): Glucose, ½ h (0.70–0.45); 1 h (0.59–0.34); 2 h (0.52–0.33); 3 h (0.52–0.10); 4 h (0.50–0); 5 h (0.20–0). Lactose (hydrolysis index), 1 h (0.79–0.48); 2 h (0.84–0.23); 3 h (0.77–0.05); 4 h (0.38–0); 5 h (0.25–0). Maltose (hydrolysis index), 1 h (0.38^{-1}); 2 h (0.36^{-1}); 3 h (0.28^{-1}); 4 h (0.32^{-1}); 5 h (0.45^{-1}). The results revealed impairment of absorption and prolongation of transit times for all three sugars during diarrhea, lactose being maximally impaired. At equal molar loads, absorption of monosaccharides provided by hydrolysis was lower than when ingested free. Close correlations for volume, Na^+ and sugar were observed with preponderance of high Na^+ (low vol, low sugar points in recovery), and low Na^+ (high vol, high sugar points in diarrhea). (APS)

[1] Maltose recovery data still incomplete.

25 *Postnatal Development of 24-Hour Rhythms in Pineal Hydroxyindole-O-Methyltransferase (HIOMT) and Salivary Gland Norepinephrine (NE).* ROBERT Y. MOORE, RUTH ANN SMITH*, JEAN A. WEAVER* and NICHOLAS A. VICK*, Dept. of Ped. and Med., Univ. of Chicago School of Med., Chicago, Ill.

The pineal gland contains NE and a unique enzyme, HIOMT, which forms melatonin. Levels of NE and activity of HIOMT show a 24-hour rhythm; they are high at the end of a dark period (8 a.m.) and low following a light period (7 p.m.). Maintenance of the norepinephrine rhythm appears to be critical for maintenance of the HIOMT rhythm. Unlike some 24-hour rhythms, the NE and HIOMT rhythms are dependent upon information about environmental lighting carried to the pineal by sympathetic nerves arising in the superior cervical ganglion. Other tissues innervated by superior cervical ganglion, i.e., the salivary glands, also show a 24-hour rhythm in NE content. The time course

of the postnatal development of these rhythms is not known and was investigated. Pregnant rats were placed in diurnal lighting (lights on 8 a.m. to 7 p.m.). Infants were killed on postnatal days 1, 3, 6, 8, 10, 16, 21 and 35 at 8 a.m. and 7 p.m. Salivary glands from each were assayed for NE content and pineals for HIOMT activity. Salivary glands showed a normal adult NE level and a clear rhythm by day 10. The pineal HIOMT levels did not approach adult activity until day 21, when a significant rhythm appeared. This provides further evidence that pineal rhythms and function are initiated and maintained by the sympathetic nervous system. (SPR)

26 *Reticuloendothelial Galactosidase and Glucuronidase in the Neonatal and Germ-Free Rat.* JOHN R. ESTERLY*, ALFRED C. STANDEN* and BJARNE PEARSON*, Fort Detrick, Frederick, Md. (introduced by Gerard B. Odell).

The histochemical demonstration and localization of beta-D-galactosidase (GAL) and beta-D-glucuronidase (GLCR) were compared to the morphologic differentiation of several lymphoid tissues in prenatal, developing neonatal, and germ-free rats. The thymus had a relatively mature histologic structure by 7–10 days of age, and maturation of the neonatal spleen and intestinal lymphoid tissue had occurred by the third week. Differentiation in peripheral lymph nodes was subsequently found between one and six months of age. These organs were markedly underdeveloped in adult germ-free rats, whereas their appearance was more nearly mature in the 'conventionalized' animals. The GAL activity of the thymus was absent or minimal in the fetal and newborn rat. Its appearance paralleled the histologic development. GLCR was present at birth, but decreased during the next few days and reappeared coincident with the GAL. In contrast, GAL and GLCR reactivity in intestinal mononuclear cells preceded the morphologic changes. In the spleen and peripheral lymph nodes, increases in both enzymes paralleled histologic maturation in the neonatal, adult, germ-free and conventionalized animals. These findings indicate that morphologic appearance may be a useful index of the relative activity of these lysosomal enzymes in developing lymphoid tissues. (SPR)

27 *Patterns of Enzyme Development in the Hepatic Endoplasmic Reticulum.* L. F. SOYKA*, Department of Pediatrics, Stanford University School of Medicine, Palo Alto, Cal. (introduced by G. M. McKhann).

Two microsomal drug metabolizing systems and four electron transport enzymes in 9,000 g supernates from fetal to 90-day-old rats have been studied to explore the biochemical correlates of the impared ability of newborns to metabolize drugs. Activity per kg body weight was employed to express results since drugs are often administered on this basis. This denominator also incorporates changes occurring in liver weight per unit body mass and protein content per unit of liver. Relative liver weight decreased after birth, reaching a nadir at ten days after birth and increasing 2× by 45 days. Hydroxylase activity was absent in the fetus, reached a peak by 30 days, and declined. Demethylase was detectable in the fetus and increased > 10× by 45 days. NADPH cytochrome c reductase was found in the 3 g fetus and showed little increase until day 30. NADH cytochrome c reductase activity fell after birth but then increased 60×, achieving maximum activity at 45 days. Diaphorase (2,6-dichloroindophenol) activity was similar for NADPH and NADH. Activity fell from day 1 to 3, as with NADH reductase, and increased to a maximum at 30 days. In summary, all enzymes studied reached maximum activity by 30–45 days of age, with decline thereafter. Sex differences did not follow a regular pattern, but were found by 45 days in some (M>F demethylation, F>M NADH reductase) but not in all. The results demonstrate that the activity of enzymes localized in the same membrane and participating in a chain of oxidative reactions exhibit different developmental patterns.

(Supported by USPHS Grant 7 R01-03063.) (SPR)

28 *A Sequential Analysis Demonstrating Increased Mortality Following Gastrostomy in Low Birth Weight Infants.* SHAKUNTHALA VENGUSAMY*, JOHN F. RAFFENSPERGER*, ROSITA PILDES*, HARRY D. LEVINE* and MARVIN CORNBLATH, Univ. of Illinois Col. of Med. and Cook County Hospital, Chicago, Ill.

A sequential controlled study was conducted to compare the effects of gastrostomy feedings with routine oral feedings on survival to 21 days in infants between 750–1250 g birth weight. A reduction in mortality of 20 % was used to test for a significant difference between the two groups. One hundred and twenty-two infants were randomly placed in a control or gastrostomy group at 48 to 72 hours of age. Each gastrostomy infant was paired with a control infant of similar weight group (I = 750–1000 g, II = 1001–1250 g) and of the same sex. All infants were given I.V. fluids on admission to the premature nursery and similar graduated feedings at comparable ages (12 hours after gastrostomy). Clinical characteristics were similar in both groups. Of 54 pairs that were matched when the study was concluded, 34 were identical in outcome and did not contribute to the analysis, whereas 20 were discordant with only one of the pair surviving (table).

Group	Similar pairs Lived	Died	Discordant pairs – Gastrostomy	Survivors Control	Total
I	6	3	2	6	17
II	25	0	5	7	37
Total	31	3	7	13	54

From these data, it was concluded that survival was significantly higher in the control group. Specific causes of death including hemorrhage and infection were more frequent in the gastrostomy group than in the control group. (APS)

29 *Effect of Colchicine on Cellular Turnover and Enzymatic Activity of Intestinal Mucosa.* JOHN J. HERBST*, RUTH HURWITZ*, PHILIP SUNSHINE and NORMAN KRETCHMER, Stanford Univ. School of Medicine, Palo Alto, Cal.

Diarrhea is a well-recognized toxic manifestation of prolonged colchicine administration. This investigation was designed to study the effects of this drug on specific aspects of intestinal physiology, e.g., enzymatic activities and cellular proliferation. Forty hours after a single injection of colchicine (1 mg/kg) into rats 30 days of age there was an 85 % decrease in the activity of intestinal invertase, which returned to normal within 72 hours.

When colchicine (4 mg/kg/day) was administered in drinking water there was a 50 % decrease in activity of

intestinal invertase after 3 days of imbibition. The animals had mild diarrhea, but their body weights were comparable to those of normal controls. Histologically, slight broadening of the villi and an increase in the number of goblet cells were noted. Within 72 hours after cessation of colchicine administration, activity of intestinal invertase returned to normal. There was a slight decrease in the activity of aspartate transcarbamylase in the villi and crypts, but the activity of uridine kinase was unchanged from control animals. Two hours after a pulse of H^3-thymidine, 36.2 % of the cells were labeled in the crypts as compared to 35.8 % in the controls.

These data indicate that the change in activity of intestinal disaccharidase following colchicine cannot be merely a reflection of arrested cellular proliferation but must also affect cellular differentiation. These experiments can serve as useful models to study the environmental acquisition of deficiencies in digestive enzymes.

(Supported by USPHS grants HD-02147 and 5T01-HD-49.) (SPR)

30 *Unusual Insulin Secretion Characteristic of Cystic Fibrosis of the Pancreas.* S. HANDWERGER*, J. ROTH*, P. GORDEN* and P. A. DI SANT'AGNESE, National Institutes of Health, Bethesda, Md.

Cystic fibrosis, with or without impaired glucose tolerance, was characterized by insulinopenia following oral glucose but normal insulin release following other stimuli. In 31 patients, oral GTT was normal or slightly impaired in 64 %, moderately impaired in 26 %, and severely impaired in 10 %. Following oral glucose, insulin secretory patterns (immunoassay) revealed that (1) patients with normal or slightly impaired tolerance had a prompt but subnormal rise in insulin; (2) patients with moderately impaired tolerance had a delayed as well as subnormal response; and (3) patients with severe intolerance had a negligible rise. When glucagon or tolbutamide was infused at the height of the insulin response to oral glucose, serum insulin promptly increased 100 % or more in all cases, irrespective of blood glucose changes. Other studies showed (1) much less impaired glucose and insulin responses to intravenous glucose as compared to oral glucose; (2) normal insulin tolerance; (3) normal HGH responses to hypoglycemia; and (4) no effect of phentolamine on insulin secretion. The glucose intolerance is not classical diabetes; family history, characteristic complications and postmortem signs of diabetes were absent. In contrast to the cystics, young patients with diabetes mellitus and hypoinsulinemia failed to secrete further insulin when glucagon, tolbutamide, or arginine was infused at the height of the insulin response to oral glucose. We conclude that the glucose intolerance in cystic fibrosis is due to a defect in the mechanism whereby *oral* glucose stimulates insulin release. (SPR)

31 *Intermittent Glycinemia and Methylmalonic Aciduria —A New Syndrome?* GRANT MORROW III*, LEWIS A. BARNESS, VICTOR H. AUERBACH and ANGELO M. DIGEORGE, Dept. of Ped., Hosp. Univ. of Pennsylvania, Univ. of Pennsylvania School of Med., Children's Hosp. of Philadelphia and St. Christopher's Hosp. for Children and Temple Univ. School of Med., Philadelphia, Pa.

Until recently, excessive urinary excretion of methylmalonic acid (MMA) was found only in pernicious anemia (PA) or asscociated with other forms of vitamin B_{12} deficiency. OBERHOLZER *et al.* (Arch. Dis. Childh. *42:* 492 [1967]) reported two cases of methylmalonic aciduria associated with chronic metabolic acidosis. The metabolic defect is in the conversion of MMA to succinate.

We have studied three patients with massive excretion of MMA without vitamin B_{12} deficiency who demonstrated abnormal urinary glycine excretion patterns in addition to periods of intermittent hyperglycinemia (3–5 mg % as compared to greater than 5 mg % in classic glycinemia). One of OBERHOLZER's patients also demonstrated an abnormal glycine spot in the urine. In our patients, MMA excretion ranged from 3 to 6 g/day. Two of the three patients developed neutropenia and thrombocytopenia during their acidotic episodes. Ketoacidosis by itself, as seen in diabetic children, does not increase MMA excretion. The urines of three children with documented classic glycinemia were tested and MMA excretions were normal. The association of abnormal amounts of MMA in the urine with intermittent plasma glycine elevations differentiate this variant of hyperglycinemia from the classical form and perhaps also from the disorder reported by OBERHOLZER *et al.* These findings may constitute a new syndrome.

(Supported in part by USPHS grants AM-02231, FR-240, HD-2870 and FR-75.) (SPR)

32 *A Metabolic Defect in Glucose Utilization by Achondroplastic Cartilage.* THOMAS H. SHEPARD, BENJAMIN C. MOFFETT* and LOUIS R. FRY*, Univ. of Washington School of Med., Seattle, Wash.

In spite of many animal models, no metabolic lesion has been demonstrated previously in achondroplasia. The affected newborn rabbits (ac/ac) resemble human achondroplastics by their shortened long bones, enlarged skulls and brains, and the histologic appearance of the metaphyses, but differ in that the mode of inheritance is recessive.

Heterozygote (Ac/ac) animals were bred; approximately 25 % of the offspring were easily identified as achondroplastic (ac/ac). Cartilage with bone from newborn dwarfs and normal littermates was carefully cleaned and placed in organ culture with one of the following isotopes: (1) ^3H thymidine (2) $^{35}SO_4$; and (3) uniformally labelled ^{14}C-glucose. After 24-hour exposure, the explants were counted in liquid scintillation or fixed for tissue radioautographs.

The isotope incorporation from the ^{14}C-glucose was significantly higher in the ac/ac cartilage than in the controls (ac/ac $= 222 \pm 26$, normal $= 139 \pm 34$, df 33, $t = 3.02$, $p<0.01$). No differences in ^{35}S or ^3H incorporation have been found.

From tissue radioautographs, the achondroplastic cartilage incorporated more ^{14}C activity than controls, but the striking difference was that the achondroplastic cells located in the central part of the cartilage were more heavily labelled than the similarly located cells in the control. The distribution of grains ^{35}S and ^3H was not qualitatively different.

One possible interpretation of these findings is that a defect in energy metabolism requiring greater glucose supply may become manifest in the central avascular areas of cartilage. As this same area is the source of cells for linear growth, the result could be the marked shortening of the achondroplastic long bone. (SPR)

33 *Type II Hyperlipoproteinemia in a Family with Hypercholesterolemia and Premature Coronary Artery Disease.* EVERETT W. LOVRIEN*, MANUEL R. MALINOW* and ROBERT D. KOLER*, Univ. of Oregon Medical School, Crippled Children's Division, O.R.P.R.C., Portland, Ore.

Two siblings, at the ages of 5 and 9 years were evaluated after their father suddenly expired at the age of 32 from acute coronary insufficiency. Pedigree analysis revealed 22 relatives had died of heart attacks before the age of 45. Plasma cholesterol in the children was 472 and 486 mg % respectively. Triglycerides were 41 and 78 mg %. Electrophoresis of plasma proteins with subsequent oil-red-O stain for lipid revealed elevation of β-lipoprotein. Using antiserum to human β-lipoprotein, agar immunoelectrophoresis was normal. The α-lipoprotein, pre-β and chylomicron fractions were normal. This type of lipoprotein abnormality has been classified as Type II by FREDRICKSON and LEES (New Engl. J. Med. *276:* 34 [1967]).

One-hundred-and-thirty members of the family were examined; 24 individuals had elevation of cholesterol and β-lipoprotein. The earliest age detected was 6 months; 10 individuals were less than 16 years of age. The earliest death from heart attack was age 27 years. The 9-year-old propositus had arcus juvenilis; some individuals had tendinous xanthomas, abnormal EKGs and calcified abdominal aortas. Males and females were equally affected. The pattern of inheritance was an autosomal dominant. Of 67 individuals at risk, 32 were affected.

By correlating plasma cholesterol levels and lipoprotein patterns, affected members can be identified early in the pediatric age group before they develop signs of coronary disease. Treatment has been started in this family with low cholesterol diet and cholestyramine. (SPR)

34 *Regulation of Beta and Delta Chain Synthesis in Sickle Cell Anemia.* HAIG H. KAZAZIAN, JR.* and HARVEY A. ITANO*, National Institutes of Health, Bethesda, Md. (introduced by Barton Childs).

A normal human erythrocyte contains 40 times as much β chain of hemoglobin A ($\alpha_2\beta_2$) as δ chain of hemoglobin A_2 ($\alpha_2\delta_2$). The basis of this difference was investigated with the use of reticulocytes from individuals with sickle cell anemia, whose erythrocytes contain 30 times as much β^S chain of hemoglobin S ($\alpha_2\beta^S{}_2$) as δ chain. Ribosomes and supernatant hemoglobins were isolated from reticulocytes that had been incubated with ^{14}C-labeled amino acids. Peptides obtained from tryptic digests of incomplete, or nascent, polypeptide chains attached to ribosomes and globin were purified and assayed for radioactivity. Since the amino-terminal tryptic peptides, δT1 and β^ST1, respectively, of δ chain and β^S chain are structurally different, they can be individually assayed. The nascent chains of the ribosomal preparations contain 0.01–0.02 as much δT1 as β^ST1. Polypeptide chain synthesis proceeds from the amino-terminal amino acid to the carboxyl-terminal amino acid. Hence, nearly all ribosomes making a polypeptide on a messenger RNA will contain the amino-terminal tryptic peptide. Thus, the number of active δ-chain-associated ribosomes is assumed to be 0.01–0.02 that of β^S-chain-associated ribosomes. The labeling of tryptic peptides from supernatant hemoglobins A_2 and S indicated that the rate of δ chain synthesis is 0.005 that of the β^S chain. These data suggest that a lower concentration of δ chain messenger RNA (mRNA) is the major cause of the slower rate of δ chain synthesis. As presently understood, an operon of δ and β chain genes would require that the synthesis of δ mRNA be no less than that of β^S mRNA. Thus, these data provide further evidence against the existence of such an operon. (SPR)

35 *MDH Isozymes in Ascaris—Biological and Diagnostic Significance.* DAVID S. ZEE* and WILLIAM H. ZINKHAM. The Johns Hopkins School of Medicine, Baltimore, Md.

The demonstration of extraintestinal parasites in host tissues may be difficult and at times impossible; e.g., the detection of *Toxocara canis* larvae in tissues of patients with visceral larval migrans. Other investigators have shown that the physical and chemical properties of host and parasite enzymes are often different. Using *Ascaris suum* ("pig Ascaris") as an experimental model, the properties of malate dehydrogenase (MDH) were studied and compared with those of the host. Starch gel electrophoresis revealed four bands of MDH activity in *Ascaris*; one mitochondrial and three supernatant forms. Thermostability and pH optimum curves of the three supernatant isozymes differed, the middle isozyme exhibiting intermediate properties. Observations on the ontogeny and tissue distribution of the supernatant isozymes, dissociation and recombination experiments and the detection of an electrophoretic variant suggests that the supernatant isozymes are dimers composed of subunits under separate genetic control. Electrophoretic differences exist between the MDH isozymes of pig tissues and *Ascaris suum* as well as between human tissues and *Toxocara canis*. When a 1:20,000 dilution of *Ascaris* homogenate was added to an equal volume of pig liver homogenate, the MDH isozymes of the parasite were still observed on the starch gel pattern. Studies are in progress to determine the minimum number of larvae that can be detected in tissues of guinea pigs experimentally infected with *Ascaris suum*. (SPR)

36 *The Cytogenetic Effect of Exposure to LSD in utero.* MAIMON M. COHEN*, KURT HIRSCHHORN* and WILLIAM A. FROSCH*, State Univ. of New York, Buffalo, Mt. Sinai Hosp., New York, and Bellevue Hosp., New York, N.Y. (introduced by Ronald G. Davidson)

The induction of chromosomal aberrations by the psychotomimetic hallucinogen, lysergic acid diethylamide (LSD-25), has been demonstrated both *in vitro* and *in vivo*. Such damage could result in the induction of neoplasia in the "user", in a teratogenic effect on the developing fetus, and in a genetic effect through chromosmal aberrations in gametes. In this study the chromosomes af 12 infants exposed to LSD at various times during fetal life were investigated. Also included were their mothers and 7 other adult "users". The control group consisted of 6 children (matched for age and sex), their mothers and 4 other normal adults. The mean chromosome breakage rate of the controls was 1.26 % cells with breaks (range 0.00–2.40 %) as opposed to 8.72 % (range 1.30–20.30) among those exposed to the drug. Comparing only the children "at risk", a similar difference was found—1.25 % in the controls compared to 7.74 in those exposed *in utero*. A total of 8 structural rearrangements was observed among those exposed to LSD (6 dicentrics and 2 quadriradials) while none was seen in the controls. Although chromosomal

anomalies have been observed in abortuses and congenitally malformed infants, all of the children in this study are apparently healthy with no obvious birth defects. (SPR)

37 *A Novel Chromosomal Basis for Imperforate Anus (The "Cat's Eye" Syndrome).* PARK S. GERALD, CHARLES DAVIS*, BURHAN M. SAY* and JOHN L. WILKINS*, Children's Hospital Medical Center, Boston, Mass.

