Assignment of human thymidine kinase gene locus to chromosome 17 by identification of its distinctive quinacrine-fluorescence in man/mouse somatic hybrid cells. ORLANDO J. MILLER, PENELOPE W. ALLDERDICE, DOROTHY A. MILLER, WILLIAM R. BREG, and BARBARA R. MIGEON. *Coll. of Phys. and Surg., Columbia Univ., N. Y., N. Y., Yale Univ. Sch. of Med., New Haven, Conn., and Johns Hopkins Univ. Sch. of Med., Baltimore, Md.*

Human chromosomes are preferentially eliminated from man/mouse somatic hybrid cells. Two groups of workers obtained viable hybrids by mixing human cells with a murine cell line lacking thymidine kinase and growing the mixture of cells in a selective medium in which cell survival required the presence of this enzyme. The hybrid cells usually contained a single human submetacentric chromosome, whose size and shape suggested that it was a member of the E-group, either chromosome 17 or 18. This chromosome presumably carries the human thymidine kinase locus.

Chromosomes of the E-group can be readily identified in human cells by their distinctive quinacrine-fluorescence patterns. By applying this technique to metaphase figures from one of the hybrid cell lines studied by Migeon and Miller (Science 162, 1005, 1968), we have found that the submetacentric chromosome which is present has the characteristic quinacrine-fluorescence pattern of a human chromosome 17.

Human chromosome identification by differential staining. PATRICE R. CHERNAY, LILLIAN Y. F. HSU, and KURT HIRSCHHORN. *Mt. Sinai Sch. Med., City Univ. N. Y., N. Y.*

We have employed the differential staining technique of F. Arrighi and T. C. Hsu (1971) to identify individual human chromosomes. After pretreatment with RNAase, the DNA is denatured with NaOH, renatured with saline citrate buffer and then stained with Giemsa. Our studies have shown that in addition to the most distinct densely staining area of the distal 273 of the long arm of the Y chromosme, the Nos. 1, 3, 9, 11, 16 and 17 chromosomes carry a densely staining area on the long arm adjacent to the centromere, most noticeable in No. 1; No. 18 has a densely staining area on the short arm, close to the centromere. Studies of patients with trisomy 21 and trisomy 13 demonstrated that chromosomes No. 21 and 13 are identifiable. The three No. 21 chromosomes showed densely stained centromeres and two Nos. 22 showed lightly stained centromeres. The three No. 13 chromosomes in trisomy 13 had dense staining at the centromeres and on the long arms in comparison to those of Nos. 14 or 15. The basis of this differential staining technique is that renatured DNA appears better able to combine with stain than partially denatured DNA. It is apparently the repetitive DNA associated with constitutive heterochromatin which renatures most rapidly and is stained most densely. This method may be very useful in identification of structural as well as numerical chromosomal aberrations.

The origin of some bone marrow fibroblasts. KURT HIRSCHHORN, JEAN HENTEL, and JESSICA W. GRANT. *Mount Sinai Sch. of Med., City Univ. New York, N. Y., N. Y.*

An attempt was made to determine the origin of bone marrow fibroblasts which almost always appear when bone marrow aspirates and explants are grown on solid surfaces in tissue culture. Bone marrow aspirates from two individuals with chronic myelogenous leukemia demonstrating the Ph-1 chromosome and from an individual with acute leukemia demonstrating the trisomic C-group karyotype served as sources of the fibroblasts. These were analyzed for the presence of the marker chromosomes found in the leukemic cells of these patients. Over half of the dividing fibroblasts demonstrated the marker chromosomes. This positive finding indicates that at least some bone marrow fibroblasts are derived from hemopoietic stem cells. These cells should, therefore, prove useful in the study of cellular differentiation.

The value of fluorescence microscopy in studying abnormalities of G group chromosomes. LESTER WEISS and MARILYN DULLY. *Henry Ford Hosp., Detroit, Mich.*

Caspersson et al demonstrated that chromosomes stained with quinacrine mustard and examined under ultraviolet light had distinctive patterns of fluroescence. This technique has been used to study the G group chromosomes from 25 individuals.

The pattern of fluorescence of the G group chromosomes from 10 normal individuals was determined. The very bright fluoresence on the distal end of long arm of the Y chromosome, as described by others, was apparent. The 4 autosomes could be separated into 2 distinct pairs. One pair had a broad band of fluorescence encompassing ⅔ of the proximal long arm. The second pair had a small area of increased fluorescence in the region of the centromere and short arm. The trisomic chromosome in 9 patients with Down's syndrome was the one with a broad band of fluorescence on the proximal ⅔ of the long arm. Chromosome #21 is smaller than chromosome #22. In a family with a G group marker, the fluorescence technique made identification of the marker chromosome, as #21, possible.

