Insulin stimulates the rate of protein synthesis and has a marked effect on RNA metabolism. Previous work on diabetic insulins has shown that serum derived juvenile diabetic insulin is abnormally resistant to degradation by insulinase and that pancreatic diabetic insulin has a decreased capacity to stimulate the incorporation of glycogen into rat diaphragms. The purpose of this study was to examine the possibility that diabetic insulin is abnormal in its action on RNA synthesis. A series of cultures are inoculated simultaneously with 5×10^5 log phase diploid mouse fibroblasts of 3T6 strain. Growth is allowed until day four at which time the medium is replaced with fresh medium containing 0.5 mc of uracil-2-C^{14} (S.A. 20 mc/millimole). Pancreatic insulins from normal and adult diabetics were prepared by acid-ethanol extraction and added to the tissue culture in a concentration of 1000 μ units/per ml of nutrient medium. After a 48 hr. incubation period, the cellular monolayer is subdivided by conventional chemical methods and the C^{14} activity of the RNA fraction is estimated.

	Diabetic (5)				
No. plates	2	2	1	2	2
Mean C.P.M./plate	5541	3980	6683	5496	7285
	Normal (2)				
No. plates	2	2			
Mean C.P.M./plate	58941	45469			
	S.D.			P*	
Normal pancreas	52205 $\pm$ 10,050			< 0.001	
Diabetic pancreas	5797 $\pm$ 1,136				

(SPR)

16 *Hormonal Regulation of Bilirubin Excretion by Rat Liver.* LAWRENCE M. GARTNER, JUDITH GLUCK* and IRWIN M. ARIAS*, Albert Einstein College of Medicine, New York, N.Y.

In hypophysectomized (hypox) or thyroidectomized (thyrox) rats, the hepatic capacity to excrete bilirubin decreased progressively, reaching the maximal reduction, 40 % of normal, 7 days post-hypophysectomy and 18 to 25 days post-thyroidectomy. Both the volume of bile secreted and the concentration of bilirubin in bile were reduced. Hepatic uptake of bilirubin *in vivo* was unaffected by hypophysectomy but was reduced to 64 % of normal by thyroidectomy. Neither hypophysectomy nor thyroidectomy affected bilirubin glucuronide formation by liver homogenate *in vitro*. In hypox rats, the excretory defect was corrected after 6 days treatment with porcine growth hormone (2.0 mg/day) or thyroxine (1.25, 2.5 or 10.0 μg/day). In thyrox rats the excretory defect was corrected after 6 days treatment with thyroxine (2.5 or 5.0 μg/day). Treatment of thyrox rats with porcine growth hormone (2.0 mg/day/6 days) restored hepatic excretory capacity to 71 % of normal.

Although neither hypophysectomy nor thyroidectomy altered hepatic glucuronyl transfereas activity *in vitro* when bilirubin served as glucuronide acceptor, when o-aminophenol (OAP) served as glucuronide acceptor hepatic glucuronyl transferase activity declined gradually to 25 % of normal 7 days post-hypophysectomy and 18 days post-thyroidectomy. In hypox rats, the OAP conjugation defect was corrected after 22 days of thyroxine administration (10.0 μg/day), whereas 6 days treatment with either growth hormone or thyroxine failed to correct the OAP conjugation defect.

These studies reveal that growth hormone and thyroxine are important in the regulation of hepatic bilirubin excretion and suggest that the synthesis of ethereal (e.g. OAP) and ester (e.g. bilirubin) glucuronides may be catalyzed by different glucuronyl transferases. (SPR)

17 *Urinary C21 Corticosteroid Sulfates in the Urine of Premature Infants Pre and Post ACTH.* JACQUES R. DUCHARME, GILLES LEBOEUF and THOMAS SANDOR*, Depts. of Ped. and Med., Univ. of Montreal and L'Hôp. Ste. Justine, Montréal, Canada.