SCHACHENMANN *et al.* described a possible new syndrome which has colloquially been called the Cat's Eye syndrome. In its complete form this syndrome consists of mental retardation, colobomata, preauricular fistulae, congenital heart disease, urinary tract malformation and imperforate anus associated with the presence of an extra, acrocentric chromosome slightly smaller than a G. Two new kindreds with this chromosomal disorder have now been studied and the identification of this collection of malformations as a new syndrome has been confirmed. In family No. 1, the propositus is a mosaic although he exhibits the complete syndrome. The parents are chromosomally normal and no other immediate member of the family has any malformations. In family No. 2, the propositus has mental retardation, congenital heart disease and imperforate anus —over 90 % of his cells have the extra chromosome. Three other members of the kindred are mosaic for this chromosome disorder; two of them have imperforate anus (and no other major defect) and the third is phenotypically normal. This latter family emphasizes the unusual behavior of this chromosome, since a mosaic grandparent has transmitted the abnormality to his mosaic grandchild through a daughter who is apparently chromosomally normal. The occurrence of a single congenital malformation in association with a chromosome abnormality supports the contention that microscopically invisible chromosome changes might be the basis for some 'isolated' congenital defects. (APS)

38 *The Role of the Y Chromosome in Females with Mosaicism.* LILLIAN Y. HSU*, KURT HIRSCHHORN*, ARTHUR GOLDSTEIN* and RALPH E. MOLOSHOK, Departments of Pediatrics and Obstetrics and Gynecology, Mount Sinai School of Medicine, New York, N.Y.

Individuals with XY/XO mosaicism present a wide range of phenotypic manifestations including Turner's syndrome, intersexes and so-called male Turner's syndrome. An XYY sex chromosome constitution has been shown to cause increased stature and mental defect. Two cases of XYY/XY/XO mosaicism studied by us were found to have markedly different phenotypic manifestations. The first patient is a 38-year-old 6′ 3″ tall female, with primary amenorrhea, lack of secondary sex characteristics, and a history of a previously excised right gonadoblastoma. Chromosome studies from blood, fibrous tissue from the region of the gonadoblastoma, and from a streak gonad on the left showed XYY/XY/XO mosaicism with a predominance of XY in the blood and the fibrous tissue, but with mostly XO from the streak. The second patient is a 13-year-old child with short stature and underdeveloped secondary sex characteristics who has been raised as a female. She was born with ambiguous external genitalia with an enlarged clitoris and a urogenital sinus. Laparotomy at 10 months revealed a uterus, tubes, a testis on the left which was removed and an unidentified gonad on the right which showed no ova but possibly some ovarian stroma on biopsy. Chromosome studies from blood showed XYY/XY/XO mosaicism (XY>XO> XYY). These patients will be compared to others to demonstrate the phenotypic variability of the presence of Y chromosomes in females. (APS)

39 *Autoradiographic Studies of D Chromosomes.* GERALD E. BLOOM* and PARK S. GERALD, Children's Hospital Medical Center, Boston, Mass.

Autoradiographic studies using tritiated thymidine have been done for 19 patients with D group (13–15) chromosome abnormalities. The specific chromosomes affected in each case were identified to determine if members of the D group were randomly involved in the various conditions studied. The results of these investigations were combined with those of 22 similarly studied patients from the literature. The results from the total of 41 patients are as follows. All six patients with the D_1 trisomy syndrome involved chromosome 13. The five D/D centric fusion translocations occurred between chromosomes 13 and 14. Thirteen D/G centric fusion translocations, with one exception, involved chromosome 14. A ring D chromosome was identified as number 13 in three patients and number 14 in two. Reciprocal translocations between a D and a chromosome in another group involved chromosomes 13 (three patients), 14 (one patient); and 15 (two patients). Prominently satellited D chromosomes (two patients) were both found to be number 15. Short arm deletions (two patients) occurred in chromosomes 13 and 15. It is apparent that the specific D chromosomes involved in certain D chromosomes abnormalities (D_1 trisomy, D/D and D/G centric fusion translocations) are not randomly determined. Evidence for satellite association as a cause of this nonrandomness was sought from autoradiographic studies of normal D chromosomes but could not be found. The nonrandomness may result because the mechanism producing the abnormality affects only certain D chromosomes. Alternatively, but much less likely, all D chromosomes may be involved in the conditions studied, but only certain combinations may persist because of selection. (SPR)

40 *Karyotypes of Consecutive Newborns.* HERBERT A. LUBS* and FRANK H. RUDDLE*, Depts. of Ped., Med. and Biol., Yale University, New Haven, Conn. (introduced by Charles D. Cook).

The chromosomes of 4,500 infants born between October 1967 and October 1968 and 1,000 of their mothers are being analysed. The primary purpose of the study is to effect a quantitative, statistical and clinical definition of chromosomal normality in man, but a number of other types of information can be gained from these data in the course of the study. Although 30 cells in each individual will be studied subsequently by quantitative technics, including a FIDAC scanner, two conventional karyotypes are being prepared initially and form the basis for this report. The present study is addressed to the problem of assessing the clinical role of chromosomal studies in the neonatal period. Close to 1 % of the infants have had a chromosomal abnormality, but only one has been clinically abnormal, an infant with D trisomy. Preliminary analysis of the clinical data, including birth weight, the presence of minor anomalies and the various factors in the parents felt to predispose to chromosomal abnormalities has not yielded a set of criteria which would enable the identification of a group of newborn infants with a high prob-

ability of having a chromosomal abnormality. If this group of disorders is to be diagnosed in infancy, therefore, routine chromosomal studies on each infant appear to be necessary. Twenty per per cent of the newborns showed a 'normal variant' in their karyotypes. These variants have been reported to be present in increased frequency in congenital heart disease and other groups of patients. Statistical analysis of the frequency of congenital anomalies, abnormal pregnancies and gross chromosomal abnormalities in these families is currently in progress in the first thousand infants. The relatively unbiased mode of ascertainment of this group of newborns with 'normal variants' should permit a final assessment of their significance. (SPR)

41 *Alpha-1-Globulin Deficiency in Familial Infantile Liver Disease.* H. SHARP*, E. FREIER* and R. BRIDGES*, Univ. of Minnesota Medical School, Minneapolis, Minn. (introduced by W. KRIVIT).

A deficiency of α-1-antitrypsin in two siblings with portal cirrhosis prompted the following investigation. They were identified by a virtual absence of the α-1-globulins by cellulose acetate electrophoresis. The studies by Eriksson and his collaborators have shown that some 90 % of the α-1-globulins on electrophoresis are accounted for by the α-1-antitrypsin, a glycoprotein with a molecular weight of 60,000. This is the same protein that is absent in these patients. Eriksson and others have previously described an autosomal recessive form of emphysema appearing in adults that is also associated with a deficiency of this glycoprotein. We have now studied a total of six families containing seven children with this syndrome. Three other infants in these kindred died of liver disease prior to this study. All the patients studied had an absence of the α-1-globulin, a level of antitrypsin inhibitor of less than 0.4 mg/ml of serum and an almost complete absence of the α-1-antitrypsin by immunoelectrophoresis using specific antisera. Six of the nine siblings of these patients so far examined have had levels of antitrypsin in the heterozygote range (0.40–0.80). The parents studied have all been in the heterozygote range. The other forms of cirrhosis that we have studied (secondary to biliary atresia, cystic fibrosis, tyrosinemia, familial cystic disease of the liver and kidney, familial biliary cirrhosis of unknown etiology, chronic active hepatitis) have all had normal or increased levels of antitrypsin. The biochemical and clinical data indicate an autosomal recessive move of inheritance at the present time. (APS)

42 *Role of Macrophages in Interferon (IF) Production in Vivo.* A. LOWELL GLASGOW, Univ. of Rochester School of Med. and Dent., Rochester, N.Y.

IF is an important factor in host resistance to virus infections. Clearance of virus from the blood by phagocytic cells and demonstration of IF production by these cells suggest that they may make a contribution to the 'interferonemia' observed in many viral infections. Peritoneal macrophages were collected, exposed to chickungunya virus (CV) *in vitro*, washed, counted, and $4–6 \times 10^6$ cells were inoculated IP into recipient C3H mice. To control for IF production in recipients by nonadsorbed virus in the inoculum, other groups of mice received an inoculum with cells: (1) removed by centrifugation; (2) disrupted by sonication. An aliquot of macrophages + CV was incubated *in vitro* and fluid harvested at 18 hours for IF assay. Serum IF levels (U/ml) at 6–10 hours after inoculation are summarized:

	No. 1	No. 2	No. 3
Macrophages + CV	1200	2000	1900
Macrophages + CV (supernatant)	340	—	—
Macrophages + CV (sonicate)	250	500	600
Macrophages + CV (maintained in tissue culture)	< 100	240	460

Three- to five-fold higher levels of IF were found in the serum of mice receiving a transfusion of IF-producing macrophages. Maintenance of the observed levels of IF in serum (1200–2000 U/ml) is particularly significant in view of the fact that the half-life of exogenous IF is only 5–8 minutes and the total production of IF *in vitro* was < 100–450 U/ml in 6–8 ml. These data suggest: (1) macrophages may be a major site of production of serum IF; (2) production of IF under *in vitro* conditions may not reflect the true *in vivo* interferon-producing capacity of a cell. Studies are in progress to determine if the transfusion of IF-producing macrophages may be utilized in the treatment of viral infections. (SPR)

43 *Proportion of Total Serum IgM-Immunoglobulins that is Specific Antibody Following Typhoid Immunization of Children.* WILLIAM A. ALTEMEIER, III*, JOSEPH A. BELLANTI and EDWARD L. BUESCHER*, Walter Reed Army Inst. Res. and Georgetown Univ. School of Med., Washington, D.C.

Following primary immunization with *Salmonella* vaccines, the majority of serum antibody activity is associated with IgM-immunoglobulins. The amount of antibody protein produced and its contribution to total serum IgM are unknown and form the basis for the present studies.

A total of 8 children received either 1 or 2 injections of typhoid vaccine. Within 10 days, total serum IgM, measured by quantitative precipitation, reached peak concentrations averaging 170 % of preimmunization levels; IgG and IgA changed relatively little. By 28 days IgM usually approximated initial levels. The quantity and specificity of IgM typhoid antibody were measured by comparing serum IgM levels before and after absorption with various types of *Salmonella*. Analysis of 6 sera obtained 7 to 14 days postimmunization revealed 22 to 46 mg of IgM/100 ml serum could be absorbed by the vaccine strain of *S. typhosa*. This represented 18 to 33 % of all serum IgM and 45 to 86 % of the IgM increase induced by immunization. The specificity of this antibody to the O, H, and Vi antigens of *S. typhosa* was studied by absorbing with *Salmonella* of varying antigenic compositions. The vaccine strain possessing O, H, and Vi antigens was most effective, *S. typhosa* H901 with O and H was intermediate, and *S. typhosa* 0901 with only O removed the least IgM. These data suggest that routine typhoid immunization increases serum IgM and that most of the increase is specific antibody. (SPR)

44 *Characterization and Isolation of Reaginic Antibodies to Wheat Gliadin in the Serum of a Wheat-Sensitive Subject.* DOUGLAS C. HEINER, GERALD B. GOLDSTEIN*, LAWRENCE GOODFRIEND* and BRAM ROSE*, Div. of Immunochemistry and Allergy, Royal Victoria Hosp., Montreal, Canada.

A subject with marked hypersensitivity to ingested wheat had reaginic antibodies in high titre (1:10,000) and a positive Prausnitz-Kustner test was elicited with either 2×10^{-6} mg of alpha gliadin intradermally or one slice of bread by mouth. Reagin, like IgG, was dispersed throughout DEAE cellulose fractions including the initial pH 8.0, 0.01 M phosphate eluate which contained only IgG, and not IgA, IgM or IgD. Gliadin was copolymerized using either ethylene maleic anhydride or ethyl chloroformate to produce immunosorbents with retained antigenic activity. Serum was exposed to three different gliadin immunosorbent preparations and, after exhaustive washing, biologically active specific reagin was eluted from each immunosorbent using 2M NaCl or 2M KI. No immunoglobulins could be detected by immunochemical analysis of the eluates but injection of washed immunosorbent-reagin complexes into three separate rabbits resulted in the production of antibodies to IgG in each. No antibodies to IgA, IgM, IgD, albumin, transferrin or ceruloplasm were produced. Antibodies to IgE were assayed by ISHIZAKA and were found to be absent. Antibodies to completement B_1C) were produced in low titre. The findings indicate that reaginic antibodies can be selectively adsorbed to, and eluted from, immunosorbents and suggest the possibility that some reagins to foods may not belong to the IgE class of immunoglobulins. (SPR)

45 *The Implications of Specific Infectious Susceptibilities in Immune Deficiency States.* VINCENT A. FULGINITI*, Univ. of Colorado Med. Center, Denver, Colo. (introduced by C. Henry Kempe).

Infections in children with deficient cellular immunity differ markedly from those encountered in children with deficient antibody production. Some insight into normal specific defense mechanisms may be gained by consideration of these differences. Fifteen children with deficient cellular immunity associated with immature development of the thymus have been studied. Eight of these had normal immunoglobulin production (thymic dysplasia) and seven very low or absent immunoglobulins (thymic alymphoplasia). The infectious disease patterns in the two groups was almost identical; extreme susceptibility to gram-negative enteric bacilli (13/15), *Candida albicans* (12/15), viruses (9 of 13 adequately studied) and the protozoan, *Pneumocystis carinii* (three definite and three possible). In addition, three of the children had peculiar microabscesses in the liver and GI tract; two in association with a presumed sporozooan. Where applicable, delayed dermal hypersensitivity and *in vitro* lymphocyte stimulation with specific antigens were tested and found absent in this group of children despite intensive exposure to the corresponding infectious agents. In contrast, among five patients with congenital or aquired hypogammaglobulinemia (HGG), infections were predominantly due to pyogenic organisms, principally the *pneumococcus* and *hemophilus influenzae*. All of the children with HGG had intact cellular immunity and underwent viral infections without incident and did not become infected with *Candida albicans*. These observations suggest that cellular immunity functions in defense against *Candida albicans* and some viruses and may play a role in gram-negative enteric and *Pneumocystis carinii* infections. (APS)

46 *Lymphasthenia; Normo-Thymic Lymphoid Dysfunction.* HEINZ J. WITTIG*, JOSEPH R. LANCASTER, JR.* and ENID F. GILBERT*, West Virginia University School of Medicine, Depts. of Pediatrics, Surgery and Pathology, Morgantown, W.Va. (introduced by William G. Klingberg).

A syndrome is described, characterized by the absence of thymus-related lymphocytic functions in the presence of a morphologically normal thymus and a heretofore unclassified type of dysgammaglobulinemia. A 3-year-old boy, with healthy parents and six healthy siblings, was admitted with a history of marked growth retardation, recurrent bronchopneumonia, long-standing *Candida* infections, a chronic anemia, and failure to respond to gamma globulin therapy. He failed to develop skin-responsiveness to dinitro-fluorobenzene after repeated attempts of sensitization, lacked isoagglutinins, and had no typhoid antibodies after repeat immunizations. His platelets were normal. He had normal IgG, markedly elevated IgA and decreased IgM. Intraperitoneal implants of thymic tissue in millipore chambers and intravenous infusions with fetal liver cells failed to restore his immunologic competence. He died suddenly.

Autopsy revealed a grossly and histologically normal thymus, but hypoplasia of Peyer's patches and of appendiceal lymph follicles. The spleen showed few but normal malpighian corpuscles. Thyroid and parathyroid tissues were normal. Thymus and peripheral lymph nodes showed PAS and pyronine positive reticulum cells and occasional plasma cells. The thymic implants were replaced by loose vascularized fibroconnective tissue with small aggregates of lymphocytic elements. Hypoplastic, nonfunctioning peripheral lymphoid tissue in the presence of a morphologically normal thymus suggests cellular unresponsiveness as the basic mechanism for the immunologic defect. (APS)

47 *Cellular Immune Defect: Immunologic Reconstitution by Allogeneic Bone Marrow.* REBECCA H. BUCKLEY*, ZOLTAN J. LUCAS*, BRACK G. HATTLER, JR.*, CHESTER M. ZMIJEWSKI* and D. BERNARD AMOS*, Duke Univ. School of Med., Dept. of Ped., Durham, N.C. (introduced by James B. Sidbury, Jr.).

Defective cellular immunity was detected in a 10-year-old girl with severe chronic mucocutaneous moniliasis by demonstration of: (1) delayed cutaneous anergy to a standard panel of antigens and to repeated applications of DNCB; (2) prolonged homograft survival; and (3) failure of cultured peripheral lymphocytes to incorporate ^{3}H-uridine normally when stimulated by phytohemagglutinin. Intravenous amphotericin B had been given every 5–12 months since the age of 4 years, but skin lesions would return within approximately 2 months of discontinuing therapy. Following a 16-day course of intravenous amphotericin B, we attempted immunologic reconstitution of the patient by the intravenous infusion of 3.42×10^9 white (predominantly bone marrow) cells from her antigenically similar father (determined by histocompatibility testing). Six months following the marrow infusion, the patient's skin remained free of monilial lesions except at the nail-beds; minimal oral thrush was present on the buccal mucosa; positive delayed hypersensitivity reactions could be demonstrated to *C. albicans* and to two other antigens; and her peripheral lymphocytes demonstrated normal blastogenesis *in vitro*. Delayed hypersensitivity was still intact when last tested at 11 months following the marrow transfusion. The patient has remained free of significant moniliasis for 17 months and her height has advanced from the 3rd to greater than

the 10th percentile for age. The clinical improvement observed following allogeneic marrow transfusion in this chronically ill child indicates the potential usefulness of the approach in the treatment of immunologic deficiency disease in the future. (SPR)

48 *Alteration of the C'1q Component of Complement (C') in Agammaglobulinemia (AGG) Syndromes: An Inborn Error Reflected in the C' System.* H. GEWURZ*, R. J. PICKERING*, C. L. CHRISTIAN*, G. NAFF*, R. SNYDERMAN*, S. E. MERGENHAGEN* and R. A. GOOD, Univ. of Minnesota, School of Medicine, Minneapolis, Minn.

Recent studies of the C' system in diseases associated with repeated infections have revealed a selective deficiency of the C'1 component in AGG syndromes. C'1 is a macromolecule which consists of three subcomponents. The first subcomponent to interact with antibody—C'1q—is itself a gamma globulin which, like rheumatoid factor, can agglutinate IGG-sensitized latex particles. Hence, the serum concentration of C'1q g-globulin was determined in the several AGG syndromes. C'1q was assayed by both the immunoprecipitin assay of HANAUER and CHRISTIAN and by adaptation of the agglutination assay of EWALD and SCHUBART. In both series, mean C'1q titers were greatly depressed in lymphopenic AGG of the Swiss type (27 % and < 10 % normal, respectively) and moderately depressed or normal in other forms of AGG (77 % and 46 % normal, respectively). 'Properdin' titers also showed the pattern of marked deficiency in Swiss-type AGG with normal or near-normal values in other forms of AGG. Normal titers of C'4 and C'2, and normal or elevated C'1q titers in six other diseases associated with repeated infections, suggested this represented a deficit of C'1q synthesis. Whether this apparent synthesis deficit is primary with the lymphoid tissues, or follows a regularly-occurring secondary damage to an alternate site such as the intestine, is not yet clear. Our finding normal C'1q levels in the DiGeorge syndrome suggests the latter possibility. In either case, we conclude that the marked reduction of C'1q and 'properdin' can aid in the early detection of Swiss-type AGG. (APS)

49 *Anaphylaxis and Antibody to Aggregated Gamma G Globulin in an Individual With Acquired Hypogammaglobulinemia.* ELLIOT F. ELLIS* and CHRISTOPHER S. HENNEY*, Univ. of Colorado School of Med. Denver, Col. (introduced by William Hathaway).

Anaphylaxis after intramuscular gamma globulin is a rare occurrence. We have investigated such an episode in a 17-year-old male with acquired hypogammaglobulinemia. A unique finding was the presence of an antibody which precipitated with immunological identity to rheumatoid factor (RF) on Ouchterlony analysis using aggregated human gamma globulin (HGG) as antigen. This antibody, however, differed from 'classical' RF in a number of important respects. It reacted neither with native HGG nor with gamma globulins of other species when studied by antigen binding techniques. The majority of the antibody was shown to be of the G species, further distinguishing it from classical RF. No anti-Gm antibodies were demonstrable in the patient's serum. IgE was not detectable by gel diffusion precipitin analysis nor did the patient's serum block an induced Prausnitz-Kustner reaction. The mechanism of anaphylaxis, therefore, did not appear to involve anaphylactic antibody of the IgE species. Two other possible explanations for the reaction could not be excluded: (1) antigen-antibody interaction involving IgG aggregates found in commercial gamma globulin preparations and the IgG antiaggregate antibody found in the patient's serum; (2) inadvertent intravascular administration of aggregate containing commercial gamma globulin with the biologically active aggregates being responsible for the reaction. (SPR)

50 *The Wiskott-Aldrich (W-A) Syndrome, A Defect in Antigen Processing or Recognition?* R. MICHAEL BLAESE*, WARREN STROBER* and THOMAS A. WALDMANN*, National Institutes of Health, Nat. Cancer Inst., Bethesda, Md. (introduced by Paul A. di Sant'Agnese).