Chromosomes from 2 phenotypic males with XX sex chromosomes were examined. The brightly fluorescent region of the long arm of a Y chromosome was not found translocated on any part of the genome. These data plus morphologic considerations indicate that if any Y material were present, it could only be short arm DNA. This is further evidence for male determinants being located on the short arm of the Y chromosome.

Quinacrine mustard staining and UV microscopy is a new technique that enables us to identify specific chromosomes and regions within chromosomes.

CARDIOLOGY

Assessment of systemic and pulmonary baroreceptor function in intact and unanesthetized fetal and newborn lambs. ELLIOT SHINEBOURNE, EERO VAPAAVUORI, ROBERT WILLIAMS, MICHAEL HEYMANN, and ABRAHAM RUDOLPH. *Cardiovas. Res. Inst., Univ. of California, San Francisco, Calif.*

Baroreceptor responses have been observed in exteriorized fetal lambs, but there have been no quantitative studies of changes with maturation in fetuses in utero. In 9 fetal and 3 newborn lambs an inflatable balloon catheter was passed from the femoral artery into the descending aorta. In 7 other fetuses inflatable balloons were placed around the pulmonary artery (PA) or the aortic isthmus. Vinyl catheters were positioned in a brachial or carotid artery, and catheters and ECG leads were exteriorized. Arterial pH, PCO_2 and PO_2 were normal in all studies. Reflex bradycardia in response to blood pressure elevation by balloon inflation was measured repeatedly for several weeks. Baroreceptor sensitivity was expressed as the regression coefficient of the beat-to-beat relationship between systolic (SP) and pulse (PP) pressure, and the subsequent R-R interval (R-R) or heart rate (HR). In over 100 observations we found: (1) elevation of systemic but not main PA pressure elicits reflex bradycardia; (2) reflex bradycardia could be elicited in all animals but was frequently absent in fetuses less

than 120 days gestation; (3) reflex bradycardia was blocked by atropine but not influenced by propranolol; (4) in fetuses the regression coefficients of SP or PP vs. R-R increased with gestational age, but not when SP or PP was correlated with HR because of the lower initial HR in older fetuses; (5) lambs had significantly higher regression coefficients for all correlations than fetuses. We have not yet evaluated if baroreceptor responses depend on gestational age and initial HR in combination.

Cardiovascular responses to autonomic blockade in intact fetal and newborn lambs. EERO K. VAPAAVUORI, ELLIOT A. SHINEBOURNE, ROBERT L. WILLIAMS, MICHAEL A. HEYMANN, and ABRAHAM M. RUDOLPH. *Cardiovas. Res. Inst., Univ. of California, San Francisco, Calif.*

Sympathetic and parasympathetic activity have been studied in exteriorized fetal lambs but not in intact fetuses in utero. We placed vinyl catheters in a systemic artery and hindlimb vein in 20 fetuses from 92 days of gestation to term and also in 3 newborn lambs. The animals were allowed to recover from surgery for 2–3 days and then studied daily for several weeks. Maternal and fetal pH, PCO_2 and PO_2 were normal in all experiments. The blood pressure (BP) and heart rate (HR) responses to selective intravenous injection of propranolol (1 mg/kg), practolol (1–2 mg/kg), atropine (1.0 mg/kg), phentolamine (0.1 mg/kg), phenoxybenzamine (5 mg/kg) and tyramine (0.25 mg/kg) were recorded. The adequacy of autonomic blockade was confirmed by absence of response to acetylcholine (5–15 μg/kg), isoproterenol (0.1 μg/kg) or methoxamine (50 μg/kg). Changes in HR from resting levels without significant BP change were observed as follows: propranolol 5–25% decrease, practolol 8–22% decrease and atropine 0–64% increase. Response to beta-adrenergic blockade did not vary significantly with gestational age. Phentolamine and phenoxybenzamine decreased systolic BP 2-14 and diastolic BP 2-12 mm Hg in 19/21 animals and tyramine increased systolic BP 7-60 and diastolic BP 10-46 mm Hg in 7/7 animals. No significant difference in sympathetic responses were observed in fetal and newborn lambs, indicating HR and BP to be under similar autonomic control at all ages studied.