We have studied the urinary excretion of cortisol (F), cortisone (E), tetrahydrocortisol (THF), tetrahydrocortisone (THE), 6β-hydroxycortisol (6β-OHF) and corticosterone (B) in premature infants. Each specimen was extracted sequentially for free, β-glucuronidase and solvolysis liberated steroids; each metabolite was identified by constant isotope ratios, and quantitated by a double-isotope derivative assay. In 3 normal prematures studied within the first 48 hours of life, 6β-OHF, THF, F and B were mainly recovered as free or sulfated compounds. THE and E were mainly sulfurylated. In a 4th infant, with respiratory distress syndrome, studied similarly, 6β-OHF was mainly sulfated while THF was mostly as a glucuronoside. THE and B were equally glucuro and sulfo-conjugated while F and E were equally recovered in free or sulfated form. In a pool of 8 premature infants studied from two weeks of age, prior to ACTH, approximately 60 % of F and E were sulfo-conjugated, while a larger proportion of B was in the sulfate form. 6β-OHF and THF were predominantly excreted as sulfates, while THE was mostly unconjugated. Post ACTH, there was a considerable increase of all steroids and glucuro-conjugation seemed generally enhanced except for F. These studies suggest that: 1. in resting state, most C21 steroids are excreted unconjugated or conjugated with sulfuric acid, in accord with limited glucuro-conjugation; 2. some limitation in reduction of Ring A of the steroid molecule exists since more unreduced metabolites are recovered than in older children and adults; 3. glucuro-conjugation can be enhanced under stress or ACTH, suggesting that glucuro-conjugation is not maximal in resting state; 4. these metabolic particularities persist at least up to four weeks of age. (SPR)

18 *A New Form of Congenital Adrenal Hyperplasia.* MARIA I. NEW* and RALPH E. PETERSON*, Cornell Univ. Medical College, New York, N.Y. (introduced by W.W. McCrory).

A new form of adrenal hyperplasia in a 12-year-old boy is being described. The unique features are: 1. classical signs of primary hyperaldosteronism, i.e. mild hypertension, hypokalemic alkalosis, low plasma renin, hypervolemia and fixed hyperaldosteronism unaffected by sodium restriction or excess; 2. low normal plasma levels of cortisol, corticosterone but elevated plasma aldosterone; 3. elevated plasma ACTH; 4. low normal urinary free cortisol, 17OH, 17KS, pregnanetriols which respond poorly to ACTH; 5. normal rise of plasma testosterone to chorionic gonadotrophin; 6. marked decrease of aldosterone production and marked fall in elevated blood pressure following treatment with glucocorticoids; 7. after 6 months of prednisone therapy blood pressure and aldosterone response to restriction and administration of sodium remain normal. Turnover rates of corticosterone (B), desoxycorticosterone (DOC), cortisol (F), and desoxycortisol

(S) studied under conditions of: sodium restriction and excess; ACTH stimulation; Metyrapone inhibition and Decadron suppression in 2 normotensive siblings and father reveal that only the patient demonstrates deficiency in cortisol secretion but the hypertensive mother has an abnormal aldosterone regulation. On the basis of steroidal data obtained, a partial 17-hydroxylase defect in the adrenal and not the gonad appears to explain the syndrome best. This form of hypertension is noteworthy because it is alleviated by medical treatment and may be misdiagnosed as primary hyperaldosteronism.

Baseline values	Normal for size	Patient	Twin sister	Brother
Urine aldosterone	up to 14 μg/d	17–26	12	9.5
Plasma aldosterone	3–15 mμg %	24	–	–
Plasma ACTH	0.3–0.7 mu %	1.5	0.3	0.4

(SPR)

19 *Familial Growth Hormone (GH) Deficiency.* BAGHER M. SHEIKHOLISLAM* and ROBERT S. STEMPFEL, Jr., Duke University Medical Center, Durham, N. C.

Isolated GH deficiency has been observed in a number of instances, but its occurrence as a recessively inherited defect has been previously documented only in adult members of two families. The present study concerns a family of 9 in which the father and 4 siblings had growth retardation, truncal obesity, facial infantilism and impaired glucose tolerance. Plasma GH responsiveness to insulin-induced hypoglycemia and to bacterial pyrogen administration was absent in those whose growth was retarded, but indirect studies of thyrotropin and adrenocorticotropin demonstrated the presence of these pituitary factors in all members of the family. Human GH administration to the 18-year-old, dwarfed, sexually infantile, female sibling resulted in objective changes in sexual maturation, alterations in fat distribution and greatly accelerated growth. The similarly treated 9-year-old male sibling experienced marked increase in the rate of statural growth, but remained sexually infantile. No discernable change in urinary corticoid responsiveness to adrenocorticotropin and no measurable change in gonadotropin excretion occurred during the therapeutic period. Studies of glucose tolerance and plasma insulin responsiveness in 4 of the dwarfs revealed an increase in peripheral resistance to insulin during GH administration. It is concluded that this form of familial dwarfism is related to GH deficiency and that the defect is monotropic. The patients described clearly illustrate the natural course of the condition, wherein delayed adolescence is the rule rather than the exception. Observations presented support the possibility that GH alters responsiveness of endocrine end-organ tissues. Serious doubts are raised regarding the validity of diagnoses which exclude hypopituitarism in patients with retarded growth on the basis of eventual sexual maturation. (APS)