Immunological studies were carried out in 11 patients with the W-A syndrome (thrombocytopenia, eczema and recurrent infection). Delayed hypersensitivity responses were absent when tested with PPD, mumps, SKSD, Tricophyton and *Candida* skin tests, and DNCB sensitization despite the fact that the patients had mean circulating lymphocyte levels of 2300/mm^3 and normal *in vitro* lymphocyte transformation to phytohemagglutinin. Measurement of serum immunoglobulins generally showed normal γG, high γA and γD, and low γM levels. Turnover studies with I^{131} γM showed that the low γM levels were due to decreased synthesis. Natural antibodies to blood group antigens and five serotypes of *E. coli* were strikingly diminished. In addition, antibody responses to the polysaccharide antigens, pneumococcal polysaccharide types I and II, blood group substances, and Vi antigen were markedly depressed. It is, therefore, likely that the low γM level is secondary to diminished responses to polysaccharide antigens which normally sustain the γM levels. Poor antibody responses compared to controls were also obtained with a variety of bacterial, viral and protein antigens including polio, diphtheria toxoid, brucella and tularemia vaccines. Both γG and γM antibodies were produced when there was a detectable response. We conclude that the W-A patients have a broad immunological defect involving both humoral and cellular responses. A broad defect of this type, in association with near normal levels of functional lymphocytes and adequate immunoglobulin levels, suggests a disorder in antigen processing or recognition; i.e. a disorder of the afferent limb of immunity. (SPR)

51 *Beta Adrenergic Blockade and Diabetes: Acute Studies and Long-Term Therapeutic Trial.* LESTER BAKER*, ROBERT KAYE, AVNER BARCAI* and NASIR HAQUE*, Children's Hospital of Philadelphia, Pa.

Previous studies demonstrated an increased ketone sensitivity to epinephrine (E) infusion in juvenile diabetics. There did not appear to be a good correlation between the rate of FFA rise and the ketone elevation. To explore these relationships further, acute beta adrenergic blockade was induced in seven diabetic children by I.V. MJ 1999, a sulfonamidophenethanolamine compound. MJ 1999 prevented any rise in glucose, FFA and ketones during E challenge.

Two diabetic girls who required hospitalization every 3–4 weeks for ketoacidosis were further studied. Loss of control appeared to be correlated with emotional upset. Stress interview resulted in a rise of glucose at three times the rate seen in a control interview, and a 10-fold increase in the rate of FFA rise. Urinary E excretion increased 3-fold. Beta adrenergic blockade

prior to stress interview resulted in no rise of glucose or FFA, although urinary E doubled. Trial of long-term oral MJ 1999 in these children has thus far resulted in no hospitalizations for almost 3 months. These results suggest: (1) E may be an important humoral mediator affecting diabetic control during emotional arousal; (2) blockade of the rise in glucose and FFA is possible with MJ 1999, and is effective when challenged by exogenous E or by emotional stress; (3) long-term beta adrenergic blockade therapy of the diabetic who is prone to frequent acidosis shows great promise, and further evaluation is warranted. (SPR)

52 *Studies in Insulin Secretion: A Comparison of Normal Newborns and Infants of Gestational Diabetic Mothers.* ROSITA S. PILDES*, ROBERT HART*, RICHARD WARRNER* and MARVIN CORNBLATH, Univ. of Illinois Coll. of Med. and Cook County Hosp., Chicago, Ill.

Infants of gestational diabetic mothers (IGDM) assimilate intravenous glucose more rapidly than normal newborns; this has been attributed to hyperinsulinism. In the adult, ingested glucose is a more potent stimulus to insulin secretion than intravenous glucose. Therefore, islet cell function was examined in 11 normal and 8 IGDM by means of oral glucose tolerance tests (2 g/kg). All newborns were less than 24 hours old and were fasted for at least 4 hours prior to the test. Changes in peripheral venous blood glucose, plasma immunoreactive insulin (IRI), growth hormone (GH) and free fatty acids (FFA) were measured at 30-minute intervals for 2 hours. In both groups, fasting blood glucose (63 versus 60 mg/100 ml) and GH (68 versus 64 mμg/ml) were the same. In the IGDM, corresponding IRI levels were high (14 ± 2 versus 7 ± 1 μU/ml, $p = <0.05$) and FFA, low (1.27 ± 0.04 versus 1.62 ± 0.15 mEq/l, $p = <0.05$). At ½ hour, IRI levels were significantly higher and glucose, lower in the IGDM. Blood glucose values remained significantly higher in the normals throughout the test. In contrast, IRI values were identical at 1 hour and higher in the normal at 1½ and 2 hours. Total 2-hour increments of IRI were 103 ± 23 in normal and 63 ± 14 μU/ml in IGDM. These observations suggest that the improved glucose tolerance characterizing IGDM may be attributed to an augmented basal insulin secretion and a brisk response to a glycemic stimulus, whereas the diminished tolerance in the normal newborns is associated with a delayed insulin release similar to that of the adult onset diabetic. The fall (20 %) in FFA was similar in both and changes in GH were variable. (SPR)

53 *The Influence of Microorganisms upon Intestinal Absorption of Carbohydrates in Premature Germfree Swine.* JACK W. LUKEMEYER* and JAMES J. SCHAFFER*, Dept. of Ped., Indiana Univ. School of Med., Indianapolis, Ind. (introduced by Morris Green).

Over 150 germfree piglets derived by hysterotomy from specific-pathogen-free Minnesota 3 sows, 3 to 5 days prior to term, have been studied. Littermates were randomly distributed among several $24'' \times 24'' \times 60''$ plastic isolators maintained in the same room regulated at 93F. Tolerance tests, involving mono- and disaccharides, cow's milk, or infant formulae, were conducted in piglets of various ages which had received: (1) no organisms, (2) selected genera of viable organisms, or (3) nonviable organisms. Animals were monitored for reducing substances in the blood by means of an autoanalyzer connected to an umbilical catheter in the piglet through pickup tubing mounted in walls of the isolators. Blood glucose (glucose oxidase), osmolar, and electrolyte values were determined routinely. In general, tolerance curves for germfree piglets were of the prediabetic type. Osmolality and electrolyte data paralleled total reducing and not glucose oxidase values. Although both types were effective, live organisms were more efficacious than killed organisms for inducing a shift from abnormal to normal patterns of absorption and utilization. Both the rate and magnitude of the 'inductive shift' were dependent upon the genus and number of organisms introduced, the test substance employed, and the age of the animal. Among 10 genera of bacteria tested, *Escherichia*, *Lactobacillus* and *Candida* were the most efficient in favorably modifying absorptive phenomena. Studies are in progress to evaluate the influence of selected microorganisms on carbohydrate absorption and utilization in premature infants.

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54 *Evidence that Acrodermatitis Enteropathica (AE) is a Disease of Fatty Acid Metabolism.* RALPH CASH* and C. K. BERGER*, Children's Hospital of Michigan and Sinai Hospital, Detroit, Mich. (introduced by Charles F. Whitten).

AE is a rare disease of infancy of undetermined etiology with distinctive epidermal lesions, malabsorption and growth failure. Because the clinical state resembles that of fat-deficient puppies, serum fatty acids were analyzed in a critically ill 3-month-old male with AE Arachidonic, an essential fatty acid (EFA) was deficient, 1.4 % of the TFA (N: 5–12), while its precursor, linoleic, was present in excess, 19.1 %. The serum also contained saturated and unsaturated fatty acids (tentatively identified) of chain length 18, 20, 22 and 24, not normally found in human serum. Oral arachidonate, while raising the level of serum arachidonate, failed to elicit a clinical or biochemical change. I.V. fat elicited a dramatic clinical response (suggesting an absorptive defect), with the disappearance of the 'abnormal' fatty acids, with the exception of a C20 with five double bonds, an acid capable of correcting the dermal lesions of EFA-deficiency in animals. The fatty acid derangement has been confirmed in an adult with AE, in whom clinically successful Diodoquin therapy caused lessening or disappearance of the 'abnormal' fatty acids. These observations suggest an explanation for the efficacy of human milk in AE, in that certain fatty acids found in cow's milk inhibit the synthesis of arachidonic acid. Our data indicates that AE is associated with distinctive alterations of the serum fatty acids, particularly the EFA. This is the first time that defective interconversion of the polyunsaturated fatty acids has been demonstrated in humans. (SPR)

55 *Maternal Phenylketonuria: Implications on Growth and Development.* WILLIAM K. FRANKENBURG* and BURRIS DUNCAN*, University of Colorado Medical Center, Denver Col. (introduced by Henry K. Silver).

Eight non-PKU offspring of phenylketonuric mothers are presented. All of the offspring were mentally subnormal and had DQ/IQ scores lower than their mothers. A review of reported 'normal' offspring of phenylketonuric mothers reveals that of 63 non-PKU offspring of phenylketonuric mothers (i.e., children

born to women having documented phenylalanine levels above 20 mg/100 ml with urine specimens giving a positive ferric chloride reaction), only one had a measured IQ score above 90. Of the eight non-PKU offspring (who are assumed to be heterozygous for PKU) given an oral phenylalanine loading test, five had phenylalanine-tyrosine ratios in the normal nonheterozygous range. It therefore appears that the one-hour phenylalanine-tyrosine ratio fails to discriminate between the normal nonheterozygote and the individual heterozygous for PKU. Growth data from the above cases and 13 additional non-PKU offspring demonstrated uniform intrauterine and postnatal growth retardation. The cases reported to date emphasize the importance of ruling out maternal phenylketonuria in all families with 'familial mental retardation' or 'familial microcephaly'. (APS)

56 *K.K.: A New Red Cell Membrane Defect.* WILLIAM KRIVIT and JAMES T. LOWMAN*, University of Minnesota, Minneapolis, Minn.

Red cell membrane defects are present in acanthocytosis, paroxysmal nocturnal hemoglobinuria and spherocytosis. Our patient, K.K. a 5-year-old Caucasian lad, has a different type of membrane defect. Severe hemolysis and splenomegaly noted in first month of life has required 40 admissions for transfusions despite multiple therapy and splenectomy. Marked peripheral blood (7–10/100 white cells) and marrow normoblastosis (70 %) continues with minimal reticulocytosis. The following were all within normal limits: osmotic fragility, acid hemolysin, hemoglobin determination on cellulose acetate, paper and starch gel, nonhemoglobin protein electrophoresis on starch gel, complete glycolytic and hexose monophosphate shunt enzymes, glucose consumption and glucose 2-C^{14} utilization and intermediate carbohydrate metabolites, urinary and bone marrow porphyrin studies, Coombs tests direct and indirect. The intracorpuscular defect was clearly indicated by Cr^{51} half-life survival of 6 days of his cells in normal recipient and converse time of 18 days. Abnormal autohemolysis (10–15 %) was corrected by glucose, ATP, inosine but not heparin. In aerobic condition, this correction was not equivalent to anaerobic tests. Mechanical fragility was twice normal. The phospholipid percentage partition of red cell membrane was abnormal as follows:

Phospho-glyceride	Choline	Ethanol-amine (PE)	Serine	Sphingo-myelin
Normal	29.2±1.5	27.5±1.5	14.8±1.7	25.4±1.4
K.K.	35.1–37.8	23.6–24.6	9.2–10.7	23.9–25.2
After incubation	37.8–39.4	12.7–17.2	4.6– 6.0	30.9–36.0

The marked loss of PE on incubation indicates an increase in susceptibility to oxidation. Conceptually, a lack of appropriate antioxident or inherent susceptibility of lipids to normal peroxidation may be the mechanism involved in this new entity. (APS)

57 *Hypermethioninemia and Elevation of Other Free Amino Acids in Infants on High Protein Intakes.* HARVEY L. LEVY*, PHYLLIS M. MADIGAN*, ROBERT A. MACCREADY* and JOHN D. CRAWFORD, Massachusetts General Hosp. and Harvard Med. School, Boston, Mass.

Markedly elevated levels of methionine in blood have been observed in homocystinuria and in various forms of hepatic disease such as that seen in tyrosinosis, galactosemia or neonatal hepatitis. These methionine elevations are relatively constant, are present with normal protein intake and cannot be permanently abrogated by only a reduction in dietary methionine. In the course of blood amino acid screening of large numbers of young infants, marked methionine elevations have been detected in 18, aged 5–9 weeks, each of whom was ingesting at least 7 grams of protein per kilogram per day. In each instance there was no evidence of either liver disease or of an inborn error of metabolism. Plasma methionine as measured by ion exchange chromatography ranged from 4.7 to 18 mg % (upper limit of normal 0.6 mg %), while most of the other free amino acid levels were elevated to at least twice the normal values. Of the latter, tyrosine was frequently the most strikingly elevated. Blood urea nitrogen levels ranged from 22 to 32 mg % (upper limit of normal 21 mg %) and serum alkaline phosphatase levels ranged from 19.5 to 28.2 Bodansky units (upper limit of normal 14 BU). The urine amino acid pattern was that of a moderate generalized aminoaciduria. In each subject, reduction of protein intake to approximately 3 g/kg/day resulted in a return of all values to normal. Several mothers reported lessened vomiting and reduced irritability in the infants. In one subject, reinstitution of the high protein intake reproduced the amino acid abnormalities which again reverted to normal upon the ingestion of lower amounts of protein. (APS)

58 *Glycogen Metabolism in Erythrocytes of Glycogen Storage Disease.* SHIMON W. MOSES*, REUBEN CHAYOTH*, STANLEY LEVIN*, ELA LAZAROVITZ* and DAVID RUBINSTEIN*. Dept. of Ped. Research, Kaplan Hosp. Rehovath and Central Negev Hosp., Beer-Sheva, Israel (introduced by Norman Kretchmer).

Evidence of active glycogen metabolism is being presented in both normal and glycogen-rich erythrocytes taken from patients with type III glycogen storage disease. Activity of all enzymes catalyzing the reactions required for glycogen synthesis and degradation have been demonstrated in mature erythrocytes. Using cells from patients with type III glycogen storage disease, it was demonstrated that the glucosyl units are first incorporated in the outer tiers, then transferred to the core where they tend to accumulate due to the absence of amylo-1,6-glucosidase. The glycogen-rich cells have a higher rate of glucose utilization which is not reflected by an increased lactate production. The rate of ^{14}CO production from glucose-1-^{14}C during incubation was increased in the abnormal cells when methylene blue was added.

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59 *Studies of the Control of Renal Gluconeogenesis.* JULIAN IRIAS* and ROBERT E. GREENBERG, Dept. of Pediatrics, Stanford Univ. School of Medicine, Palo Alto, Cal.

Renal gluconeogenesis has recently been related to certain aspects of renal function. Several approaches have been used in the present study to elucidate control mechanisms. Rates of gluconeogenesis in rat renal cortex slices were determined by measuring glucose release into incubation media under aerobic conditions.

Role of calcium ion: Gluconeogenic rates are increased 2–3 times by Ca^{++} (initially reported by KREBS *et al.*: Biochem. J. *86:* 22 [1963]). Glucose production increases linearly with $[Ca^{++}]$ until a maximum is reached at 5 mEq/l, decreasing at higher $[Ca^{++}]$. At a normal serum concentration of ionized Ca^{++}, 2.5 mEq/l, an intermediate rate is observed, suggesting that the calcium effect may represent a regulatory mechanism. If slices are incubated in a calcium-free medium for 30 min and calcium is then added to a concentration of 5 mEq/l, a maximal gluconeogenic rate is obtained without lag. The calcium effect is demonstrable when either pyruvate of dihydroxyacetone is used as substrate, implying a site of effect above the level of triose phosphate.

Developmental analysis: With pyruvate as substrate, minimal glucose was produced by slices from newborn animals; by four days of age, the rate was half that of slices from adult rats. By contrast, dihydroxyacetone supported gluconeogenesis equally well in renal cortex from newborn and adult rats, suggesting that the step limiting development of gluconeogenesis is below the level of triose phosphate. The activity of phosphoenolpyruvate carboxykinase increases at a much faster rate than that of other gluconeogenic enzymes in kidney during the first four days after birth. (SPR)

60 *Salicylate Intoxication—Treatment with Potassium Citrate.* WILLIAM T. DOBBINS*, Children's Hospital, Denver, Col. (introduced by Alan K. Done).

The primary treatment of salicylate intoxication consists of the rapid removal of salicylate from the body. This is best accomplished by the diuresis of an alkaline urine. Use of sodium bicarbonate as advocated by OLIVER, WHITTEN and others has not been entirely successful since many patients with severe acute and chronic salicylate intoxication have shown a resistance to alkalization of the urine. In addition, the administration of large amounts of sodium bicarbonate has produced complications of hypernatremia, alkalosis, tetany and hypokalemia. Based on the premise that the resistance to alkalization was due to a relative potassium deficiency, a study was undertaken to determine the effectiveness of large amounts of oral potassium citrate in promoting the production of an alkaline urine in children with salicylate intoxication. Children ranging in age from 3 months to 8 years with severe acute, and chronic salicylate intoxication were treated with various fluid regimens, with and without potassium citrate. The combination of: (1) I.V. fluids (44 mEq $NaHCO_3$, 35 mEq KCl in 1000 ml 5 % glucose) 2000 ml/M^2/8 h; (2) $NaHCO_3$–3 mEq/kg by push and 1.5 mEq/kg q15 min × 3 if needed to obtain an alkaline urine; and (3) potassium citrate, orally or by nasogastric drip, 200 mEq/M^2/8 h produced the best results, namely: (1) All patients had a rapid diuresis of an alkaline urine. (2) Blood levels of salicylate decreased an average of 70 % in 8 h. (3) Salicylate clearance averaged 20 mg/kg/h. (4) None of the patients developed complications. (SPR)

61 *Hepatic Failure in 8 Cases of Reye's Syndrome.* ALLEN D. SCHWARTZ*, PETER R. HUTTENLOCHER* and GERALD KLATSKIN*, Depts. of Ped. and Med., Yale-New Haven Hospital, New Haven, Conn. (introduced by Charles D. Cook).

Between December 1965 and January 1968, the authors have observed eight patients with acute encephalopathy and hepatic dysfunction, a syndrome consistent with that described by REYE *et al.* in 1963. All presented in winter months; ages ranged from 3 ½ months to 12 years. All had a history of recent mild illness; two had chicken pox and six had mild upper respiratory infections. They began to have recurrent vomiting followed by rapid neurological deterioration with delirium progresing to coma, rigidity and decerebrate posturing. None had icterus but hepatic enlargement was noted in seven. Five were noted to have abnormalities in respiration and a respiratory alkalosis was demonstrated in the three patients who had blood gas determinations. Spinal fluid cell and protein determinations were normal. All eight patients had laboratory evidence of hepatic damage with elevated serum glutamic oxaloacetic transaminase levels. Only two had blood glucose levels under 40 mg %. Blood ammonia levels were obtained in the last three patients and were markedly elevated with values as high as 1022 μg % (normal 50–150). Prothrombin time was done in six patients and was 50 % or less in five. One child had rising antibody titers to influenza A_2. There were three deaths and one living child is severely neurologically damaged. The remaining children have recovered fully. The last three patients, two of whom died, were treated with cleansing enemas, neomycin sulfate orally and by enema, steroids and repeated exchange transfusions. These cases demonstrate that severe hepatic failure may occur in Reye's syndrome and they suggest that ammonia intoxication may contribute to the neurologic impairment. However, irreversible cerebral injury occurs very rapidly and it is as yet unknown whether vigorous treatment of the hepatic failure can improve the survival. (APS)

62 *Spongy Degeneration of the Brain (Von Bogaert-Bertrand Type)—Probable Generalized Metabolic Disorder.* W. MELLMAN, A. TENENHOUSE* and P. GIAMBETTI*, Depts. of Pediatrics, Biochemistry and Neurology, Univ. of Pennsylvania School of Med., Philadelphia, Pa.

Two siblings have been diagnosed as having spongiform degeneration of the brain. One is a 7-year-old boy with spastic quadriplegia, macrocephaly and optic atrophy. His brain and muscle biopsies were examined by both light and electron microscopy. The brain reveals the atypical astrocytic mitochondria described in this disease. The muscle shows atrophy of a neurogenic type.

The younger sibling now 13 months has had a presumptive diagnosis of this disorder. At 10 weeks she lacked head control and was hypotonic. A muscle biopsy at this time, while on a 300 mg calcium intake, contained 4–5 × control levels of calcium (960 μg/g). At 5 months she still lacked head control and she was placed on a 40 mg calcium intake. Two weeks later she had head control, improved muscle tone, and social responsiveness. This clinical improvement lasted for only about 3 weeks, despite continuation of the diet. She then lost head control and has made no progress since then; she has neither spasticity nor macrocephaly at 13 months.

The calcium content of her muscle 3 weeks after starting the low calcium intake was 296 μg/g, at 8 months was 191, and at 12 months was 93. The serum calcium was always within the normal range. At one year she showed bone demineralization and low serum phosphate. At this she was returned to a normal calcium intake, and 2 weeks later her muscle calcium was 763.

This disease appears to be associated with altered evels on nonionized intracellular calcium. (SPR)

63 *The Neuropathy of Krabbe's Diffuse Sclerosis (Globoid Cell Leukodystrophy).* H. G. Dunn*, B. D. Lake*, C. L. Dolman* and J. Wilson* Department of Paediatrics, University of British Columbia, Canada, and Hospital for Sick Children, Great Ormond Street, London, England (introduced by S. Israels).

