of the mother for measurements of flow, osmolality and clearance. The glomerular filtration rate (GFR) was studied utilizing inulin ^{14}C administered by a single rapid injection. Following surgery fetal urine osmolality increased by 2–4 times that observed later in pregnancy when a steady state was established. During surgical stress urine flow was frequently less than $0.03 \text{ cm}^3/\text{min}/\text{kg}$ body weight and urine osmolality was $>350 \text{ mOsm}$. The urine osmolality stabilized 3–6 days following surgery at a value markedly hypotonic to plasma (range 65–150 mOsm) and fetal urine flow increased to approximately $0.15 \text{ cm}^3/\text{min}/\text{kg}$ —over $1,000 \text{ cm}^3/\text{day}$ in a near-term fetus. Fifteen measurements of GFR in two fetuses gave a value of $1.05 \pm 0.04 \text{ cm}^3/\text{min}/\text{kg}$ (mean $\pm$ SEM). Column chromatography on Sephadex G-10 confirmed that there was no hydrolysis of inulin by fetal tissues. Recovery of approximately 85% of inulin ^{14}C in the fetal urine over a 3-day period suggests that the fetal kidney is the primary means of inulin excretion *in utero*. The techniques permitting fetal renal function measurements in unstressed animals will be described.

- 42 *Immaturity and Enhanced Renal Clearances of Native Proteins.* LEONIDAS G. MORPHIS, PAUL M. TAYLOR and PHILIP FIREMAN, Dept. of Ped., Univ. of Pittsburgh Sch. Med. and Magee-Women's Hospital, Pittsburgh, Pa.

Renal clearances of 3 plasma proteins were studied in the mature and immature human neonate and adult to test the hypothesis that capillary permeability may be directly related to immaturity in the human, as it is in the sheep [J. Physiol. 201: 567, 1969] and dog [Amer. J. Physiol. 213: 441, 1967]. Urine was collected for 18–24 h, filtered, dialyzed and concentrated by lyophilization. Protein concentrations were measured in the serum and concentrated urine by the technique of radial immunodiffusion with specific antisera. Eighty to 90% of the 3 proteins studied were recovered from pooled human serum diluted to the approximate protein concentration of urine and subjected to the same procedures. Clearances (mean $\pm$ SD) $\times 10^{-2}$ in ml/kg body wt/24 h and the number of subjects studied (in parentheses) follow:

	Transferrin	Albumin	$\alpha 1$ -Antitrypsin
Molecular weight	90,000	69,000	45,000
Premature (9)	7.2 ± 4.1	1.6 ± 0.8	2.1 ± 0.7
Full-term (5)	1.3 ± 0.4	0.3 ± 0.1	0.3 ± 0.1
Adult (5–8)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.04

Thus, there was 1.5- to 7-fold greater clearance for full-term infants than for adults and 5- to 7-fold greater clearance for premature than for full-term infants. Protein clearances did not correlate with molecular weights.

The data suggest that the immature as compared to the mature human has greater glomerular capillary permeability, greater tubular excretion or decreased tubular reabsorption either singly or in combination.

- 43 *Intrarenal Blood Flow Distribution (IRBF) in the Puppy Using Xenon Washout and Microspheres.* PEDRO A. JOSE, ALEXANDER G. LOGAN, GILBERT M. EISNER, LAWRENCE M. SLOTKOFF, CHARLES E. HOLLERMAN and PHILIP L. CALCAGNO, Depts. of Ped. and Physiol., Georgetown Univ. Sch. of Med., Washington, DC.

Total renal blood flow measured by the ^{133}Xe washout (Xe) rose from $1.5 \text{ ml/g}/\text{min}$ at 6 wks. to 3.5 at 16 wks. of age in 30 canine puppies. IRBF analyzed from the Xe curve showed Component I (CI) increased from $1.7 \text{ ml/g}/\text{min}$ at 6 wks. to 4.6 at 16 wks. and Component II (CII) increased from 0.9 at 6 wks. to 1.1 at 16 wks. The ratio CII/CI was 0.55 at 6 wks. and fell to 0.25 at 16 wks. ($p < 0.01$). The low cortical flow was also demonstrated by ^{169}Yb microsphere method. Anatomical vascular development using silicone rubber further showed an increase in cortical size with age. Cardiac output measured by indocyanine green in 8 anesthetized puppies over an age range of 6–16 wks. remained at $90 \text{ ml/kg}/\text{min}$ while RBF in $\text{ml/g}/\text{min}$ increased. The relatively low cortical flow at 6 wks. was associated with a PAH extraction ratio (E_{PAH}) of 0.44 and filtration fraction (FF) of 0.40 . E_{PAH} increased to 0.75 at 12 wks. and FF decreased to 0.24 at 16 wks. These studies directly demonstrate for the first time underperfusion of the young puppy renal cortex as compared to the medulla. Renal functional maturation is accomplished in the canine by an increase in cortical blood flow during growth.