20 *Growth Hormone (GH) Responsiveness to Stimulation by Insulin Hypoglycemia, 2. Arginine, 3. Piromen in normal and Growth Deficient Children.* M.H. MAC GILLIVRAY*, T. ACETO, Jr.* and L.A. FROHMAN*, Departments of Pediatrics and Medicine, State University of New York at Buffalo, Buffalo, N.Y. (introduced by M.I. Rubin).

Plasma GH levels in 11 normal children, in 5 children with hypothalamic-pituitary disorders, in 8 children with idiopathic GH deficiency and in 4 children with a constitutional growth delay were examined in the fasting state and following stimulation with insulin hypoglycemia, arginine, and bacterial pyrogen (Piromen). Normal children exhibited a marked fluctuation in fasting GH levels (radioimmunoassay). An elevated prestimulatory GH value was associated with a diminished response. Of the 3 stimuli, insulin hypoglycemia resulted in the highest GH levels. In normal children, GH responses to Insulin Hypoglycemia ($\bar{x}$ 20.4$\pm$6.4) Arginine ($\bar{x}$ 15.2$\pm$4.3) and Piromen ($\bar{x}$ 9.9$\pm$2.8) were not significantly different because of the marked variability observed within each group. In children with a constitutional growth delay, both fasting and poststimulatory GH values were indistinguishable from normal. In idiopathic GH deficiency, GH values prior to stimulation ($\bar{x}$ 2.4$\pm$0.4) and after the three stimuli ($\bar{x}$ 3.6$\pm$1.0) were significantly lower than those values observed in the normal or constitutional delay groups. The data suggest 1. Insulin Hypoglycemia, Arginine and Piromen are suitable stimuli with which to evaluate GH responsiveness; 2. failure to respond to stimuli in normal children is associated with elevated resting levels of GH; 3. children with idiopathic GH deficiency have significantly lower pre and post stimulatory GH values than do the normal but do respond better to stimulation than children with pituitary-hypothalamic disorders; 4. constitutional delay in growth is not associated with abnormal GH responsiveness; 5. normal GH secretion can be diagnosed if a resting GH level 6 mμg/ml. (APS)

21 *A Genetic Variant of Human Mitochondrial Malate Dehydrogenase.* JEAN A. CORTNER* and RONALD G. DAVIDSON*, Roswell Park Mem. Inst. and State Univ. of N.Y., Buffalo, N.Y. (introduced by Mitchell I. Rubin).

The enzyme malate dehydrogenase (MDH) is known to exist in two distinct forms in most mammalian tissues. One is present in the cytoplasm as a soluble enzyme (S-MDH), and the other is bound to the mitochondria (M-MDH). Among the distinct physical and chemical properties of these two enzymes are the different patterns seen after starch gel electrophoresis and specific enzymatic staining (see diagram: usual). Recent studies have suggested that the usual minor mitochondrial bands (1–4) are conformational modifications of a single protein, band 5. We have previously reported a rare genetic variant of soluble MDH found in the red cells of one apparently normal Negro female out of 3000 individuals examined. This report concerns a new inherited electrophoretic variant of M-MDH found in either leukocyte or placental extract of 3 of 350 unselected normal individuals. The electrophoretic pattern of this variant is shown in the diagram. The two additional major bands of MDH activity have identical electrophoretic mobility with minor bands 4 and 3 of the normal mitochondrial pattern. Family studies of two of the probands show that affected individuals are heterozygous for a relatively common autosomal mutant gene. All of the individuals found to have the mitochondrial variant have normal S-MDH patterns, adding further evidence that the two intracellular forms of MDH are under the control of different genes. (SPR)