In contrast to metachromatic leukodystrophy (sulfatide lipidosis), the Krabbe type of diffuse sclerosis has not been generally recognized as being associated with a peripheral neuropathy. In the present study, six infants with Krabbe's disease were examined in this respect. Tendon reflexes were depressed in five of these six cases and gradually lost in two. Nerve conduction studies in three children repeatedly showed marked reduction of conduction velocity in motor fibres of large limb nerves; conduction in sensory fibres was also tested in two patients and found to be impaired. Biopsy of a sural nerve in two children and of a scalp nerve in another demonstrated demyelination when myelin stains, acid phosphatase preparations or electron microscopy were used. At autopsy, all six cases showed evidence of demyelination in peripheral nerves, and teased nerve preparations in two cases demonstrated that this demyelination was segmental in type. It is concluded that a peripheral neuropathy is usually present in Krabbe's disease. Peripheral nerve biopsy, usefully preceded by nerve conduction studies, may be of value in the diagnosis of this leukodystrophy. (APS)

64 *Spasmus Nutans and Congenital Nystagmus: Nosologically Separate?* P. Jayalakshmi*, T. F. McNair Scott, S. H. Tucker* and D. Schaffer*, Children's Hospital of Philadelphia, School of Med. Univ. of Pennsylvania, Philadelphia, Pa.

Infantile nystagmus (NYS) of unknown etiology may be variously diagnosed as spasmus nutans (SN), congenital nystagmus (CN), or of unknown type (NUK). To test the nosological validity of this distinction, prospective data were reviewed from birth to 8 years on a total (T) of 52 infants with NYS and 56 controls (C), from within NIH Collaborative C. P. Project (Philadelphia population—7,000 [POP]), regardless of previous diagnoses. Of these, 30 were SN [sub group (SGr) of 28 studied in greater detail], 4CN and 18 NUK. *Results.* I. Incidence of NYS higher ($p = 0.01$) in (a) Negroes (96% T; 97.5% SGr; 87% POP); (b) multigravida 3, (75% T; 71% SGr; 5.5% POP). II. The following were higher ($p = 0.01$) in T and SGr than C (1) family history (FH) of (a) NYS (10/44 T; 7/25 SGr; 1/56 C), (b) neuropsychiatric disease (NPD), (23/46 T; 14/25 SGr; 15/56 C); (2) presence in child of (a) strabismus (S), (25/52 T; 11/28 SGr; 3/56 C), (b) other neurological abnormality (NA), (17/52 T; 9/28 SGr; 1/56 C). III. Prognosis as to persistance of NYS 5 years of age in both T and SGr not significantly altered ($p = 0.1$) in regard to FH of NYS, FH of NPD, presence of S or NA, onset at birth or later. IV. In SGr, the following precipitating events were insignificant ($p = 0.1$). (1) Previous or present obstetrical complications; (2) neonatal course; (3) prematurity; (4) illnesses; (5) heat trauma. In addition, disturbed maternal child relationship (psychologist reported 4/28 SGr; 7/48 C; $p = 0.1$) and poor lighting (judged adequate on inspection for 23 of 28 children) were not operative. *Conclusion.* Regardless of initial diagnosis, SN, CN and NUK could not be differentiated by parameters studied. SN was not associated with above possible antecedents.

(Supported by USPHS grant PH 43-68-4.) (APS)

65 *The Effect of Nerve Growth Factor (NGF) on the Synthesis of a Neuronal Lipid.* Michael Graves* and Guy M. McKhann, Department of Pediatrics, Stanford University School of Medicine, Palo Alto, Cal.

Nerve growth factor (NGF) is a protein which promotes growth and differentiation of two immature nerve cell types, sensory and sympathetic nerve cells. Previous studies have emphasized that the effect of NGF is to increase anabolic processes, with a stimulation of RNA synthesis the primary event. In the present study, we have focused on the effect of NGF on the synthesis of gangliosides, a group of glycolipids which are components of neurones, particularly of synaptic membranes.

The assay system is the incorporation of 1-C^{14}-glucoseamine into gangliosides *in vitro* by the dorsal root ganglion of the 8-day chicken embryo. A concentration of NGF is used which promotes nerve fiber outgrowth by this tissue *in vitro*. Gangliosides are separated by solvent partitioning, with final identification by thin layer chromatography. Over an 8-hour incubation period, NGF promotes a 50% increase of incorporation of C^{14}-glucoseamine into gangliosides. In contrast, insulin has anabolic effects on hexose and nucleotide metabolism by ganglia but promotes neither nerve fiber outgrowth nor ganglioside synthesis.

This experimental system may be useful for the study of the relation between a growth-promoting substance with tissue specificity and the metabolism of a membrane lipid localized within that same tissue. (SPR)

66 *Abnormal Phospholipid Composition and Synthesis in PMN Leucocytes in Downs' Syndrome.* E. E. McCoy and J. L. Nance, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Va.

A shortened half-life and increased activity of multiple enzymes has been noted in PMN leucocytes of Downs' syndrome (DS). As phospholipids are important in cell structure, lipid composition and synthesis was studied in isolated PMN leucocytes from male DS patients, age 14 to 25 years, and normal young men, age 20 to 25 years. Isolated PMNs were incubated in acetate-C^{14}, lipid extracted then separated into classes and phospholipids into 5 fractions by thin layer chromatography. The total lipid content determined chemically was similar and the amount of cholesterol and triglycerides was similar in the two groups. The total amount of cholesterol ester and of phospholipid was significantly less in DS subjects ($p < 0.025$). There was significantly less phosphatidyl choline (PC), lysolecithin (LPC) and sphingomyelin (Sph) in DS PMN but similar amount of phosphatidyl serine (PS) and of phosphatidyl ethanolamine (PE). The incorporation of acetate-C^{14} into total lipid, cholesterol and cholesterol ester was similar. Incorporation by DS was markedly greater into triglycerides ($p < 0.001$) but significantly less into the phospholipid fraction ($p < 0.005$). Acetate-C^{14} incorporation into PS was less ($p < 0.005$), and into PE greater ($p < 0.025$) by DS, but similar for PC, LPC and Sph for both groups. The results show that lipid composition and synthesis in PMN of male DS patients differs from that of normal men. As phospholipids have important roles in cell function and

structure, the abnormalities of PC, LPC, PS, PE and Sph metabolism in DS may provide an explanation for a shortened half-life and increases of multi-enzyme activities previously noted in PMN leucocytes of these patients. (SPR)

67 *Fatty Acid Synthesis in the Developing Brain.* ERNESTO AEBERHARDT* and JOHN H. MENKES, UCLA School of Medicine, Los Angeles, Cal.

Mitochondria and microsomes prepared from mature rat brain incorporated malonyl-Co A and acetyl-Co A in a variety of saturated and unsaturated fatty acids by a chain elongation mechanism. Malonyl-Co A was the precursor for fatty acid biosynthesis in the microsomal system, while both malonyl- and acetyl-Co A were active in mitochondria. Microsomes incorporated malonyl-Co A into saturated fatty acids (18:0), and polyunsaturated fatty acids (22:4, 22:6). The mitochondrial acetyl-Co A system synthesized mainly monounsaturated fatty acids (20:1), with lesser amounts of saturated and polyunsaturated fatty acids.

In the developing rat the total amount of fatty acid synthesis by either system was maximal at 13 to 15 days, a time of rapid myelination. Synthesis of saturated fatty acids, mainly 18:0, by the microsomal system followed a similar development pattern, while mono- and polyunsaturated fatty acid synthesis in the microsomal and mitochondrial system did not change with maturation.

The effects of estrogens on fatty acid synthesis in mature and immature rat brain will also be presented. (SPR)

68 *Postnatal Alterations in Brain Protein Metabolism.* RICHARD J. SCHAIN, MICHAEL J. CARVER* and JOHN H. COPENHAVER*, UCLA School of Med., Dept. of Ped., Los Angeles, Cal., and Univ. of Nebraska Coll. of Med., Dept. of Neurol. and Psychiat., Omaha, Neb.

Protein turnover, as determined by incorporation of labelled amino acids into protein fractions, is more rapid in the immature nervous system than in the adult male. The present studies were undertaken to investigate these changes in the newborn miniature pig. The piglets were procured by hysterectomy at differing gestational ages or following spontaneous delivery. Tracer doses of L-($U^{14}C$) phenylalanine were administered intraperitoneally during the first few days of life. Animals were sacrificed one hour after administration of the isotope. TCA insoluble (protein) and soluble (free amino acid) fractions were prepared from liver, cerebral cortex, pons-medulla and cerebellum. The relative order of activity in the areas examined was constant regardless of age. The activity in liver protein was 4–5 fold greater than in brain areas. Incorporation of the labelled amino acid into brain protein fell 50 % or more within the first 24 hours in the spontaneously delivered piglets. Expression of the radioactivity in brain protein as a fraction of activity in liver protein revealed, while there was some decrease in incorporation into liver protein postnatally, there was a considerably greater decrease in the various brain regional areas. Premature delivery of the piglets resulted in a similar but more abrupt decrease in labelling of protein in all regional brain areas. These data suggest that a rapid decrease in brain protein turnover occurs shortly after birth unrelated to gestational age. The occurrence of a similar phenomenon in prematurely born human infants might be expected to significantly affect CNS development. (SPR)

69 *Dwarfism and Mental Retardation: the Serum Growth Hormone Response to Hypoglycemia.* S. DOUGLAS FRASIER* and FRED G. SMITH, JR., Pacific State Hosp., Pomona, Cal., Univ. of South Calif. School of Med., Los Angeles County General Hosp. and Univ. of Calif. School of Med., Los Angeles, Cal.

An association between mental deficiency and growth retardation is well recognized. In order to evaluate the possible role of abnormalities in growth hormone (GH) secretion in dwarfism associated with mental retardation, we have investigated the serum GH response to hypoglycemia in mentally retarded children. Thirty-one institutionalized test patients (height 2–6 SD below the mean for age), 15 institutionalized control subjects (height $\pm$ 2 SD from the mean for age) and a noninstitutionalized control group of 20 endocrinologically and intellectually normal children were studied. Serum samples were obtained 0, 15, 30, 60, 90 and 120 min after the i.v. administration of crystalline insulin (0.05–0.1 units/kg). Serum GH was measured by radioimmunoassay. The mean peak serum GH concentration after the induction of hypoglycemia was 26.6 mμg/ml (95 % CL 1.8–70.7 mμg/ml) in institutionalized and 18.9 mμg/ml (95 % CL 4.5–38.3 mμg/ml) in noninstitutionalized controls. The mean peak serum GH concentration was not significantly different in these two groups. The data were pooled, giving a mean peak serum GH concentration of 22.2 mμg/ml (95 % CL 3.1–52.9 mμg/ml) for control subjects. The mean peak serum GH concentration in test patients was 18.5 mμg/ml (95 % CL 1.0–50.0 mμg/ml) and did not differ significantly from that of control subjects. However, the peak serum GH concentration was <5 mμg/ml in 7 test patients (22.5 %) while the peak serum GH concentration exceeded this level in all control subjects. This difference is significant ($\chi^2 = 6.75$; $p = <0.01$). These results indicate that abnormalities in GH secretion occur commonly in dwarfism associated with mental retardation and may contribute to the growth failure observed in these patients. (SPR)

70 *A longitudinal Study of EEG and Auditory Evoked Responses During Sleep in Low Birth Weight Infants.* ELLIOT D. WEITZMAN* and LEONARD J. GRAZIANI*, Depts. of Neurol. and Ped. and Rose F. Kennedy Center, Albert Einstein Coll. of Med., New York, N.Y. (introduced by Harry H. Gordon).

Brain maturation was evaluated by a study of EEG patterns, auditory evoked responses and activity behavior during sleep in 25 low-birth-weight infants with gestational ages of 23 to 39 weeks. Algebraic summed evoked responses to clicks were recorded from the scalp EEG by a computor technique. Sequential recordings and behavioral observations were obtained at 2-week intervals until discharge from nursery and then one month later. The less mature infants spent more time in an active sleep state. As the infants matured, periods of quiet sleep occurred for longer durations and interrupted the active sleep phase more frequently. The EEG pattern of infants 23 to 26 weeks post conception age consisted almost entirely of an 'intermittent' pattern. As maturation progressed, this 'intermittent' pattern became more closely associated with periods of quiet sleep. A 'continuous' EEG pattern, appearing at approximately 29 to 31 weeks post conception, was increasingly associated with an active sleep phase. By 34 to 35 weeks the correspondence between EEG pattern

and sleep state was more constant. The first negative and the second positive wave of the evoked response had a significantly longer latency during the 'intermittent' (quiet sleep) compared to the 'continuous' (active sleep) EEG pattern in infants 34 to 44 weeks of age post conception. Wave latencies decreased with maturity of the infant and the evoked response complexity was related to both age post conception and the scalp electrode placement site. (SPR)

71 *Experimental Congenital Adrenal Cortical Hyperplasia: Existence of Vulnerable Primordium of 3β-Hydroxysteroid Dehydrogenase in the Rat Blastula.* ALLEN S. GOLDMAN, Children's Hosp. of Philadelphia, Pa.

Most teratogens are ineffective when given to the pregnant rat before the eighth day of gestation, during the development of the three germ layers. The production of a model of congenital adrenal hyperplasia due to a genetic deficiency of 3β-hydroxysteroid dehydrogenase by a C-19 substrate analog (2α-cyano, 4,4,17α-trimethyl-17β-hydroxyandrost-5-en-3-one) is due to its selective inhibition of this fetal enzyme. At term, fetuses of rats injected with analog on days 5, 6 (time of implantation of the early blastula), 7 and 8 have a highly significant degree of adrenal hyperplasia, clitoral hypertrophy, and hypospadias, and virtually no histochemical activity of the dehydrogenase in the fetal adrenal or Leydig cells. Adrenal weight per g fetal weight was significantly larger in fetuses of mothers treated prior to mating, since their birth weights were reduced. These fetuses also had a slight but significant degree of hypospadias and reduced activity of dehydrogenase. These observations indicate that analog produces the same syndrome and defect of dehydrogenase when administered considerably before either development of activity of dehydrogenase, or of differentiation of cells containing enzyme. Since it is unlikely that these effects can be explained by persistence of free analog in mother or fetus, evidence is provided for the existence and vulnerability of a molecular primordium of an enzyme in a mammalian egg or blastula. (SPR)

72 *Hybridization of Cultured Cells with Normal and Increased Folate Reductase.* JOHN W. LITTLEFIELD, Ped. Genetics Unit, Massachusetts General Hospital, Boston, Mass.

Extending earlier work by FISCHER and by HAKALA, we have selected in a step-wise clonal fashion a number of presumably pre-existing variant hamster cells with 100- to 10,000-fold increased resistance to aminopterin due to 10- to 100-fold increased folate reductase activity. The latter probably reflects an increased amount of enzyme, judging from the experience of HAKALA with another cell line, since there is no evidence for an inhibitor or activator nor any change in the sensitivity of the enzyme to heat or aminopterin. The increased amount fo enzyme is probably due to accelerated synthesis because there is essentially no change in the stability of the enzyme *in vitro* or *in vivo*.

In bacteria increased enzyme synthesis can be explained by loss of a repressor ('regulator-constitutive' mutants) or the ability to be repressed ('operator-constitutive' mutants). Since the former should be recessive and the latter codominant, several of the variant cells have been hybridized with others containing a normal amount of reductase. For this purpose, the high reductase marker was introduced into sublines lacking either thymidine kinase or inosinic acid pyrophosphorylase. In five experiments, almost all of the hybrid cells contained an intermediate reductase activity. Therefore, the variant cells may represent 'operator-constitutive' mutants; alternatively the synthesis of reductase may not be controlled by a diffusible repressor in mammalian cells. (SPR)

73 *A Bacterial Model System for Study of a New Mechanism for Mutation.* CHARLES R. ROE*, Duke Univ., Durham, N.C. (introduced by James B. Sidbury, Jr.).

An incisive test for genetic relatedness in microorganisms is the guaninecytosine (GC) content of DNA and hybridization of single-stranded DNA from different species. An irrefutable law of kinship in microorganisms is that the DNA GC content is constant for each species. A mutational event has been witnessed that conflicts with the hypothesis requiring a fixed GC content. A mutant organism obtained from a culture of *Pseudomonas testosteroni* has been studied which has a higher GC content than its wild-type. Electrophoretic studies of numerous enzymes as well as the total protein reveal all to be more acidic. Amino acid analyses reveal remarkable changes in per cent composition of many amino acids but no deletions. This organism grown under certain conditions reverts back to organisms which in all categories fall into the *P. testosteroni* group. A mechanism based on a defective DNA polymerase having altered base selectivity has fulfilled all criteria of change in the mutant organism. Reports of transitions and transversions in protein synthesis are becoming more frequent. These reports are related to single proteins while the present hypothesis suggests ordered nucleotide substitution throughout the DNA. An extensive mutation of this nature would result in three categories of organisms all having GC content displaced from the normal species value: (1) potentially lethal mutations in which several amino acids could not be utilized in protein synthesis; (2) 'normal' individuals in which all proteins would be normal in amino acid content and sequence; and (3) individuals in which all proteins would be affected and marked phenotypic changes would be present. The latter category may well have application in developmental abberations involving overwhelming phenotypic changes. (SPR)

74 *Inhibition of 3β-Hydroxysteroid Dehydrogenase (3β-HSD): Changes in Adrenal Mitochondrial Protein Synthesis which are not Effects of ACTH.* SALVADOR CASTELLS* and EDWIN D. BRANSOME, JR.*, Unit of Experimental Medicine, Dept. of Nutrition and Food Science, Massachusetts Inst. of Technology, Cambridge, Mass. (introduced by Ira K. Brandt).

Guinea pigs were treated for 4 days with a synthetic steroid inhibitor of 3β-HSD, 2α-cyano-4,4,17α-trimethyl-androst-5-en-17β-ol-3-one (CK), or depot ACTH for 4 days to 2 weeks. There were a number of differences in effect on adrenal protein metabolism. Mitochondrial proteins were of particular interest because they include steroidogenic enzymes remote from the microsomal locus of the inhibition by CK of 3β-HSD action. The adrenals were exposed to L-leucine-3H for 4 hours *in vivo*. Crude mitochondrial pellets were obtained by centrifugation at 15,000 g for 10 minutes of an 800 g cytoplasmic supernatant. Adrenal hypertrophy, as measured by adrenal weight, was more pro-

nounced in the CK-treated animals than in ACTH-treated. The specific radioactivity of mitochondrial protein was greater in the inhibited animals than in either chronic ACTH-treated or normals. When mitochondrial proteins were fractionated by acrylamide gel electrophoresis, it was evident that the inhibition of 3β-HSD led to increased labeling of some proteins and not of others. ACTH failed to reproduce such effects. In another series of experiments, endogenous ACTH production and the effect of CK on adrenal enlargement were suppressed by administration of dexamethasone, but many of the effects of CK on protein metabolism remained. These results suggest that inhibition of one of the enzymes involved in steroidogenesis may by itself cause changes in protein synthesis which cannot be completely explained by the stimulatory effect of ACTH. Thus, in adrenogenital syndromes, not only increased ACTH production, but intracellular controls of enzyme protein synthesis may also play a role in adrenocortical growth and steroidogenesis. (SPR)

75 *Changes in Bilirubin UDP-Glucuronyl Transferase During Postnatal Development.* JOSEPH KRASNER* and SUMNER J. YAFFE, Dept. of Ped., School of Med., State Univ. of New York at Buffalo, N.Y.

Activity of the enzyme, bilirubin UDP-glucuronyl transferase, is low at birth and reaches a peak (2 to 3 times greater than adult values) at 14 days of age. *In vitro* assays were carried out using hepatic microsomal preparations derived from mice of different ages. Kinetic studies under the same assay conditions showed marked differences between microsomes derived from adult and 14-day-old animals. Michaelis-Menten constants (Km), a characteristic of enzyme-substrate affinity, were $\sim 6 \times 10^{-5}$M and $\sim 2 \times 10^{-3}$M respectively at these two ages. Preparations derived from adult females had enzymic activity twice that of the male, but the Km values were the same. After dialysis of the microsomal pellet against EDTA and addition of deoxycholate, separation of the solubilized enzyme was carried out on a Sephadex G-200 column. This treatment resulted in 60–70 % solubilization of the activity contained in the original microsomal pellet. Striking changes were noted in the relative substrate affinity of the solubilized enzyme, derived from adult animals, with Km increasing to $\sim 2 \times 10^{-3}$M. In contrast, solubilization did not modify the Km value in preparations from 14-day-old animals. These findings indicate that different forms of the enzyme exist at the several stages of development studied. Alterations produced by solubilization procedures suggest a common structural form which changes its conformation during maturation. (SPR)

76 *Synchronous Changes in Activity of Pyrimidine Biosynthetic Enzymes During Embryogenesis.* ALBERTO GALOFRE* and NORMAN KRETCHMER, Stanford Univ. Medical School, Palo Alto, Cal.