- 44 *Regulation of Glucose and Ammonia Production in the Isolated, Perfused Rat Kidney.* RICHARD D. PROPPER and ROBERT E. GREENBERG, Dept. of Ped., Stanford Univ. Sch. of Med., Stanford, Calif.

Renal gluconeogenesis *in vitro* is enhanced by increased $[\text{H}^+]$. *In vivo*, both acute and chronic metabolic acidosis are associated with increased NH_4^+ excretion. Previous studies from our laboratory, using renal cortex slices, indicated that increased rates of ammonogenesis from glutamine are not dependent on corresponding changes in glucose production. The following studies have utilized perfusion of the isolated rat kidney to further investigate these interrelationships.

A modified, oxygen-saturated Krebs-Henseleit buffer, with added glutamine, was utilized as perfusate in an open system. The adequacy of this system was shown by the capacity to establish a $[\text{H}^+]$ gradient, and constancy of urine flow, glomerular filtration (C^+) and rates of gluconeogenesis for a period of two hours.

Glucose production and urinary ammonia excretion were markedly increased when the pH of perfusate was 7.1 as compared to 7.7 . However, total ammonia production (effluent + urinary) was decreased at the acid pH. Addition of α -ketoglutarate or pyruvate to the perfusate led to expected increases in gluconeogenesis while total ammonia production decreased. Addition of glucose to the perfusate had no effect on total ammonia production from glutamine.

These studies indicate the following: (1) urinary ammonia excretion is not directly correlated with total ammonia production; (2) enhanced ammonia production is not an obligate consequence of increased glucose formation; (3) rates of ammonogenesis are regulated by changes in concentration of Krebs cycle intermediates.

- 45 *A Relation Between α -Ketoglutarate Utilization and Excretion of Ammonia, Titratable Acid, Sodium Reabsorption and Renal Gluconeogenesis in Man.* J. METCOFF, J. E. LEWY, K. SCHARER, M. ORT, G. RUIZ and T. YOSHIDA, Michael Reese Hosp. and Med. Center, Dept. of Ped., Chicago, Ill.

In vivo utilization of the renotropic substrate α -ketoglutarate (α -Kg) is associated with a high RQ

and augments the glutamate pool. The glutamate pool influences glutaminase-I activity and NH_4 production. *In vitro* studies show that α -Kg may contribute to titratable acid (TA) formation, and to renal gluconeogenesis (RGP). RGP has been coupled with ammoniogenesis. The present studies in man demonstrate that these several processes may be interrelated through metabolism of α -Kg. Trans-renal extractions of α -Kg, glucose, oxygen and PAH with measurements of inulin and PAH clearances, Na , NH_4^+ and TA excretions were measured in 6 children with acyanotic congenital heart defects and 4 children with normal renal functions, recovering from glomerulonephritis, during an infusion of $\text{Na } \alpha$ -Kg. The urinary pH rose from 5.2 to 6.1. α -Kg infusion increased RGP, fractional Na reabsorption (R_{Na}), and TA, but decreased NH_4^+ excretion. Statistically significant correlations were found for renal α -Kg utilization directly with RGP, R_{Na} , QO_2 and, inversely, with NH_4^+ excretion. RGP also was inversely correlated with NH_4^+ excretion. QO_2 was correlated with R_{Na} , but could account for only 60–80% of α -Kg utilized. Increased R_{Na} was associated with increased glucose utilization. These observations indicate that the renal concentration and metabolism of α -Kg might regulate pathways leading to urinary acidification or Na reabsorption, as well as to RGP. The latter may be a metabolic control device for Na reabsorption. (Supported by NIH grant HD 168-03,04.)

- 46 *Net Acid Balance in Growing Infants.* JAMES C. CHAN, POUL A. KILDEBERG, KUND ENGEL and ROBERT W. WINTERS, Dept. of Ped., Columbia Univ. Coll. of Physicians and Surgeons, New York, NY.