Catecholamine uptake and storage of the newborn rat heart during post-natal development. GERALD F. ATWOOD and NORMAN KIRSHNER (Intr. by Madison S. Spach). *Duke Univ. Med. Ctr., Durham, N. C.*

The ability of atria, removed from Sprague-Dawley rats at ages 1–21 days, to take up and store catecholamines was studied to correlate this activity with the previously observed physiologic immaturity of the cardiac sympathetic nervous system in the neonate. The cardiac tissue was incubated in Krebs-Henseleit bicarbonate buffer containing 10^{-5} M iproniazid and 10^{-7} M H^3-norepinephrine (H^3NE). Total uptake and subcellular distribution at 1, 4, 7, 14, and 21 days were determined at various time intervals between 5 and 30 minutes. Uptakes at O°C. served as controls.

Uptake rates were expressed on a per gram wet weight basis. There was a small amount of uptake observed during the first post-natal day (0.515 nanagrams/minute) which increased only slightly by 4 days of age (0.683 ng/min). The most significant change occurred between 4 and 7 days (115%). Uptakes at 7, 14, and 21 days were similar to adult values. Since reserpine effectively blocks uptake of catecholamines into the storage granule, 10^{-5} M reserpine was added to the incubation media to determine the role of the granule in the previously observed uptake. Reserpine inhibited the 30 minute uptake 57% during the first day of life increasing to 70% inhibition by 7 days of age. The uptake observed in the microsomal (granular) fraction showed similar developmental patterns and effect of reserpine. These studies suggest a marked inability of the newborn rat heart to take up and store norepinephrine due to either decreased number of storage vesicles or an immature uptake mechanism in the granule.

Energy production in the developing heart. ROBERT WELLS, BURTON E. SOBEL, and WILLIAM F. FRIEDMAN. *Univ. of Calif., San Diego Sch. of Med., La Jolla, Calif.*

The influence of growth on myocardial energy metabolism is not clear. Mitochondria are the main source of production of ATP in cardiac muscle. Accordingly, mitochondria from the hearts of 9 fetal and 7 newborn lambs, and 9 adult sheep were isolated in KCl-albumin-EDTA media, studied polarographically, and compared biochemically. No age-related differences were found in P/O ratios, a measure of efficiency of ATP production, with either succinate or glutamate as substrate; or in ATPase activities, in the presence or absence of DNP. However, mitochondria from fetal and newborn animals had significantly increased maximum O_2 consumption/mg protein in the presence of ADP (state III respiration) (0.19 ± 0.01 S.D. and 0.17 ± 0.01 μatoms/min.) compared to the adult (0.10 ± 0.01, $p < 0.001$). Thus, increased respiratory control ratios, a measure of the dependence of respiratory rate on ADP, were increased in the fetus (12.9 ± 0.6) and newborn (15.6 ± 2.6) compared to the adult (9.5 ± 0.5 $p < 0.001$). O_2 consumption in mitochondria uncoupled by DNP, was highest in the fetus and newborn (0.30 ± 0.02 and 0.29 ± 0.02 μatoms/mg protein) compared to the adult (0.16 ± 0.01, $p < 0.01$). These augmented respiratory rates in mitochondria from the youngest hearts may reflect increased electron transport, a view consistent with the finding of 56% of 65% greater cytochrome oxidase activities in fetal and newborn heart mitochondria, respectively, when compared to the adult. Thus, age-dependent differences exist in cardiac energy metabolism that are of potential importance to our understanding of myocardial function in the perinatal period.

Alteration of fetal pulmonary vasculature by maternal hypoxia. STANLEY J. GOLDBERG, RICHARD A. LEVY, BIJAN E. SIASSI, and JOANNE BETTEN. *Univ. of Arizona, Tucson, Ariz., UCLA, and USC Schools of Med., Los Angeles, Calif.*

A syndrome of pulmonary vascular obstruction in the newborn human in which massive right to left shunting occurs through the ductus arteriosus has been recently described. In an effort to simulate the syndrome in an animal model, pregnant rats were maintained in atmospheres containing 13%, 40% and 20% oxygen. The remainder of the atmosphere was nitrogen. In all other respects, pregnant rats were treated the same. Pregnant rats were removed from their experimental atmospheres during delivery so that all newborns were delivered into room air. Newborn rats were sacrificed at birth, the fourth, fifth, tenth, twelfth, thirteenth and twenty-first days of life. The entire lung was sectioned and stained so that intima and media could be easily distinguished. The medial to diameter ratio of all arteries between 50–150 microns (total = 474) were measured according to the technique of Wagenvoort. The mean ratio of neonatal arteries of progeny of hypoxic mothers was significantly thicker for each size group than those born of control or hyperoxic mothers. Thickening