The relevance of the *de novo* pathway for pyrimidine biosynthesis to the formation of nucleic acids and consequently to the phenomenon of growth stimulated this investigation. Previous work from this laboratory showed that the activity of aspartate transcarbamylase was elevated in the heart and liver of the fetal rat and it decreased progressively during development. The chick was chosen as the experimental animal in the present study because of the relative ease in determining the stage of embryogenesis and also because determinations could be made at very early stages of development. Two enzymes of the pathway, aspartate transcarbamylase (ATCase) and dihydroorotase (DHOase) were assayed at intervals of 3 days in the liver, heart, brain and intestine of the chick embryo from the 7th to the 19th day of embryogenesis. The most elevated enzymatic activities were observed in the earliest stages. These values decreased with increasing embryonic age, apparently in relation to the relative rate of growth of each organ. High activities were detected in liver and intestine while lower activities were encountered in brain and heart. Preliminary work indicates the presence of dihydroorotate dehydrogenase and carbamyl phosphate synthetase in these embryonic tissues. The developmental patterns of activities and the magnitude of these activities for ATCase and DHOase are distinctive for each organ, although within the individual organ these characteristics are strikingly similar for both enzymes. In unicellular organisms this pathway has been shown to be regulated allosterically. Similar phenomena may exist during development.

(Supported by USPHS Grant HD 02147-02 and the W. K. Kellogg Foundation.) (SPR)

77 *Effect of Caloric Restriction on Regional Growth of Rat Brain.* IRVING FISH* and MYRON WINICK, Dept. of Pediatrics, Cornell Univ. Med. School, New York, N.Y.

We have previously reported that caloric restriction during the first 21 days of life decreases the rate of cell division in rat brain. However, neither normal cellular growth in specific brain regions nor the effects of caloric restriction on this growth have been studied. DNA content reaches a maximum in brain stem by 13 days and cerebellum by 17 days. In cerebral cortex, DNA content is still increasing at 21 days. In hippocampus, a discrete rise in DNA content occurs between 14 and 17 days. Protein content increases more rapidly than DNA in cerebrum and brain stem and more slowly in cerebellum. Thus protein/DNA increases in cortex and brain stem and decreases in cerebellum. These data demonstrate that cell number reaches a maximum in different areas at different times and protein/cell increases in certain areas and decreases in others. Caloric restriction from birth results in a marked decrease in cerebellar DNA by eight days of age, whereas no effect can be demonstrated on cerebral cortex until 14 days. The major early effect on brain stem is a reduction of total protein and hence the protein/DNA ratio. Thus caloric restriction effects the various regions of brain differently. Cell division is interfered with first in cerebellum and only later in cortex and brain stem. Protein/cell is reduced most markedly in brain stem. These data highlight the importance in future studies of dividing the brain into component regions. (SPR)

78 *Development of Ornithine-Ketoacid Aminotransferase Activity in Mammalian Liver.* NIELS C. R. RAIHÄ* and M. KEKOMÄKI*, Dept. of Med. Chem. and Children's Hosp., Univ. of Helsinki, Finland (introduced by Norman Kretchmer).

Ornithine functions as an obligatory substrate for urea synthesis in the liver and as a precursor of polyamines. The first step in ornithine catabolism is catalyzed in mitochondria by ornithine-ketoacid aminotransferase (OKT; EC 2.6.1.13). This study was undertaken in view of the marked changes which occur in urea and polyamine synthesis during development. In human liver a high fetal level of IKT was followed by much lower activity postnatally. In the rat, on the other

hand, the activity was low during fetal life, with a transient peak around birth. After the 14th postnatal day, activity increased to adult level within a week. Five mg/100 g body weight of triamcinolone i.p. induced OKT activity to adult level in 24 hours when given postnatally before the natural increase of activity. Puromycin inhibited completely this effect. Fetal or adult animals did not respond to glucocorticoid. In adult animals, the enzyme activity was markedly increased by high protein, and depressed by lack of protein and/or arginine in the diet. It is suggested that the peak of OKT activity around birth is a developmental increase caused by some unknown factor and not influenced by exogenous glucocorticoids, the second increase an adaptive one due to a change in the balance between intake and utilization of protein and amino acids. This can be altered by glucocorticoids (SPR)

79 *Biochemical Similarities of Childhood and Adult Cystinosis.* JERRY A. SCHNEIDER* and J. EDWIN SEEGMILLER*, National Institutes of Health, Bethesda, Md. (introduced by R. Rodney Howell).

Individuals with the adult form of cystinosis (AC) have corneal, conjunctival and bone marrow deposits of cystine crystals which are indistinguishable from those observed in children with cystinosis (CC). Unlike CC, which is invariably fatal, AC is entirely benign, with no demonstrable renal defects. It is hoped that studies of AC will increase our understanding of CC.

The free-cystine content of both leukocytes and cultured skin fibroblasts from three unrelated patients with AC was markedly greater than normal (NL, see table). In all cases, the values fell between those for heterozygotes (HET) and homozygotes for CC.

When either leukocytes or fibroblasts from AC patients were subjected to subcellular fractionation, three observations were made which had also been true for these tissues in CC: (1) three-quarters of the free-cystine was found in the granular (acid-phosphatase-rich) fraction; (2) almost twice as much cystine was recovered from the granular fraction if it was first treated with Triton X-100; and (3) hypotonicity caused a greater release of cystine than acid phosphatase from this fraction, suggesting the cystine is not trapped in lysosomes. Thus, the principal difference between CC and AC was content of intracellular cystine, which was 80–100 times normal in CC and 30–50 times normal in AC. (SPR)

Free-cystine (μmole ½ cystine/gram protein)

Pt.	*Leukocytes* Mean	(Range)	No. of patients	*Fibroblasts* Mean	(Range)	No. of patients
CC	6.44	(3.98–13.1)	9	8.40	(6.64–10.6)	6
HET	0.49	(0.01–0.89)	9	0.34	(0.12–0.72)	4
NL	0.08	(0.01–0.17)	10	0.07	(0.01–0.15)	9
AC	2.24	(0.96–3.60)	3	3.32	(1.54–5.09)	3

80 *Embryonic Growth Retardation in the Chick Secondary to Mechanically Induced Heart Defects.* IRA H. GESSNER, Univ. of Florida Coll. of Med., Gainesville, Fla.

An experimental method for producing a spectrum of predictable congenital heart defects in the chick has been described. This method utilizes temporary mechanical manipulation of the primitive heart tube as the etiologic agent. Defects produced include a small or large ventricular septal defect, a large ventricular septal defect with an overriding aorta, and double outlet right ventricle.

Current studies have shown that significant growth retardation is present in the embryos with heart defects which is not seen in sham operated controls. In addition, the growth retardation is more severe in specimens with double outlet right ventricle than in those with less severe abnormalities. Growth retardation is general, affecting linear measurements as well as weight, although weight seems slightly more retarded. On the 20th day of incubation, the weight of 15 experimental animals was 79 % of that of 20 sham operated controls. Crown-rump length of experimentals was 85 % of control values.

The etiology of the growth retardation is undetermined. Since the defects are produced by purely mechanical means, it is the heart defect itself which in some way leads to interference with embryonic growth. Total egg weights during incubation indicate that experimental eggs lose more weight during incubation than do controls. This might suggest higher metabolic activity since only water vapor and gases can be lost through the shell. However, the differences are too small to account for more than a portion of the total growth retardation. (SPR)

81 *Plasma Progesterone in the Perinatal and Neonatal Period.* PATRICIA W. CONLY*, THEODORE MORRISON*, DOUGLAS H. SANDBERG* and WILLIAM W. CLEVELAND, Univ. of Miami School of Med., Miami, Fla.

This study was designed to investigate progesterone metabolism at delivery and in the neonatal period. Concentrations of progesterone were measured in maternal, cord and infant's plasma. The competitive protein-binding radioassay developed by MURPHY (J. clin. Endocr. *27:* 1167 [1967]) was employed. The sensitivity of the method allows measurements of mμg/ml concentrations of 12 women at delivery ranged from 46 to 387 with a mean of 158. By 2–3 days post partum the concentrations had dropped to 1–24 mμg/ml. Cord plasma from the same subjects had much higher concentrations ranging from 435–2000 mμg/ml with a mean of 1130. In the infants, levels of progesterone declined rapidly. There were 4–32 (mean 17) on the first, 3–15 (mean 7) on the second, and 0–11 (mean 3) mμg/ml on the third day of life. Concentrations of progesterone in 27 prematures varying in birth weight from 1350–2470 g ranged from 2–34 mμg/ml on the first day, 1–5 (mean 2) on the second day, and 2–4 on the third and fourth days. Concentrations in six infants with respiratory distress syndrome were not different from other prematures. Maternal and cord levels of progesterone in one case of abruptio placenta were remarkably low. The concentration in the mother's plasma at delivery was 28 mμg/ml and that in cord blood 88. These studies indicate that blood from the placenta contains extremely high concentrations of progesterone. Lower levels in the peripheral venous blood from the mother may represent dilution. In both mother and baby, levels of progesterone decline rapidly following delivery. (SPR)

82 *Plasma FSH Levels in Normal Children and in Children with Various Pituitary or Gonadal Disorders.* DON S. SCHALCH*, MICHAEL F. BRYSON* and LOUYSE A. LEE*, Univ. of Rochester School of Medicine and Dentistry, Rochester, N.Y. (introduced by Gilbert B. Forbes).

Plasma FSH levels have been determined by radioimmunoassay in a number of normal children ranging in age from birth to adolescence, and in children with various pituitary or gonadal disorders. At birth, the mean FSH level (32.9 mμg/ml) in normal infants was markedly elevated. Subsequently, plasma FSH concentrations dropped to low but readily detectable levels in both boys (2.6 mμg/ml) and girls (1.5 mμg/ml), and showed a gradual but significant rise ($p<.05$) to the time of adolescence. In menstruating girls, the mean FSH level during the follicular phase of the menstrual cycle (3.3 mμg/ml) was significantly higher ($p<.001$) than the level during the luteal phase (1.4 mμg/ml). All demonstrated a mid-cycle peak in FSH (8.6 mμg/ml), while only half had a second peak (8.3 mμg/ml/ which occurred during the mid-follicular phase. Eight children with various degrees of precocious sexual development had normal plasma FSH levels. In contrast, the mean FSH level in 7 patients with primary gonadal failure (3 Turner's, 2 Klinefelter's, 1 testicular feminization, 1 anorchia) was markedly elevated (14.6 mμg/ml), consistent with the absence of gonadal hormone suppression. Evidence that the negative feedback system was still intact was provided by the finding that plasma FSH dropped to normal levels in some of these subjects after testosterone or estrogen administration. No detectable plasma FSH was found in 2 subjects with hypopituitarism and 1 with a hypothalamic tumor. These studies reveal the dynamic nature of the pituitary-gonadal axis and also the usefulness of this immunoassay in the diagnosis of pituitary and gonadal disorders. (SPR)

83 *The Relationship of Plasma Luteinizing Hormone (LH) Concentration to Gonadal Function.* A. W. Root*, T. Moshang, Jr.*, A. M. Bongiovanni and W. R. Eberlein, Univ. of Pennsylvania School of Med. and Children's Hospital, Philadelphia, Pa.

Plasma LH concentrations were determined by double antibody radioimmunoassay in 201 subjects. In 34 normal males, 0.17–13 years, LH was <2.5 mIU/ml (2nd IRP-HMG) in 28 and 2.5–11 mIU/ml in 6. In 15 males, 13–18 years, LH was 2.8–11 mIU/ml in 7, and was not detectable in 8. LH was <2.5 mIU/ml in 11/14 females less than 10 years, and 3–14.8 mIU/ml in 12/15 early pubertal premenarchal females (10–14 years). LH was not measurable in the plasma of 24/33 males (3–16 years) with constitutional delay in growth and development and ranged from 2.7–9.8 mIU/ml in the others. In 18/20 subjects with hypopituitarism, LH was <2.5 mIU/ml. Of 22 children with true sexual precocity, premature pubarche or premature thelarche, the LH concentration was <2.5 mIU/ml in 18; in 2 children with sexual precocity and intracranial tumors, LH was 9.5 and 5.8 mIU/ml. LH was 15.8–22.5 mIU/ml in 4/8 children (0.08–12 years) with Turner's syndrome, while in 4/5 older patients (12–18 years) LF was 9.5–47.3 mIU/ml. Plasma LH ranged from 7–63.4 mIU/ml in 9 males (17–47 years) with Klinefelter's syndrome and 4.5–36.3 mIU/ml in 4 males (11–16 years) with anorchia. In 4 male pseudohermaphrodites (0.25–15 years), LH was 6–11 mIU/ml. The intravenous administration of estrone depressed elevated plasma LH levels in patients with Turner's and Klinefelter's syndromes and maintained suppression for more than 12 hours. The presence of circulating LH in some normal children and elevated levels in the child with hypogonadism indicate activity of the pituitary-gonadal axis in the prepubertal state. (APS)

84 *Constitutional Male Sexual Precocity-Testosterone Production Rates (TPR) and Therapy.* Alvro M. Camacho* and Robert L. Trouy*, Univ. of Tennessee Coll. of Med., Memphis, Tenn. (introduced by James N. Etteldorf).

Urinary TPR's (mg/24 h) in 5 males (ages 2 to 7 years) with constitutional sexual precocity (CSP) ranged from 25 to 100 % of normal adult males (av. 5.8). Biweekly injections of 150 to 250 mg of 17α-hydroxy-6α-methylprogesterone acetate (6-MPA) were given for 10 to 80 weeks. TPR's were determined 4 to 6 days after the last injection. In patients C.V. and J.H., the TPR fell from 5.8 to 0.2 and from 1.0 to <0.1; in C.V., twelve days after the last injection of 6-MPA, the TPR was similar to the control level; it was increased in both to greater than control levels with the simultaneous administration of chorionic gonadotropin and 6-MPA. C.V. was treated with 6-MPA for 80 weeks and there was a deceleration of linear growth and skeletal maturation; TPR was 1 ½ times the control value 6 months after discontinuing 6-MPA. In R.G., M.M. and D.I., there was a definite decrease in the TPR while receiving 6-MPA (from 7.8 to 3.8, 9.3 to 3.1, 4.9 to 1.4) but not to the level attained in C.V. and H.J. Six months of treatment in R.G. did not decelerate linear growth and skeletal maturation; data are incomplete in M.M. and D.I. In C.V., H.J. and D.I., the percentage of injected testosterone excreted as free and glucuronide was altered by 6-MPA; there was a 53 %, 206 % and 650 % increase. *Conclusions:* (1) TPR is increased in males with CSP and is decreased by 6.MPA. (2) Variable suppression of TPR by 6-MPA indicates the importance of TPR's in monitoring the effect of the drug; urinary testosterone excretion may not correlate with the production of testosterone in males receiving 6-MPA. (3) Increased dosages and/or more frequent administration of 6-MPA should result in further lowering of TPR. (4) The data suggest that 6-MPA may be effective in the treatment of boys with this disorder. (SPR)

85 *The Effects of the Administration of Beta-Hydroxybutyrate (BOHB) on the Levels of Insulin, Glucose and Glycerol.* Liliane Loridan* and Boris Senior*, Department of Pediatrics, New England Medical Center Hospitals, Boston, Mass. (introduced by S. S. Gellis).

Past studies have shown a reduction in levels of glucose and free fatty acids (FFA) in response to ketones, an effect generally attributed to stimulation of insulin secretion. Since increased insulin secretion as a normal response to starvation appears paradoxical, the effects of ketone administration were reexamined. The subjects were six adults, four children with a history of hypoglycemia with ketosis and two control children. After an overnight fast, BOHB 15 g/m² was infused intravenously. The study was repeated in the children following induction of gluconeogenesis by 2 days of a low carbohydrate-low calorie diet and a 15–20-hour fast. Sequential samples of blood were assayed for glucose, glycerol, FFA, immunoreactive insulin (IRI) and BOHB. In response to BOHB: (a) Glucose levels fell in all, following the overnight fast, but to a greater degree in the children (decrement 29.7 mg per cent versus 5.5 mg per cent). (b) During induced gluconeogenesis, glucose levels barely decreased (2.8 mg per cent). (c)

Glycerol levels fell substantially in all. (d) No increase in levels of IRI was found. (e) The patients and control children did not differ in their responses. The results indicate a direct reduction of lipolysis and lowering of glucose levels by BOHB. Ketones appear physiologically in response to carbohydrate deprivation at a time when the glucose level is maintained by gluconeogenesis. By providing fuel for muscles and brain they may actually spare glucose. Thus ketones appear to accompany hypoglycemia and not cause it. (APS)

86 *Diazoxide (D) and Hypoglycemic States of Infants.* THEODORE W. AVRUSKIN*, JOHN F. CRIGLER, JR., and DENNIS SLONE*, Harvard Med. School and Tufts Univ. School of Med., Boston, Mass.

The effect of D (15 mg/kg/day for 5–16 days) alone or with ACTH-gel or Naturetin on multiple daily fasting and postprandial blood sugars (BS-mg %), fasting serum immunoreactive insulin (IRI-μU/ml) and growth hormone (IRGH-mμg/ml) and the response of BS, IRI and IRGH to glucose, glucagon, tolbutamide or l-leucine has been studied in three infants with persistent idiopathic hypoglycemia (IH) and an 18-month-old girl of a diabetic mother (IDM) with hypoglycemic seizures. Two of three infants with IH showed no significant changes in fasting BS and increased IRI and IRGH or in BS and IRI responses to tolbutamide and l-leucine (patient 1) or glucagon (patient 2) when D was given. BS decreased and clinical symptoms increased when Naturetin was added (patient 1) and ACTH-gel dose reduced (patient 2). The third infant with IH (BS 30 ± 2.0, mean $\pm$ standard error) and abnormal BS and IRI response to tolbutamide became euglycemic (57 ± 1.9) during D and remained so after D was discontinued. Fasting IRI and IRGH increased to elevated levels in the post-D period (17 ± 3.8; 10 ± 2.5). The effect of D in patient 3 is uncertain. The IDM showed a significant ($p<0.001$) change in fasting BS (63 ± 2.5, 75 ± 2.1, 65 ± 1.9) and IRI (5.7 ± 0.4, 2.9 ± 0.7, 4.0 ± 0.7) with no change in IRGH on addition and withdrawal of D, respectively. Repeated tests before and during D showed significant attenuation of l-leucine and tolbutamide-induced hypoglycemia and IRI release and a temporary decrease of glucose-induced IRI associated with an appropriate change in BS. Hypoglycemic infants vary considerably in their responses to D, a fact of therapeutic as well as possibly etiologic importance. (SPR)

87 *Action of Insulin and Growth Hormone on Cell Growth.* DONALD B. CHEEK, and JOAN E. GRAYSTONE*, Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Md.

This study was designed to show: (1) The action of insulin on muscle mass, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and skeletal collagen, and on liver, weight, protein, RNA, DNA, zinc (Zn), manganese (Mn), and enzymes glutamic dehydrogenase (GDH) and glutamic oxalacetic transaminase (GOT) (transaminase) in the intact rat from 26 to 38 days postnatally. Controls were given an ad lib diet. (2) The action of insulin on hypophysectomized rats 38–49 days as contrasted with hypophysectomized rats receiving GH or no treatment. These hypophysectomized groups received the same food/100 gram body weight as did intact rats (calorie restricted). It was found that insulin in presence of pituitary raises food efficiency, increases muscle growth (more and bigger cells) but total liver protein is the same. Without the pituitary, insulin raises food intake, food efficiency and weight, but there is no growth of muscle or carcass, only growth of liver. Here RNA, protein, Zn, Mn and GDH per unit DNA are markedly increased (for size or age mate) ($p<0.001$). By contrast in pituitary insuffiency (untreated) liver RNA, GDH, Zn and Mn are markedly decreased per unit DNA (size mates) ($p<0.001$). GH does not increase enzymes, trace metal, protein, or RNA per unit DNA of liver. Calorie restriction produces a picture similar to that of a hypophysectomized insulin treated rat. (APS)

88 *Plasma Growth Hormone after a Single Intramuscular Injection.* S. DOUGLAS FRASIER*, GERTRUDE COSTIN*, SHUN M. LING* and SOLOMON A. KAPLAN, Los Angeles General Hosp., Children's Hosp. and Univ. of South Calif. School of Med., Los Angeles, Cal.

Efficient utilization of human growth hormone (HGH) requires administration of the minimum dosage which will achieve an adequate clinical response. Dosage has not been based on the plasma concentrations achieved after HGH administration. We have followed the plasma HGH concentration, measured by radioimmunoassay, over 48 hours after the initial IM injection of 1 or 2 mg HGH (NPA lot No. S-4) in 5 hypopituitary children. Patients received 25-60 μg HGH/kg body weight. Detectable levels of HGH (5.2–29 mμg/ml) were observed at 15 minutes. The plasma HGH concentration increased rapidly to a peak of 65–158 mμg/ml at 2 or 4 hours and slowly fell over the next 8–10 hours. Twelve hours following injection, the HGH concentration was 1–12 mμg/ml. Plasma HGH was 3 mμg/ml or less in all patients at 24 hours. Between 24 and 48 hours the plasma HGH concentration was 1.6–3.5 mμg/ml. The pattern of plasma HGH concentration following an IM injection is clearly different from the pattern of physiologic diurnal variation in plasma HGH. These observations suggest that present dosage schedules of HGH are pharmacologic and that more efficient use of HGH may result from schedules which better approximate physiologic growth hormone secretion. (SPR)

89 *Pituitary Hormone Concentration in the Stunted Head-Irradiated Rat.* H. DAVID MOSIER, JR., and REGINA A. JANSONS*, University of California, Irvine; College of Medicine, Los Angeles, and Illinois State Pediatric Institute and the Department of Pediatrics, University of Illinois College of Medicine, Chicago, Ill.