Methods recently developed by KILDEBERG *et al.* [Acta paed. scand. 58: 321, 1969] permit the quantitation of net acid balance (NAB) in growing infants for the first time. NAB consists of the difference between net acid intake (NAI) and urinary net acid excretion (NAE). NAI, defined as the daily net gain of non-carbonic, non-metabolizable acid from all extrarenal sources, consists of contributions from G-I absorption, sulfuric acid production, organic anion (OA^-) excretion, skeletal mineralization (Ca^{++} balance) and soft tissue growth, each of which can be measured or approximated. Daily NAB was determined in 3 groups of 6 to 12 growing healthy premature infants fed 100–120 Cal/kg/d of either PM60/40R (group A, 50 periods), Similac^R (group B, 28 periods), or a standard cows milk formula (group C, 28 periods). All formulae contained surprisingly large loads of undetermined anion [$\text{UA} = (\text{Na}^+ + \text{Ca}^{++} + \text{Mg}^{++}) - (\text{Cl}^- + 1.8\text{P})$], being lowest in A and highest in C, but since fecal UA approximated oral intake of UA in all groups, net absorbed UA (potential base) was small. Sulfuric acid production was low in A and B and higher in C. Acid load due to urinary OA^- excretion was significant and similar in all groups, while that due to skeletal growth was proportional to Ca^{++} intake. Despite differences in the amount and pattern of NAI, NAE closely matched NAI so that NAB was not different from zero in any group. These results show that normal growth is accompanied by a relatively high level of acid production, the pattern and amount of which can be altered in response to type of dietary intake. They also suggest a heretofore unrecognized role of the G-I tract in defense against potential base load of commonly used formulae.

- 47 *Ontogeny of Amino Acid Metabolism in Mammalian Kidney.* K. BAERLOCHER, C. R. SCRIVER and F. MOHYUDDIN, McGill Univ., Montreal Children's Hosp. Research Inst., Montreal.

Mammalian kidney transports imino-acids and glycine at physiological concentrations on three types of membrane sites: a low-Km proline system, a low-Km glycine system and a 'common' high-Km system. Our recent studies (in press) show that absence of the two low-Km systems accounts for postnatal iminoglycinuria in mammals. Further studies with newborn rat kidney reveal additional ontogenetic features. *Uptake:* The low-Km proline system becomes active 1 week after birth. The low-Km glycine system is not active until the third week, indicating independent genetic control of these sites. *Efflux:* Reduced efflux rates explain increased internal accumulation of soluble amino acids in newborn kidney, at high and low internal concentrations. Efflux rates evolve with age in a manner suggesting that control of this membrane function is segregated from the control of uptake. *Catabolism:* Rates of oxidation (measured by $^{14}\text{CO}_2$ formation) and conversion of intracellular L-proline and glycine to other soluble metabolites, are lower in newborn kidney compared to mature kidney, while protein incorporation rates are enhanced in the former. These events do not account for the differences between newborn and adult kidney in net transport of substrate. Ontogeny of iminoglycine metabolism in kidney thus involves independent regulation of influx, efflux, incorporation and oxidation. (Supported by grants from MRC, Canada.)

- 48 *Uptake and Metabolism of L-Proline by Human Kidney Cortex.* MYRON GENEL, PHILIP G. HOLTZAPPLE and STANTON SEGAL, Children's Hosp. of Philadelphia, Pa.

A specific aminoaciduria involving glycine and the imino acids, proline and hydroxyproline, has been found in patients with defective proline catabolism (hyperprolinemia I and II) and in patients with renal imino-glycinuria, a selective defect in tubular reabsorption. To further elucidate the transport mechanisms of these amino acids, we have studied the uptake and metabolism of ^{14}C labeled L-proline in cortex slices of 'normal' human kidney obtained from surgical specimens. Metabolism of proline was considerable, with 80% or more of the calculated uptake recoverable as $^{14}\text{CO}_2$ during a short incubation over a wide substrate range (0.065 to 50 mM/L). Further, only 33–50% of tissue radioactivity was recovered as proline, the proportion increasing with increasing media concentration. Of the remaining tissue label, the majority was recovered as glutamic acid with proportions increasing with time to 75% at steady state conditions. Formation of glutamic acid was markedly inhibited by anaerobiasis. Uptake of proline demonstrated saturable kinetics, with two apparently distinct concentration dependent systems—high capacity, low affinity at high proline concentrations and low capacity, high affinity at low concentrations. Kinetics of oxidation followed an almost identical pattern. Glycine was a non-competitive inhibitor of proline uptake in the 'high' system and competitive in the 'low'; however $^{14}\text{CO}_2$ production was unaltered by glycine inhibition. Although kinetics of proline uptake could be determined in human kidney cortex, the data indicates that extensive metabolism precludes formation of a true concentration gradient and emphasizes a possible role of proline catabolism in the process of tubular reabsorption.