The mechanism responsible for growth failure resulting from irradiation of only the heads of two-day-old rats has been investigated. Previous studies have shown that the pituitary is reduced in size to a greater extent than is the body weight in the stunted rat. Administration of bovine growth hormone (Li), thyroxin or both, fails to correct the growth impairment. In this experiment somatotropin, gonadotropin and thyrotropin were measured in the pituitaries of irradiated and control rats at 23, 42 and 121 days of age using the tibial epiphyseal width, the combined ovarian weight and 24-hour thyroidal I^{131} uptake in young hypophysectomized rats as indices. Controls were injected with saline. The results showed essentially that the concentration of these pituitary hormones are not reduced in the pituitaries of the irradiated rats. In fact, in 121-day-old females the pituitary concentration of gonadotropin was about twice that of controls. Thus, although there

is a reduction of pituitary size, the findings suggest that mechanisms for pituitary hormonal production are intact in the stunted irradiated animals. (SPR)

90 *Growth Hormone Responsiveness in Fetal Malnutrition.* DONALD A. HILLMAN, MARILYN M. LI* and ELEANOR COLLE, McGill Univ., Montreal Children's Hosp. Research Inst., Montreal Canada.

From a study of growth hormone responsiveness in 200 children admitted for the investigation of growth failure, the birth histories revealed that 15 % were significantly underweight for their gestational age—fetal malnutrition (FM). The HGH response following insulin, lysine vasopressin and arginine in the FM babies was compared to a control group of children of similar age and sex whose growth rate was normal. The HGH responses to insulin induced hypoglycemia were as follows:

	Fasting	20 min	40 min	
FM	5.3 μg/ml	11.2	17.8	
Control	2.4	3.73	8.1	
P <	0.02	0.01	0.005	
	Fasting	60 min	90 min	120 min
FM	5.3 μg/ml	15.4	12.0	6.7
Control	2.4	8.09	5.21	5.2
P <	0.02	0.05	0.01	n s

In addition, arginine stimulation of four FM and four normal newborn infants in the first three days of life, revealed in both groups high levels of HGH, irregular responses to arginine and no signicant difference between the two small groups. In some FM children, plasma cortisol response to hypoglycemia and LVP was exaggerated, and increases in plasma insulin in response to arginine were minimal. However these changes were not regularly observed. It is concluded that the growth failure observed in FM cannot be ascribed to HGH deficiency. The reason for the higher HGH levels is unexplained and is not related to differences in blood glucose in the two groups. (SPR)

91 *An Inherited Kinetically Aberrant Isozyme of Erythrocyte (RBC) Pyruvate Kinase (PK) Responsible for Hereditary Hemolytic Anemia.* DENIS R. MILLER*, DONALD E. PAGLIA*, WILLIAM N. VALENTINE*, MARJORIE A. BAUGHAN*, CLAUDE F. REED* and O. ROSS MCINTYRE. Univ. of Rochester, N.Y., Univ. of California, Los Angeles, Cal., and Dartmouth, Hanover, N.H., Medical Schools (introduced by Robert J. Haggerty).

Atypical cases of heritable hemolytic anemia have been noted which conform clinically and biochemically to anemia of PK deficiency except for apparently adequate quantities of RBC PK activity. The reported patients complied with all criteria for PK deficiency including Dacie type II autochemolysis patterns, diminished and unstable RBC ATP, increased 2,3-diphosphoglycerate levels, the absence of spherocytosis, hemoglobinopathies or other demonstrable enzyme deficiencies and a partial but significant response to splenectomy. Further investigation of five such anomalous cases in two unrelated families demonstrated a kinetically aberrant isozyme of PK (PK_2) with remarkable inefficiency at lowered concentrations of its basic substrate, phosphoenolpyruvate (PEP). Michaelis constants for PEP were tenfold greater than control values but no kinetic abnormality was evident for adenosine diphosphate. Differences in pH optimum and functional stability of the isozymes were also evident. Leukocytes were unaffected. Family studies revealed paternal heterozygosity for quantitative PK deficiency of the usual type (PK_1). Clinically normal maternal relatives and some siblings demonstrated intermediate deviations in PK kinetics. Phenotypic expression in the propositii required simultaneous inheritance of both the PK_1 and PK_2 genes. Both genetic defects were traced through three generations and transmission conformed to autosomal recessive modes in the propositii. (SPR)

92 *Iron Supplementation: Effect on Iron Stores.* W. K. SCHUBERT*, S. SEGER* and F. SCHNEEMAN*, Children's Hospital Research Foundation, Clinical Research Center, Babies' Milk Fund Association, Cincinnati, O. (introduced by Edward L. Pratt).

Iron supplementation for normal infants remains controversial; studies of its effect on storage iron are not available. Twenty-two newborn infants with no evidence of hematologic disease by history or physical examination and with hemoglobin concentrations of over 16 grams % were assigned at random to two groups, one fed an iron-supplemented prepared formula containing 12 mg of iron per quart and the other the same formula without iron. These groups were part of a larger study of 118 infants in whom identical studies, except for tissue iron evaluation, were done. Nutritional management, including introduction of strained foods and iron-fortified cereal at 6 weeks of age, and routine well-baby care were performed by the same clinician. Prepared formulas were fed for 12 months; thereafter, homogenized milk was fed. Tissue iron was estimated by sideroblast counts of bone marrow smears obtained at 3-month intervals during the first 18 months of life. Sideroblast counts were lower in the non-supplemented group beginning at 6 months of age, and were significantly lower at 9, 12, and 15 months of age ($p = 0.01$, 0.01, 0.05); significance was not present at 18 months of age. Hemoglobin, hematocrit, MCV, MCH, MCHC, TIBC and per cent saturation were significantly lower from the third month through 18 months of age in the non-supplemented group. Serum iron was also lower in the non-supplemented groups and significantly lower ($p = 0.01$) between the sixth and fifteenth months. Between 6 and 18 months of age of the 60 patients in the non-supplemented group, 35 had hemoglobins below 10 grams %; 7 of these were below 8 grams %. In the iron-supplemented group, 8 had hemoglobins below 10 and none had hemoglobins below 8 grams % ($p = 0.01$). These findings suggest that in hematologically normal children, supplemental iron in prepared formula prevents depletion of iron stores and iron deficiency anemia. (APS)

93 *Anticoagulant Properties of Stored Pooled Normal Human Plasma.* GEORGE R. HONIG*, C. F. ABILDGAARD, A. LINDLEY*, E. N. FORMAN*, S. P. GOTOFF, J. WOLINSKY* and I. SCHULMAN. Univ. of Illinois Coll. of Med., Chicago, Ill.

Recent observations in our laboratory reveal that stored plasma, widely used in treatment of shock, diarrhea and burns, has potent anticoagulant activity. Hematologic studies on an infant admitted for shock disclosed striking prolongation of a variety of coagulation tests which suggested heparin effect (prothrombin time, partial thromboplastin time, thrombin time, in

the face of normal levels of specific coagulation factors, fibrinogen and fibrinolysin). Further history revealed that the child had received a large volume of stored, pooled normal human plasma in the emergency room. The same plasma produced a marked anticoagulant effect on normal plasma, *in vitro,* with the results indicating inhibition of the thrombin-fibrinogen reaction. Similar results were obtained with 16 different lots of stored plasma from five different sources. Biochemical and immunologic studies revealed that the plasma was devoid of fibrinogen but contained large amounts of fibrin degradation products. Treatment of the plasmas with rabbit IgG antihuman fibrinogen completely removed the anticoagulant activity. Infusion of stored plasma into rabbits produced a defect identical to that found in the original patient. It has long been known that storage of purified fibrinogen results in degradation of the protein into split products which have anticoagulant activity. That this occurs on storage of whole human plasma has not been appreciated. Therapeutic use of the plasma may in some circumstances produce laboratory abnormalities suggesting severe coagulation disorders and may, in individuals being treated for hemorrhagic shock, contribute to further bleeding. (SPR)

94 *Studies on Fetal Hemoglobin Synthesis in the Newborn: Its Application in Determining Gestational Age.* ARLISS H. TUTTLE, deceased, SERGIO A. DE LAMERENS* and DANIEL M. LANE*, Univ. of Tennessee Coll. of Med., Memphis, Tenn. (introduced by James N. Etteldorf).

A modification of the technique of KLEINHAUER *et al.*, with reticulocyte staining previous to the acid elution, was used to determine the relative concentrations of adult and fetal hemoglobins within the mature erythrocyte and the reticulocyte of newborns. Calculation of these concentrations was determined by the method of GARBY *et al.* Since reticulocytes are recently formed cells with a short half-life, concentrations of fetal hemoglobin within these cells should reflect its rate of synthesis at the time of sampling.

Ninety-six newborns (including 13 sets of twins), of gestational ages varying from 27 to 40 weeks, were studied. In infants of less than 35 weeks' gestation, fetal hemoglobin concentrations in the mature erythrocytes and in the reticulocytes were similar. After 35-week gestation, there was an abrupt and progressive drop in the percentage of fetal hemoglobin in the reticulocytes.

This technique can be used in determining gestational age and could serve as a new parameter to investigate the maturity of the newly born. (SPR)

95 *Isolated Defect of Gastrointestinal Absorption of Folic Acid Resulting in Megaloblastic Anemia, Associated with Mental Retardation and Cerebral Calcification.* PHILIP LANZKOWSKY*, MARION E. ERLANDSON and ALLAN I. BEZAN*, Cornell University Medical College, New York City, N.Y.

Folic acid deficiency associated with a generalized malabsorption of other nutrients is a well recognized entity. A syndrome consisting of isolated malabsorption of folic acid (FA) has been identified in a child who presented at 3 months of age with megaloblastic anemia, diarrhea and mouth ulcers. She has been followed for 17 years and has shown normal physical development, gross mental retardation and basal ganglia punctate calcification. The megaloblastic anemia has been shown to be due to specific gastrointestinal malabsorption of dietary or physiologic amounts of FA with normal absorption of glucose, xylose, fat and vitamin B_{12}. Barium studies of gastrointestinal tract and microscopy of the jejunal mucosal biopsy were normal. On regular diet without supplemental FA she develops anorexia, weight loss, buccal ulceration, anemia, leukopenia and thrombocytopenia, bone marrow megaloblastosis, increased chromosomal breakage with structural changes including dicentric chromosomes and quadriradial configurations, increased urinary FIGLU excretion, reduction in plasma, RBC and CSF folate to Ong/ml (L. Casei method). FA 250 μg orally did not correct the anemia and she continued to deteriorate hematologically whereas 250 μg FA intramuscularly resulted in a reticulocytosis within 7 days and return of the above parameters to normal. Reversal of normal plasma/CSF folate ratio with normal plasma folate levels indicated impaired folate transport into CSF. Return and maintenance of normalcy could also be achieved by 40 mg FA orally daily. No FA antagonists in serum were demonstrated. (APS)

96 *The Mechanism of Action of Chemotherapeutic Agents in Acute Lymphoblastic Leukemia.* B. C. LAMPKIN*, T. Nagao* and A. M. MAUER, Dept. of Pediat., Univ. of Cincinnati Coll. of Med., Cincinnati, O.

The effect *in vivo* of chemotherapeutic agents in acute lymphoblastic leukemia is poorly understood. However, knowledge of their effects is essential to proper use of these drugs for therapy. For this study three types of chemotherapeutic agents, corticosteroids, vincristine (VCR) and cytosine arabinoside (CA) were evaluated. Their effect on marrow blast cells was followed by serial measurements of H_3T labeling index (LI), mitotic index (MI), and volume of buffy coat (BC). Studies with prednisone were done in four children; hydrocortisone in seven; VCR in seven and CA in three. A highly significant decrease in LI was seen in all patients after prednisone and in five of seven patients 24 hours after hydrocortisone. In the latter group, a striking decrease in BC was found 1 to 24 hours after therapy and a significant decrease in the MI 24 to 48 hours after therapy. After VCR, the MI increased 4 to 14 times at 12 to 24 hours. In five patients the MI was still increased at 48 hours. The LI was decreased at 48 hours. After CA, the MI and LI were decreased markedly by 4 hours but after 24 hours the LI had returned to pretreatment levels. The MI was still decreased. From these results it would appear that (1) corticosteroids inhibit DNA synthesis and thereby secondarily decrease the number of cells in mitosis; (2) VCR arrests cells in mitosis and thus removes cells for subsequent DNA synthesis; (3) CA inhibits both DNA synthesis and mitosis; and (4) corticosteroids lyse lymphoblasts. The time relationship of these effects is important in designing treatment regimens. (APS)

97 *Comparison of Exchange Transfusion and Deferoxamine in the Treatment of Acute Iron Poisoning* N. MOVASSAGHI*, G. G. PURUGGANAN* and S. L. LEIKIN, Research Foundation, Children's Hosp. of the D. C. and Dept. of Ped., George Washington Univ. School of Med., Washington, D. C.

Acute iron poisoning in children continues to be a serious problem but, because of the lack of an effective therapy, severe cases of iron poisoning can be fatal. The efficacy of iron removal by deferoxamine (DF), exchange transfusion or a combination of DF and ex-

change transfusion was studied in dogs with acute iron poisoning. A lethal dose of ferrous sulfate was administered by gastric tube to 3 groups of dogs. In group 1, two hours following iron administration, DF 200 mg/kg was given intravenously over a one-hour period. Group 2 underwent exchange transfusion three hours after iron administration, and group 3 received DF and an exchange transfusion two and three hours, respectively, following iron administration. The amount of iron removed was measured in the exchanged blood and in the urine collected while the animal was alive or for a period of six hours. The mean quantity of iron removed was 486 μg, 12,755 μg and 12,641 μg in groups 1, 2 and 3 respectively. Two dogs survived in the group receiving exchange transfusion but none in the DF or combined treated group. Exchange transfusion appears to be a more effective and rapid method than DF alone for removal of iron in the treatment of severe iron poisoning. The addition of DF to exchange transfusion does not significantly increase the amount of iron removal nor does it improve the survival. (SPR)

98 *Detecting Hemolytic States from Carboxyhemoglobin Levels.* ROLF R. ENGEL*, F. LEE RODKEY* and CARL E. KRILL*, National Naval Medical Center, Bethesda, Md. (introduced by John A. Anderson).

During the catabolism of heme there is a molecule of CO generated for each molecule of bilirubin formed. In demonstrating increased endogenous CO formation from accelerated hemolysis, it is important to separate out the effect of variable exogenous exposure. The carboxyhemoglobin (COHgb) level in 229 normal children ranged from 0.6 to 3.9 % saturation with a mean of 1.3 % (SD 0.47 %). Twenty children with hemolytic diseases had from 1.3 to 2.6 % of their circulating hemoglobin saturated with CO. The 6-fold range observed in normal children is largely due to variable ambient exposure, since 226 comparisons between nonsmoking adults who were breathing the same gas mixture for extended periods always gave COHgb values which differed by less than 0.3 % saturation. By comparing the blood CO content of a suspect hemolytic patient with a normal sibling or other control individual who has been exposed to the same exogenous CO level for several hours, it is possible to obtain a qualitative index of increased endogenous CO production. Children with known hemolytic disease had COHgb levels which were at least 0.5 % saturation higher than their matched controls. Because fetal hemoglobin was found to have a lower affinity for CO than adult hemoglobin, caution must be exercised in comparing a newborn or thalassemia patient with an individual who has primarily adult hemoglobin or hemoglobin S. The assay for COHgb can be done in 10 minutes by gas chromatography and requires only 0.1 ml of whole blood. Unlike the reticulocyte count, abnormal results can be expected before the erythropoietic response. (SPR)

99 *The Mechanism of Erythrocyte Sickling and Hemolysis in Sickle Cell Anemia.* JAMES G. WHITE, Univ. of Minnesota Medical School, Minneapolis, Minn.

Despite precise definition of the molecular defect in sickle hemoglobin (HbS), the fundamental mechanism by which reduced HbS causes distortion of susceptible erythrocytes has not been determined. One theory suggests that sickling is due to the intracellular crystallization of HbS, and another that distortion may be due to tactoid formation, i.e., bundles of parallel rods. Fresh blood from two patients with HbSS, and one with HbSC were sickled by exposure to low O_2 tension or sodium bisulfite, fixed initially in glutaraldehyde at 37° C and processed for study in the electron microscope. Thin sections of sickled cells revealed cytoplasmic elements never observed in normal red cells. Mildly distorted cells contained an irregular meshwork of fibers varying from 65 A° filaments to 180 A° rods. Crescentric sickle cells contained bundles of 180 A° rods generally oriented in the long axis. Chilled sickled cells were disc shaped and contained no filaments or rods. Colchicine and vinca alkaloids inhibited sickling and rod formation. Sickled erythrocytes were hemolyzed in a tissue homogenizer under conditions which did not destroy normal cells. The parallel bundles of rods broke down into an irregular meshwork of 65 A° filaments, then dissolved as swelling increased. Crystals of hemoglobin formed in normal blood cells did not resemble the tactoids in sickled cells. These findings indicate that sickling is due to a sol-gel transformation and not to intracellular crystallization of HbS. The degree of cell distortion is dependent upon the extent of rod formation and association in parallel bundles. Rods appear to develop from 65 A° filaments of HbS, and hemolysis reverses the process of rod formation. (SPR)

100 *An Increase of Antihemophiliac Globulin (AHG) in a Hemophiliac with Acute Lymphoblastic Leukemia.* JOHN D. BOUHASIN*, St. Louis Univ. School of Med. and Cardinal Glennon Mem. Hosp., St. Louis, Mo. (introduced by Arthur E. McElfresh).

Elevations of AHG levels in patients with acute leukemia have been reported previously, but a rise of AHG in a hemophiliac with leukemia has not been reported.

A four-year-old male with AHG deficiency of moderate degree (5 % AHG conc.), presented with acute lymphoblastic leukemia in April, 1966. Bleeding did not occur after the bone marrow aspiration or from injection sites. AHG assays initially showed 75 % and 100 % of normal AHG concentration.

AHG levels on two occasions during remission were 20 % and 49 % of normal. During a recent relapse, the AHG concentration rose to 97.5 % of normal. Preliminary work with cultured lymphocytes reveals definite AHG activity in the cells.

In view of the previously reported elevation of AHG nonhemophiliac patients with acute leukemia, the recent splenic perfusion studies, and our findings, it is postulated that the lymphocyte is the site of production of AHG. (SPR)

101 *Transmission of an Encephalitogenic Agent from Patients with Subacute Sclerosing Panencephalitis (SSPE) to Ferrets.* M. KATZ*, L. B. RORKE*, W. S. MASLAND*, H. KOPROWSKI* and S. H. TUCKER*, The Wistar Institute and Depts. of Pediatrics, Pathology and Neurology, University of Pennsylvania, Philadelphia, Pa. (introduced by A. M. Bongiovanni).

Ferrets were inoculated with brain material from two children with SSPE. After an incubation period of five months, the ferrets exhibited ataxia, spastic gait and weight loss. Histopathologic examination of their brains revealed cortical gliosis, structures suggestive of intranuclear eosinophilic inclusion bodies and mild meningitis. Secondary passage into new ferrets reproduced the disease in three months and its form

was more intense and included myoclonic jerks. Histopathologic picture revealed an intense meningoencephalitis of a characteristic viral type. White matter, brain stem and spinal cord were involved to a greater extent than gray matter, and cerebellum was spared. There was no demyelination. Electroencephalograms showed patterns compatible with progressive encephalitis, culminating in slow wave configuration and burst-suppression activity resembling that seen in SSPE. Neither serologic analyses nor tissue culture inoculation has yielded thus far any clue to the identity of this infectious agent. Its prolonged latency in the experimental animals and the subacute course of their disease suggest that this agent belongs among slow viruses. (APS)

102 Studies of the Altered Reactivity to Measles Virus in Killed Vaccine Recipients. VINCENT A. FULGINITI, JAMES H. ARTHUR* and C. HENRY KEMPE, Univ. Colorado Med. Center, Denver, Colo.

Children who have previously received killed measles virus vaccine (KMV) may demonstrate an altered reactivity to subsequent live virus exposure. The exact mechanism is unclear. To clarify the immunologic reactivity of such vaccines, 145 children who received KMV in 1961–1962 were evaluated for serum antibody and delayed hypersensitivity and compared to children who experienced only natural measles or only live virus immunization in the past. The following data were obtained

Group	Number	Serum HIA $\leq$1:8	Skin induration = 1.0 cm Killed virus	Live virus
Measles only	10	0	0	0
Live vaccine only	10	0	0	0
KMV	145	95 (66 %)	142 (98 %)	119 (82 %)

In a second experiment, nine of the seronegative KMV children were bled six weeks later and given 0.5 ml of live measles virus vaccine. All nine had developed serum HIA titers of 64–512 *after* the skin test and *prior* to the vaccine administration. None reacted adversely to the vaccine nor changed their serum titer. By comparison, 15 children studied in 1967 who developed severe atypical measles 5–6 years after KMV administration had serum HIA titers less than 1:8 at the onset of their disease. These collective data suggest that KMV recipients develop delayed hypersensitivity which may be responsible for their altered reactivity, but the latter is only manifest if serum antibody titer is low or absent. It appears as if intradermal virus inoculation may boost antibody titer without severe sequelae. (SPR)

103 Clinical Comparison of Highly Modified and Conventional Strains of Attenuated Rubella Viruses. HARRY M. MEYER, JR., PAUL D. PARKMAN, THOMAS E. HOBBINS*, H. ELLIOTT LARSON* and HOPE E. HOPPS*, National Institutes of Health, Bethesda, Md.

Successful clinical trials with attenuated rubella virus (HPV-77) led to further efforts at virus modification to determine whether one could develop a strain which would not be shed in the nasopharynx. This report describes a clinical comparison of HPV-77 vaccine grown in green monkey kidney (GMK) with seven other attenuated preparations. HPV-77 derived vaccines were produced by our laboratory in chick embryo cells (CE) and in GMK; by Philips Roxane, Inc. in dog kidney cells (DK) and by Merck, Inc. in duck embryo cells (DE). Merck, Inc. also produced Benoit strain vaccines at three passage levels. None of 112 persons inoculated with the eight attenuated viruses experienced vaccine-associated illnesses and there was no communicability to 90 contacts. HPV-77 vaccines in GMK, DE and DK and Benoit C vaccine induced antibodies in 91 to 100 % of participants. Benoit D, HPV-77 propagated in CE and Benoit E seroconverted 83 %, 75 % and 29 %, respectively. Antibody titers were 2- to 8-fold lower in persons given the three less immunogenic vaccines and antibody appearance was delayed. Virus excretion was generally characteristic of the vaccines. Even with the less immunogenic vaccines produced from 'over-attenuated' strains, virus was shed in 59 % of persons developing antibody. Pharyngeal tissue appears to be a preferred site for attenuated rubella virus multiplication. While data presented here indicate that some virus shedding may be expected with any effective live rubella vaccine, communicability appears unlikely. Fully immunogenic HPV-77 derived vaccines have been administered to 553 persons without infection of any of their 537 intimate contacts. (SPR)

104 Fluorescent Antibody Test for Cytomegalovirus Macroglobulin. JAMES B. HANSHAW, HARVEY J. STEINFELD* and CHERIE J. WHITE*, Univ. of Rochester School of Med. and Dentistry, Strong Memorial Hosp., Dept. of Ped., Rochester, N.Y.

REMINGTON [1967] demonstrated toxoplasma macroglobulin in the serum of infants with congenital toxoplasmosis by an indirect fluorescent antibody test. The application of this method to the detection of cytomegalovirus (CMV) macroglobulin could provide a rapid and practical test for active infection in early infancy. A drop of the test serum is placed on an encircled smear of trypsinized CMV infected WI-38 cells. (Uninfected cells on the same slide act as serum controls.) Following a 30 min incubation period at 20° C, the slide is washed and a drop of fluorescein-conjugated goat antihuman γM antiserum is added to each cell area. The smear is again incubated for 30 min, washed, and mounted. Positive smears, characterized by fine-lined, brilliant, blue-green fluorescence in the perinuclear area, were found in all of 26 virus-positive infants (0–6 mos.). Cytomegalovirus macroglobulin could be demonstrated in infected infants irrespective of the γM or CF antibody level. In contrast, infants with rubella, toxoplasmosis were negative, as were uninfected infants with maternally acquired CMV antibody or elevated γM levels. These data suggest that the cytomegalovirus macroglobulin test is a specific and sensitive indication of active CMV infection in early infancy. (SPR)

105 Echo Virus Type 9 Infection and Tuberculin Sensitivity. S. BERKOVICH, E. M. SMITHWICK* and M. STEINER*, Dept. of Ped., State Univ. of N.Y., Downstate Med. Center, Brooklyn, N.Y.

A number of viral infections can depress delayed skin sensitivity to tuberculin. Among these agents, measles is most consistently effective. Following attenuation, however, this effectiveness is largely lost. During measles, the altered skin reaction is associated with a significant decrease in the *in vitro* lymphocyte response

to tuberculin. In addition, sensitive lymphocytes infected *in vitro* with wild measles fail to respond as expected to tuberculin stimulation. Both effects may be due to changes induced in the lymphocyte by the measles virus. The question arises whether other viral agents that proliferate in cultures of peripheral lymphocytes also depress skin and lymphocyte reactivity to tuberculin. Answer to this query was provided by study of an outbreak of Echo 9 disease that occurred in our tuberculosis ward during August, 1966. The skin tests of 14 children with virologic and/or serologic evidence of infection remained strongly positive. The virus strain recovered has been tested in peripheral lymphocyte cultures obtained from eight other tuberculous children and has not significantly depressed the reactivity of their lymphocytes to tuberculin. In contrast to the measles virus, therefore, Echo 9 does not exert a detectable effect on tuberculin sensitivity *in vivo* or *in vitro*. Apparently, the ability to proliferate in peripheral leucocytes, a property shared by both agents, is not the viral factor responsible for changes in skin and lymphocyte reactivity to tuberculin. (SPR)

106 *Antibiotic Therapy in Chronic Granulomatous Disease of Childhood (CGDC).* HAROLD W. LISCHNER* and THEODORE R. LAMMOT III*, Temple Univ. School of Med. and St. Christopher's Hosp. for Children, Philadelphia, Pa. (introduced by Victor C. Vaughan III).

Recurrent purulent infections in CGDC are due to an inborn defect in intracellular lysis of bacteria after their phagocytosis (HOLMES *et al.*: Lancet *1:* 1225 [1966]). There is no satisfactory therapy. The failure of usual treatments is understandable, since the granulocytes of children with CGDC can protect phagocytized bacteria from the action of a wide variety of antimicrobial agents even after the granulocytes are no longer viable (J. Reticuloendothelial Soc. *4:* 431 [1967]). A rational approach to palliative therapy may however be possible. *In vitro* data suggest that phagocytic vacuoles may protect bacteria after disruption of the cell, and may sometimes undergo rephagocytosis. Resting-state bacteria maintain their antimicrobial sensitivity in such vacuoles and, *in vitro* at least, are susceptible to antimicrobial action upon final rupture of the vacuole. If bactericidal antibiotics were present at the site and time of phagosomal rupture in the body, reinfection might be prevented. This approach, using continuous therapy, appears to have been successful at least temporarily in two brothers with classic CGDC. One boy had severe osteomyelitis and multiple cutaneous ulcers, the other a huge deep tissue abscess and liver abscesses or scars demonstrated by ^{131}I rose bengal scan. The acute lesions, due to coagulase negative *Staphylococcus aureus*, responded slowly to vigorous therapy. Subsequently pencillin has been administered continuously for nine months. For the first time since early infancy the boys have appeared healthy and had no recurrences or new lesions, in spite of the fact that the same penicillin-sensitive organism still hibernates in the protective environment of at least one boy's phagocytic cells.

(Supported by USPHS grant FR-5624.) (SPR)

107 *Impaired Pneumococcal Phagocytosis in Sickle Cell Disease.* ROBERT H. DRACHMAN* and JERRY A. WINKELSTEIN*, Johns Hopkins Univ. School of Med. Baltimore, Md. (introduced by David H. Carver).

Children with sickle cell disease (SS disease) appear to be far more susceptible to severe pneumococcal disease, such as meningitis, than are those with normal hemoglobin. Their often fulminant course is reminiscent of pneumococcal infections in splenectomized children. The parallel is consistent with the known pathologic changes in SS disease which markedly affect the spleen. Serum opsonizing activity for the pneumococcus in SS disease was measured with a phagocytic test using peripheral leukocytes from normal individuals incubated in test and control sera. The percent phagocytosis in SS disease serum was 6.8 % $\pm$ 3.2 (SD), while for control children it was 37.6 % $\pm$ 14.4. Activity disappeared from both control and test sera when heated. Total hemolytic complement activity of SS disease sera was comparable to that of normal individuals. Leukocytes from patients with SS disease were indistinguishable from those of normal controls when incubated in normal serum. The deficiency of opsonizing activity may account for the impaired clearance of blood-borne pneumococci in SS disease. (SPR)

108 *Corynebacterium Diphtheriae Skin Infections and the Epidemiology of Diphtheria in the South.* MARK A. BELSEY*, MICHAEL SINCLAIR*, RUTH RODER* and DOROTHY LEBLANC*, Tulane Univ. School of Public Health and Tropical Medicine, New Orleans, La., and the National Communicable Disease Center, Atlanta, Ga. (introduced by Margaret H. D. Smith).

C. diphtheriae infections of skin lesions (SL), though not as frequent as nasopharyngeal and throat (NPT) infections, may be as important as the latter in the epidemiology of diphtheria. NPT and SL carrier prevalence of 7.8 % and 2.8 % were found among 1070 healthy individuals, 263 of whom had skin lesions, in six population groups. SL carrier levels ranged from 0.7 % in nonendemic areas to 18.9 % among case contacts. The total and NPT *C. diphtheriae* carrier prevalences were higher in 11 households with SL carriers compared to 22 NPT carrier households; infection persisted longer in SL carrier households. Twenty-one of the 38 individuals with SL *C. diphtheriae* infections had NPT infection, the strains being identical at both sites.

C. diphtheriae in skin lesions probably represents a skin equivalent of the respiratory carrier state; they have been found at every stage in a wide variety of lesions, and appear not to influence the spontaneous healing of lesions. Autogenous SL to NPT transmission was suspected as being causally related to several diphtheria cases. All other patterns of transmission were also noted. However, SL to SL, and SL to NPT transmission, is more consistent than NPT to NPT transmission with the seasonal incidence of diphtheria in the South.

Artificially acquired immunity, determined by history or antitoxin assay by the hemagglutination technique, was unrelated to either SL or NPT *C. diphtheriae* infection. Age was inversely related to NPT and SL infection (reflecting natural immunity). (SPR)

109 *Nursery Routines and Staphylococcal Colonization.* C. P. S. WILLIAMS* and T. K. OLIVER, JR., Dept. of Pediatrics, Univ. of Washington, Seattle, Wash.

Nurseries were opened at the University of Washington in September 1961, providing an opportunity to study coagulase-positive staphylococcal (staph) colonization for an extended period of time. The number of

infants admitted per year to the newborn nursery has risen from 814 in 1962 to 1374 in 1967. Average stay is four days. The anterior nares are cultured at discharge, as a monitor of staph colonization. For the first 20 months, care included caps and gowns for all personnel, masks for all except full-time nursery nurses, hairnets for female personnel, hand scrubbing with brushes using hexachlorophene on entering nursery, and handwashing with hexachlorophene between handling of infants. Infants were bathed with hexachlorophene in the delivery room and daily thereafter. The colonization rate for first 20 months in the newborn nursery was 4.2 %. After that time, sequential changes in routine were made: caps, masks, hairnets were discontinued, initial bathing deferred until infants achieved thermal stability, medical students and parents given free access to the nursery, brushes in handwashing discontinued, and finally, gowns discontinued except when actually handling infants outside an incubator, all with no rise in colonization and virtually no disease. Adjacent to the newborn nursery is a 16-unit intensive care nursery for low birth weight or other ill newborns, two-thirds of whom are outborn. Low colonization rates have also occurred here, despite a more extended stay and the occasional admission of outborn infants infected with staph. This study demonstrates that low staph colonization and disease levels can be maintained in nurseries for an extended period of time using careful handwashing and infant bathing with hexachlorophene. Furthermore, certain traditional elements of nursery routine may be eliminated, as long as surveillance is maintained so that rises in colonization may be detected promptly. (APS)

110 *Epidemiology of Enteropathogenic E. Coli (EEC) Infections.* MARGARET H. D. SMITH, KENNETH W. NEWELL* and JULIE SULIANTI*, Depts. of Pediatrics and Epidemiology, Tulane Univ., WHO, Geneva, Maternal and Child Welfare Service, Ministry of Health, Djakarta, Indonesia.

A prospective longitudinal study was undertaken in 57 households in Louisiana from June 1963 through June 1965, to describe characteristics of EEC-infected persons, duration of excreter state and factors influencing incidence and prevalence of infection. 8.2 % of 8,483 fecal specimens contained EEC serogroups. EEC was a total household rather than a childhood infection. However, more children than adults were infected; risk of secondary infection was higher if a child, 1–2 years of age, was the primary case. Findings suggest EEC introduction into households by foods, probably from zoonotic reservoir. (APS)

111 *Experiment in Updating Nursery Design Concepts.* SYDNEY SEGAL and EILEEN G. HASSELMEYER*, Department of Paediatrics, University of British Columbia, Vancouver, B.C., and PBIM, National Institute of Child Health and Human Development, NIH, Bethesda, Md.

Structural and legal impediments to the application of modern techniques of special care and clinical investigation were explored in an experiment in interdisciplinary communication and creativity. Participants from pediatrics, obstetrics, surgery, anesthesia, nursing, hospital administration, public health, industry, animal care, hospital architecture, as well as federal and state agencies, met for three days of interchange. Bottlenecks created by building codes had been imposed by clinical authorities of previous years and perpetuated by officials. Recommendations were for more flexibility of design to facilitate future changes, but meanwhile to redistribute bassinet space on a new basis, computed as to number of infants per given size of room, compartmentalization of nursery area specifically into close observation, intensive care, convalescence and standard full-term newborn care. Juxtaposition of labor and delivery room area, as well as pediatric services, was recommended. Attention was drawn to the possible advantages of movable walls, and to changes in air flow management designed to reduce cross-colonization and perhaps reduce the need for incubators. Functional changes included charting within each nursery, and use of the same special care nursery for postoperative management. Additional aspects will be presented to illustrate agreement as to the need for flexibility experiment in design and function, use of 'warehouse' space rather than oblong outside walls, and repeated reevaluation of the effects of future innovation.

(Sponsored by NICHD-NIH) (APS)

112 *Development of a Practical Neonatal Monitoring System for Systolic and Diastolic Blood Pressure, Heart Rate, Respiratory Rate and Temperature.* PAUL A. BYRNE* and JOHN W. BELD*, St. Louis Univ. School of Med., Cardinal Glennon Mem. Hosp., St. Louis, Mo., and Conductron Corp., St. Charles, Mo. (introduced by Arthur E. McElfresh).

Blood pressure is measured by an automatic system for auscultation of Karotkow sounds detected by a crystal microphone placed over the brachial, radial or tibial artery. A circuit activated by the Karotkow sounds controls a servo-system to inflate or deflate a cuff at the diastolic and systolic pressures. Electrical gating at a precise time interval controlled by the QRS complex eliminates many artifacts. Correlations between directly measured intraarterial blood pressures and those measured as described yield an almost perfect correlation.

	Direct aorta (mm Hg)	Indirect brachial (mm Hg)
Infant 1 (systolic)	64	62
Infant 1 (diastolic)	38	40
Infant 2 (systolic)	95	105
Infant 2 (diastolic)	48	50

Impedance pneumography is utilized in measurement of rate and patterns of respiration.

Heart rate, respiratory rate, temperature and blood pressure are read on meters calibrated appropriately and may be recorded.

Practicality and simplicity have been stressed in this system. It has been used to measure the blood pressure and/or monitor 75 infants weighing between 820 g and 5600 g (SPR)

113 *Oxygen Toxicity in Newborn Rats.* J. CRAIG, LIVIA H. REV-KURY* and G. KURY*, Harvard Medical School, Boston, Mass.

Twenty-one litters of newborn rats with appropriate controls (8 litters) were exposed to concentrations of 60 %, 80 % and 100 % oxygen for periods up to 9 days; the lower concentrations were mixed with appropriate concentrations of nitrogen. At levels of 100 % oxygen, the mothers died within 3 days with massive pulmonary edema and pleural effusions. The mothers, therefore, were removed from the 100 % oxygen for 12 hours of

each 24. The infant rats had growth retardation in comparison to the air-exposed controls. Incubation of the excised lungs of rats exposed to 100 % oxygen in tritated thymidine revealed 1/10 the frequency of labeled cells in comparison to the controls. The histologic changes in the fixed expanded lungs were evident only in the 80 % and 100 % oxygen animals after 6 and 4 days or more of exposure. These were qualitatively minimal. They consisted of overdistension of the alveolar spaces, irregular thickening of the alveolar walls and failure of growth of alveolar segments. No membranes, edema or effusions occurred. It is evident that the lung of the newborn rat is less susceptible to acute oxygen toxicity than the adult and that inhibition of lung and body growth may occur.

(Supported by grants USPHS HD 00144 and AEC [30-1] 3777.) (APS)

114 *A Quantitative Assessment of Lung Injury from Oxygen with and without Assisted Ventilation, and Air with Assisted Ventilation in Newborn Lambs.* R. DE-LEMOS*, J. WOLFSDORF*, R. NACHMAN*, J. BLOCK*, G. LEIBY*, H. A. WILKINSON*, T. ALLEN*, W. MORGAN*, A. HALLER* and M. AVERY, The Johns Hopkins Medical School, Baltimore, Md.

The pathogenesis of the pulmonary injury reported in infants and older individuals after prolonged use of assisted ventilation with oxygen is unclear, since in the clinical situation the respirator or the oxygen could conceivably be injurious or synergistic in producing injury. Fifteen lambs from 1–3 weeks of age were divided into three groups, those receiving 80+ % oxygen alone through a tracheotomy, others ventilated with 80+ % oxygen, and a third group ventilated with air from a volume-limited respirator. The following are a few of the measurements made:

	Oxygen alone	Oxygen and respirator	Air and respirator
Mean survival time (h)	36	55	86
Range	10–56	20–72	70—>100
Lung wt/body wt	0.040	0.037	0.024
Mean peak vol at a static pressure 45 cm H_2O (ml/g lung)	1.91	2.24	3.14

Radiographic and histologic findings, as well as surface film balance studies, support the trend shown in the table that no change was found with assisted ventilation with air for periods up to 100 hours; significant changes were associated with oxygen with or without a respirator, although they were of lesser severity in the caudal lobes, and tended to be patchy. (SPR)

115 *The Complications of Chronic Umbilical Vessel Catheterization.* VILDAN ERKAN*, WILLARD BLANKENSHIP* and MILDRED T. STAHLMAN, Vanderbilt Univ. School of Med., Nashville, Tenn.

Between October 1961 and June 1967, 317 newborn infants were subjected to umbilical vessel catheterization as part of a diagnostic and therapeutic regimen; 202 had hyaline membrane disease, the remainder a wide variety of serious problems. Number 5 French feeding tube catheters with platinum beads in the dead space and luer adapters were used. In 284 instances, the catheter lay in the abdominal aorta a mean of 48.8 hours; in 200 instances in the umbilical vein above the ductus venous a mean of 49.3 hours; and in 115 instances in the UV below the DV a mean of 52.1 hours. Venous catheters were used for infusion of fluids; aortic catheters for pressures and sampling sites. All infants received a penicillin 100,000 U/kg/day and chloromycetin 20 mg/kg/day without complications for duration of catheterization + 72 hours. Heparin was not used. Two hundred and thirteen survivors had had at least a six-month follow-up. One hundred and four died with 92 autopsies. Two hundred and seven esophagograms have been done on 155 survivors. Two survivors show residual complications. One has extrahepatic occlusion of the portal vein with bleeding esophageal varices, and one has asymptomatic occlusion of the right common iliac artery. One death was directly related to embolization of the superior mesenteric artery. Four infants showed localized necrosis associated with infusion of 0.3 M THAM. Three showed thrombosis of UV, DV or hepatic veins alone. Two had traumatic aortitis with adherent thrombi. One proven instance of sepsis occurred, not present at catheterization. The primary risk associated with chronic aortic catheterization in this series appears to be embolization, while the risk of venous catheterization is one of thrombosis and localized necrosis especially when the tip resides below the DV and when THAM is infused at that site. (SPR)

116 *Review of Experience with Umbilical Artery Catheterizations in the Newborn.* WILLIAM D. COCHRAN*, HEATHER T. DAVIS* and CLEMENT A. SMITH: Harvard Med. School, Boston, Mass.

In 387 infants over a 5 ½-year period, umbilical artery catheterizations were done for diagnosis, therapy or both. Two hundred and forty-nine of the infants had respiratory distress syndrome; 92 were either infants of diabetic mothers or premature infants with transient respiratory distress; 18 were 'dysmature'; 7 had severe erythroblastosis and 21 were classified as miscellaneous. Two hundred and thirteen of the infants were 2500 grams or under in birth weight. In most instances the catheter was left in place for 22–39 hours; the longest such period was 92 hours; the earliest insertion was 13 minutes and the latest 96 hours of age.

Complications (thrombosis, arteritis or perivascular hemorrhage at autopsy, and blanching of one leg or bleeding in surviving babies) occurred in 8 % of all infants. However, in only 3 % of all cases were those complications found at autopsy in potentially hazardous locations. Twenty-one per cent of the 86 total autopsies (out of 93 deaths) had one or more of the autopsy complications mentioned. No known subsequent effects have been observed following return of circulation in the group with blanched legs. (APS)

117 *Maturation of Chemical Thermogenesis in Premature Infants.* LEO STERN, ANGELES RAMOS* and JEAN LEDUC*, McGill University, Montreal Children's Hospital Research Institute, and Department of Physiology, University of Montreal, Canada.

Noradrenaline is the mediator of chemical thermogenesis in the newborn. Nine premature infants below 34 weeks gestational age at birth were studied sequentially to determine their ability to increase urinary noradrenaline excretion when changed from a 'neutral' (32–34°) to a 'cool' (25–27°) environment. On initial study, six of nine increased noradrenaline excretion on cold exposure with a mean increase of 0.350 nanog/kg/

min. Mean fall in rectal temperature for the group was 2.4° with the three nonresponding infants showing the largest fall (3–5°). When restudied two weeks later, all nine infants increased urinary noradrenaline excretion in the 'cool' zone. The increases were quantitatively greater with a mean rise of 0.717 nanog/kg/min, and mean rectal temperature fall for the group was now only 0.9° not exceeding 2.2° in any of the subjects. These findings suggest that the noradrenaline response is a major mechanism in the newborn's defence against cold and that its maturation in time parallels the development of thermal stability in the premature infant. (SPR)

118 Phospholipids in Human and Monkey Amniotic Fluid. STANLEY N. GRAVEN, Hartford Neonatal Center, Univ. of Wisconsin and St. Mary's Hosp., Madison, Wis.

Phospholipids in amniotic fluid at different gestations were studied as a possible indicator of fetal pulmonary development. Amniotic fluid was obtained from 40 mothers at gestations of 21–40 weeks. The phospholipids were extracted and separated by TLC. The phospholipid phosphorous determinations were performed.

All of the amniotic fluid samples from mothers with uncomplicated pregnancies and healthy fetuses contained significant concentrations of: (1) lecithin; (2) a fraction located below cephalin (probably a mixture of phosphatidylmono and dimethylethanolamine); and (3) a fraction that migrated with or near the solvent front (probably phosphatidic acid). The amniotic fluids contained very low concentrations of cephalin, lysolecithin and sphingomyelin. The lecithin extracted from the amniotic fluid migrated as a narrow homogenous band easily distinguished from the wide band observed for lecithins extracted from serum, egg yolk, or whole lung.

No lecithin or fraction (2), but a low concentration of fraction 3 (phosphatidic acid) was found in the amniotic fluid from a mother in labor at 34 weeks' gestation whose 1.77 kg infant developed RDS. A previous 1.93-kg-34-week infant born to this mother and a 1.80-kg-33-week infant of the mother's sister both had severe RDS.

Data on amniotic fluid from 25 monkey pregnancies at 75, 100, 125, 150 and 175 days' gestation also will be presented.

It is proposed that phospholipids of fetal pulmonary origin appear in the amniotic fluid as early as 21–24 weeks' gestation, but may be absent or in low concentration in amniotic fluid from pregnancies producing fetuses which appear to have a familial predisposition to the development of the RDS. (SPR)

119 Synthesis of Serum Albumin by Perinatal Piglets. L. A. PAGE* and R. LARDINOIS*, Dept. of Pediatrics, Stanford Univ. School of Medicine, Palo Alto, Cal. (introduced by R. E. Greenberg).

At birth the predominant serum proteins in the piglet are postalbumin (α-fetoprotein) and a prealbumin(s) recently described by this laboratory. Postnatally, there is a sharp fall in these proteins and a rise in albumin. We have examined the synthesis of albumin by perinatal piglets and slices of their livers, using immunologic techniques. The studies indicate that at least some of the rise in serum albumin reflects increased synthesis.

Intravenous or intraperitoneal injection of H^{-3} or C^{-14} labelled leucine into animals of various ages resulted in the following percentages of total serum protein label precipitable by an immunoelectrophoretically pure antiserum to porcine albumin: 2 % in three fetuses injected *in utero* and in their simultaneously injected, delivered littermate; 7–13 % in unsuckled newborns; 19 % in a 5-day-old animal; 25 ½ % in a 10-day-old animal; and 20 % in a 15-day-old animal. Preincubated slices from late fetal and unsuckled newborn animals showed only equivocal or no net synthesis of albumin, whereas slices from 10- and 14-day-old animals showed substantial synthesis (means 60–80 μg/g liver wet weight). Radioactive labelling of immune-precipitable albumin by liver slices from 5-, 10- and 14-day-old animals was 2–10 times greater than by slices from unsuckled newborns, using either tissue weight or protein as a basis. The percentage of total protein label as albumin was 1 ½–3 times greater in slices from older animals. Precipitations by antialbumin performed on double-labelled slice incubation products confirmed that the results above did not result from artifacts in the precipitation step itself. (SPR)

120 Direct, Serial Blood Sugar Determinations in Infants with Respiratory Distress Syndrome by Use of the Autoanalyzer Technique. JAMES J. SCHAFFER* and JACK W. LUKEMEYER*, Indiana Univ. School of Med., Indianapolis, Ind. (introduced by Morris Green).

Several investigators have demonstrated the favorable effects of the early administration of glucose and water in groups of high risk infants. Accordingly, a method was employed which permitted the use of an autoanalyzer for the direct determination of blood glucose levels in a group of 12 infants with respiratory distress syndrome. A sterile assembly connected to the autoanalyzer pickup tube was attached to a No. 8 polyethylene catheter inserted in the umbilical vein and samples of blood were withdrawn automatically. Bacteremia was not encountered. A solution of 10 % glucose with sodium lactate was infused via scalp vein at a rate to maintain normal glycemia and urinary flow. Favorable clinical response was manifested by a decrease in respiratory rate and cyanosis, an increase on muscular activity, and adequate urinary output (5–10 ml hourly). Various degrees of hyperbilirubinemia existed in the distressed infants; however, no exchange transfusions were required. Preliminary data indicate that the infusion of a relatively small quantity of glucose affords a rapid, significant rise in blood sugar, that glycosuria is common, and that the effect of glycogenolytic agents, e.g., epinephrine and glucogon, is unpredictable. The advantages of the autoanalyzer technique include: (1) the use of a small blood volume for determination; (2) reduced manipulation of the sick infants; (3) immediate visualization of accurate blood sugar levels; and (4) regulation of the infusion at a rate to insure normal glycemia and urinary flow. (SPR)

121 Clinical and Morphological Correlates in Acute Glomerulonephritis. JOHN E. LEWY*, LUIS SALINAS-MADRIGAL*, CONRAD PIRANI* and JACK METCOFF, Michael Reese Medical Center, Chicago, Ill.

Eighty-two biopsies and three autopsies were obtained on 49 patients with the clinical diagnosis of acute glomerulonephritis and serologic evidence of a recent streptococcal infection. Two patients died during the

acute stage of the disease and were autopsied. Forty-three biopsies were obtained during the acute phase (4–42 days after clinical onset). The severity of the histologic lesions were graded semiquantitatively from 0–4+ based on an assessment of a number of histopathologic features in the glomeruli, tubules, vessels and interstitium, and compared with concurrently estimated levels of glomerular filtration rate (creatinine clearance), blood urea nitrogen (BUN), antistreptolysin O (ASO) and total hemolytic complement titers, and semiquantitative urinalysis (Addis count). One or more follow-up biopsies associated with clinical and laboratory reevaluations were obtained on 24 patients for a period up to 5 years. In the acute phase, there was a statistically significant correlation between indices of histologic severity and impairment of creatinine clearance, and elevation of BUN. The morphologic changes did not correlate statistically with ASO or complement titers, or degree of hematuria, proteinuria, pyuria or cyllindruria. Twenty-five children were followed for two years or longer. One of these died from progressive disease and was autopsied, two revealed persistent histologic and clinical changes, and 6 revaled only slight persistent clinical abnormalities with almost complete resolution of their histopathology. Sixteen appeared to have completely recovered clinically, with morphologic resolution in all ten of these who were rebiopsied. (APS)

122 *The Morphogenesis of Renal Parenchymal Malformation.* JAY BERNSTEIN, Dept. Pathology, Albert Einstein Coll. of Med., New York, N.Y.

In previous studies we have shown that abnormalities of tubular development result from experiment hydronephrosis in immature animals. These alterations in epithelial maturation mimic very closely the histologic abnormalities seen in congenitally malformed kidneys. An extremely frequent complication of the experimental procedure, however, has been severe chronic pyelonephritis due to gram-negative bacilli. To establish that the developmental abnormalities are related to urinary obstruction rather than to chronic inflammation, similar studies were carried out in germ-free animals. Unilateral ureteral ligation in newborn rats resulted in a spectrum of histologic abnormality similar to that seen in conventional animals, except that inflammatory lesions were not encountered. The initial alteration appeared to be marked dilatation of collecting ducts and tubules, in which the epithelium retained or reverted to a primitive appearance. In the absence of tubular dilatation, even in severely hydronephrotic kidneys, primitive epithelial changes were inconspicuous. These observations support the concept that urinary obstruction during nephrogenesis and renal maturation is a major factor in the morphogenesis of parenchymal abnormalities. (SPR)

123 *Split Products of Fibrinogen in Patients with Uremia.* E. RICHARD STIEHM and CARL W. TRYGSTAD*, Dept. of Pediatrics, Univ. of Wisconsin Med. School, Madison, Wis.

The presence of split products of fibrinogen (SPF) in the serum as measured by an immunologic technique is a sensitive indicator of intravascular coagulation not detectable by other coagulation studies. Our prior studies (Midwest Soc. Ped. Research, 1967) indicated that serum from patients with several forms of renal disease have positive tests for SPF, and that their measurement provides useful diagnostic and prognostic information. We have extended these studies to include 25 children with uremia secondary to a variety of renal disorder. Sixty of 69 sera (87 %) from 20 of 25 patients (80 %) uremic patients were positive for SPF; patients with high blood urea nitrogen (BUN) had high levels of SPF. Coagulation studies were normal without evidence of fibrinolysis. Infusion of urea into a patient without renal disease resulted in a BUN of 90 mg/100 ml but no SPF in the serum. Serial studies of SPF indicate that in certain illnesses, e.g., hemolytic-uremic syndrome, anaphylactoid purpura, chronic nephritis, SPF are elevated during azotemic and disappear as the BUN returns to normal, suggesting that uremia causes the intravascular coagulation. In other illnesses, e.g., acute glomerulonephritis, lupus nephritis, SPF are elevated after azotemia disappears or without azotemia and are correlated with the activity of the renal disease, suggesting that intravascular coagulation is part of the pathologic process affecting the kidney. Heparin did not affect the SPF level in one uremic patient and may have aggrevated a preexisting bleeding tendency. (SPR)

124 *Anticoagulant Therapy of Progressive Renal Disease in Children.* ROGER C. HERDMAN*, JOHN R. EDSON* and ROBERT A. GOOD, Dept. of Pediatrics, Univ. of Minnesota Hospitals, Minneapolis, Minn.

Ten children with progressive renal disease including subacute nephritis, anaphylactoid purpura nephritis, chronic hypocomplementemic (CHN) and normocomplementemic nephritis and Wegener's granulomatosis were selected for treatment with several months of aqueous heparin (H) given subcutaneously every 8 hours in doses sufficient to keep clotting time always 2 ½ times normal; followed by long term warfarin (W) treatment in doses sufficient to double prothrombin time. In 5 cases an increase in renal function measured by inulin or creatinine clearance and in 4 cases a decrease in daily urine protein output occurred although it is not proven that this was due to therapy. Assays of circulating coagulation factors were performed. In most instances these were abnormal initially and in some instances were changed by therapy. Titers of hemolytic complement did not change except in 2 cases of CHN where they rose from 30–40 % of normal to normal during H. In such patients I^{125} B_{1C} survival was studied. Serial renal biopsies showed improvement in glomerular morphology in 3 instances by routine microscopy with disappearance of glomerular deposits of fibrin and/or immunoglobulin in 2 instances by fluorescence microscopy. The data firmly support the conclusions that H can return complement titers to normal in CHN and that in some instances quantitative abnormalities of coagulation factors can be corrected. There is suggestive evidence that in some types of renal disease anticoagulation may be worthwhile therapeutically.

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125 *Elevated Plasma Ketones, Free Fatty Acids and Insulin Levels in Renal Glycosuria.* P. W. HOUCK, A. M. GLASGOW and E. E. MCCOY, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Va.

Renal glycosuria is a disorder characterized by a low renal threshold for glucose and fasting plasma glucose (PG) within the normal range. Normal and abnormal glucose tolerance tests (GTT) have been reported in these patients. To explain the basis of abnormal GTT, the studies presented were undertaken. Simultaneous

studies of plasma ketone (PK), free fatty acid (FFA) and plasma insulin (PI) levels in the fasting state, and after glucose loading, were carried out in a three-year-old girl who has had continuous glycosuria of at least one year's duration in the presence of normal fasting PG values. During this time she has had two normal and two abnormal GTT's. In the fasting state and with normal PG values, markedly increased plasma levels of acetoacetate (PAA), β-hydroxybuterate (PβOHB), FFA, and PI were found. After glucose loading the PAA, PβOHB and FFA decreased to near normal, but PI levels remained high. These studies demonstrate that some patients with renal glycosuria and abnormal GTT have abnormalities of PAA, PβOHB and FAA metabolism, plus elevated PI levels. It is postulated that these abnormalities are a compensatory mechanism secondary to large renal losses of glucose. Treatment with frequent glucose feeding partially corrects these metabolic defects. (SPR)

126 *Identification and Analysis of Multiple Glycine Transport Systems in Isolated Mammalian Renal Tubules.* RICHARD E. HILLMAN*, ISIDORA ALBRECHT* and LEON E. ROSENBERG*. Departments of Pediatrics and Medicine, Yale University School of Medicine, New Haven, Conn. (introduced by C. D. Cook).

Studies in patients with hyperprolinemia and iminoglycinuria have indicated that the renal transport system shared by glycine and the imino acids is not the only mode of glycine transport in the kidney. *In vivo* and *in vitro* studies in rats have confirmed this impression but have not delineated the number or nature of these transport systems. In the present experiments, isolated rabbit renal tubules prepared by collagenase digestion provided a suitable tissue preparation with which to analyze glycine transport and its relation to that of other neutral amino and imino acids. At low glycine concentrations (0.01–1.0 mM), double reciprocal analysis of kinetic data revealed a K_m of 0.7 mM and a V_{max} of 2.5 mmoles/l/5 min. At higher concentrations (1–100 mM), a sharp break in the double reciprocal plot indicated the presence of a second transport system (K_m–67 mM; V_{max}–100 mmoles/l/5 min). Both systems were inhibited equally by cyanide, dinitrophenol, and by removing sodium from the incubation medium, but studies using L-analine and L-proline as inhibitors of glycine uptake provided evidence for a third transport process. Maximal inhibition of glycine uptake by proline or alanine alone never exceeded 30–40 %, whereas the combination of the two resulted in 60–70 % inhibition. About 30 % of glycine transport was not affected by any competing amino acid. We conclude that glycine is transported in the renal tubule by at least three distinct systems, one shared with the imino acids, one shared with the neutral amino acids, and one which may be specific for glycine. (SPR)

127 *Effect of Aminonucleoside (AMN) Injection on Amino Acid Incorporation into Protein of Rat Kidney-Cortex.* TAKASHI YOSHIDA*, YACOB Y. AL-UBAIDI* and JACK METCOFF, Michael Reese Hospital, Dept. of Ped., Chicago, Ill.

Since mitochondria are the major sites of intracellular bioenergetic processes, decreased oxidative phosphorylation, oxidative enzyme activities and O_2 uptake by kidney of AMN nephrotic rats suggests impaired mitochondrial energy metabolism. The effects of AMN on the incorporation of ^{14}C labeled amino acids into proteins of rat renal cortex *in vivo* and *in vitro* was used to test this hypothesis.

Leucine-U-^{14}C (10 μc/100 g body wt) was injected I-P, either 24 h or 7 days after a single i.v. injection of AMN (10 mg/100 g body wt) and 2 h prior to sacrifice of rats. ^{14}C incorporation into cytoplasmic protein was not affected 24 h after AMN injection, but markedly increased after 7 days (cpm/mg protein, mean $\pm$ SE, AMN versus control: 352 ± 29 versus 227 ± 10, $p < 0.01$). In contrast, ^{14}C incorporation into mitochondrial protein was decreased within 24 h after AMN injection (106 ± 6 versus 332 ± 35, $p < 0.01$), but increased after 7 days (256 ± 12 versus 147 ± 2, $p < 0.01$). When renal cortex slices of normal rats were incubated with glycine-2-^{14}C *in vitro*, addition of α-oxoglutarate (α-OG) to the medium increased ^{14}C incorporation into whole tissue protein (no substrate versus α-OG: 732 ± 20 versus 1728 ± 64, $p < 0.001$). ^{14}C incorporation by renal cortex slices of rats injected c AMN 24 h previously also increased (943 ± 44), and addition of α-OG increased it further (1807 ± 46). However, the increment of ^{14}C incorporation due to addition of α-OG was much less in AMN treated rats (145 ± 14 % versus 97 ± 15 %, $p < 0.05$). Thus, AMN may interfere with protein synthesis by cells of rat renal cortex, perhaps by inhibition of α-OG oxidation leading to impaired energy metabolism.

(Supported by USPHS grant AM-08951.) (SPR)

128 *The Specific Nature of Glomerular Bound β_{1C} Globulin in Nephritis: Correlation with Immunofluorescent Studies and Evidence for an in vivo Immune Reaction.* R. E. SPITZER*, A. JAMES MCADAMS and C. D. WEST, Dept. of Ped., Univ. of Cincinnati Coll. of Med., and Children's Hosp. Research Foundation, Cincinnati, O.

Immunofluorescent studies on renal biopsies have often revealed the presence of β_{1C} globulin. The specific nature of the protein seen by this technique, however, has never been determined. Utilizing fluorescein-labeled antibody directed against the three specific antigens of β_{1C}, we have demonstrated the existence of only two (A, D) of the three (A, B, D) determinant groupings normally found in native β_{1C} on the renal biopsy from a patient with chronic glomerulonephritis. The absence of the B antigen indicated that this deposited complement was not β_{1C} *per se* as formerly thought. Elution with EDTA buffered saline at pH 7.7 of a cortical-rich homogenate from the kidney of this patient and immunoelectrophoretic analysis against these same specific antisera showed not a single protein (native β_{1C}) but, instead, two separate breakdown products of β_{1C}–β_{1G} (containing the A and D determinants) and α_{2D} (containing only the D determinant). Since β_{1G} is the primary product of the reaction of β_{1C} with an immune complex, these findings are evidence of such a reaction occurring *in vivo*. Additional studies of biopsy specimens revealed that antibody to the D antigen of β_{1C} most often yields the strongest fluorescence; occasionally, it gives the only fluorescence. This would suggest that the use of labeled antibody directed against this determinant alone would be more specific and reliable than randomly tagged antisera. (SPR)

129 *Immunofluorescence of Cultured Kidney Cells Derived from Individuals with Renal Diseases.* NESRIN BINGOL* and EDWARD WASSERMAN*, Dept. of Ped., New York Med. Coll. N.Y. (introduced by Miriam Lending).

Twelve percutaneous renal biopsies were performed in patients with acute, subacute and chronic glomerulonephritis as well as lupus nephritis; biopsy specimens were processed simultaneously for light microscopy, immunohistology, electron microscopy and tissue culture.

A piece of cortex approximately 1 mm × 6 mm was chopped, washed, subjected to Trypsin and EDTA action for 20 minutes. Dissociated cells were cultured in Leighton tubes with growth media containing 30 % fetal calf serum, incubated at 37° and 5 % CO_2 in air.

One drop of cell suspension was stained with rabbit antihuman 7S gamma globulin and antihuman complement (β_{1c}–β_{1a}) labelled with fluoresceinisothiocyanate prior to culture.

Subcultures were prepared every week by 1:2 dilution and monolayers, grown on coverslips, stained with immune stains at weekly intervals.

Specific peripheral staining for 7S gamma globulin as well as for complement was observed on dispersed cells prior to culture.

Staining for antihuman 7S gamma globulin continued for several passages while staining with anti (β_{1c}–β_{1a}) disappeared rapidly.

Six control biopsies from individuals with non-immunological renal diseases did not show any immune staining on the first day or when propagated in the tissue culture under the same condition. (SPR)

130 *The Use of Azathioprine in Nephrotic Syndrome not Amenable to Steroid Therapy.* William T. Kniker*, William J. Flanigan* and George L. Ackerman*, Clin. Study Center, University of Arkansas Medical Center, Little Rock, Ark. (introduced by E. R. Hughes).

Twenty-two patients (11 children and 11 adults) with nephrotic syndrome were treated with azathioprine (Az) for periods of 2 to 31 months (average 14). All had been unresponsive to prednisone, required undue dosage, or could not tolerate the drug. Az was given daily in a dose of 3 mg/kg; steroids were used concomitantly in a third of the cases. Serial renal biopsies were obtained on each patient, the first before starting Az. Thirteen patients had proliferative glomerulonephritis, six rapidly progressive nephritis (RPN), two membranous changes, and one no histologic alterations.

Twelve cases did well, achieving sustained clinical remission and complete or nearly complete chemical remission. Two cases, both with proliferative disease, were partially improved. Eight (four proliferative; four RPN) manifested progressive disease. Six of the latter failed to respond to Az. In each of these, irreversible renal damage probably had occurred before Az therapy, as indicated by a C_{cr} below 20 ml/min/1.73 m² and/or vascular changes in the pre-Az biopsy. In one case, Az-induced pancytopenia led to fatal sepsis. In another patient, abrupt cessation of Az led to rapid renal deterioration and death. In summary, sustained remissions followed the use of azathioprine in 12 of 15 patients treated before irreversible renal damage had occurred. (SPR)

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