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ABSTRACTS

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PLENARY SESSIONS

1 *Autonomic Innervation of the Developing Heart: Pharmacological, Biochemical, and Histochemical Correlations.* W. FRIEDMAN*, C. COOPER*, P. POOL*, D. JACOBOWITZ*, and E. BRAUNWALD*, University of California, San Diego, School of Medicine, La Jolla, California (introduced by A. M. Rudolph).

This investigation was undertaken to define the autonomic innervation of the mammalian fetal heart. Isolated fetal lamb myocardium, obtained near term, was found to be equally responsive to the negative inotropic effects of acetylcholine, but supersensitive to the positive inotropic effects of norepinephrine (NE) compared with the adult. Cocaine, which blocks neural NE uptake and binding, potentiated the response of adult but not of fetal myocardium to NE; following cocaine, adult and fetal myocardium exhibited equal responsiveness to NE. This finding, and equal responsiveness of fetal and adult myocardium to isoproterenol despite fetal supersensitivity to NE, indicate that beta-receptor sensitivity is similar in fetal and adult myocardium. Cardiac NE concentrations were significantly lower in the fetal than the newborn lamb ($p<0.001$), and highest in the adult ($p<0.002$). The activities of intraneural enzymes involved in NE biosynthesis and degradation, tyrosine hydroxylase and monoamine oxidase, respectively, were lowest in fetal heart, higher in the newborn, and highest in the adult ($p<0.001$), whereas the activity of catechol-o-methyl transferase, an extraneural degradative enzyme, was highest in the fetus. Histochemical studies showed sparse catecholamine-containing neurons in fetal myocardium and a progressive increase of these nerves with age. In contrast, the extent of parasympathetic innervation of the fetal and adult hearts appeared similar. Thus, biochemical, pharmacological, and histochemical techniques describe differential development of parasympathetic and sympathetic innervation in the mammalian heart, and allow an explanation for age-dependent differences in autonomic responsiveness to the adrenergic, but not the cholinergic, neurotransmitter. (Supported by HE-12373.)

Discussion

DAVID W. SMITH (University of Washington Medical School): There being some variability from one animal to another, have you had an opportunity to look at this in the human newborn to see if the sympathetic innervation of the heart is of a comparable type and state to that which you noted in the lamb?

W. FRIEDMAN: We have not looked at this in humans. Some years ago Dr. ROBERT GREENBERG reported levels of catecholamines in human abortuses of about 0.3μg/g of cardiac tissue, which is about a fifth of the adult concentration of catecholamines.

We have examined dogs, cats, rats, and rabbits, and there are species differences with respect to when catecholamines reach adult levels. For example, in the rabbit it takes about 3 weeks after birth for adult levels of catechols to be obtained, whereas in the lamb by about age 3 or 4 days adult levels are achieved. I think that this reflects an adult density of sympathetic innervation.

In the calf there doesn't seem to be a major difference between the late fetus, early newborn, and the adult. All the other species that I have mentioned do show rather striking reductions in cardiac stores of norepinephrine early in life.

HORACE L. HODES (Mount Sinai Hospital, New York): Can you distinguish by study of the fine structures by EM any difference between the two kinds of nerve endings?

Dr. FRIEDMAN: We have not looked at ultrastructure in this species. Others have indicated that there does not seem to be the same density of core vesicles in nerve endings in the young rat. But we have not done that in the lamb.

2 *Chemistry and Histology of the Human Aorta During Growth.* FELIX P. HEALD, ANTHONY C. CHUNG* and JOHN C. HOUCK*, Dept. of Pediatrics, George Washington University Medical Center and Children's Hospital of the District of Columbia, Washington, D.C.

One hundred thirty-seven aortas were studied ranging in age from the newly born to 17 years. Chemical analysis of the aorta included phospholipids, triglycerides, free and esterified cholesterol, and ground substance (glycoprotein, etc). For chemical analysis, the tissue was pooled by age and sex. Sections were taken to include the fatty streak and adjacent normal aorta. Each section was stained with hematoxylin and eosin, oil-red-O (lipids), periodic acid Schiff (glycoproteins) colloidal iron (acid mucopolysaccharides and Masson (collogen). Results: When the lipid concentrations of 'plaque' pools are compared with those of normal pools (> 2 years) both the free cholesterol (0.348 ± 0.126 mg/m mole N) and ester cholesterol

* By invitation

(0.304 ± 0.145 mg/m mole N) concentrations are significantly higher ($p<0.01$) than the free cholesterol (0.263 ± 0.059) and ester cholesterol (0.039 ± 0.026) concentrations in the normal pool. No significant differences are noted in phospholipid and triglyceride concentrations. Beginning at 4 years, there is generalized non-specific thickening of the intima. The initial change in the fatty streak appears to be focal increase in intimal thickening with poor peripheral demarcation. There is increased mucopolysaccharide material appearing as 'extracellular lakes' with a small amount observed to be intracellular. The gross lesions stain positively for intra- and extracellular fat when they are observed to be yellow and slightly raised. The age of the lesion rather than the age of the aorta determines its appearance. There is no evidence of glycoproteins, calcification or cholesterol crystals in the aortas.

Discussion

WILLIAM T. KNIKER (University of Arkansas Medical Center): Dr. COCHRANE and I showed, some years ago, that there are at least two factors to explain why lipids, macromolecules, serum proteins, etc., collect in the intima of arteries. Such aggregations occur at areas of turbulence where there is mechanical damage to the intima which increases permeability locally. Also at the same sites, platelets and leukocytes are damaged, releasing permeability factors that enhance the deposition of materials from the serum.

There is accumulating evidence suggesting that increased serum levels of lipids are associated with increased permeability of the intima. If you believe this to be true, what are your thoughts as to the mechanism for the phenomenon?

ANTHONY C. CHUNG: I don't know whether it actually alters the membrane permeability or not. Several animal *in vitro* experiments have shown that the uptake of labeled cholesterol by the aorta is increased with the increase of cholesterol concentration in the medium. However, the uptake by the boiled aorta is similar to the unboiled so it is not clear whether the increased uptake is due to mass action or membrane permeability. On the other hand, the fact that the infiltration of cholesterol is not just a passive process has been demonstrated in that a considerable portion of the free cholesterol taken up from the medium by the aorta is esterified.

Dr. D. B. ZILVERMIT has calculated from experimental data that the cholesterol influx into the arterial wall is relatively high and that if there were no removal mechanism present, the cholesterol content would be too high in a relatively short time.

Whether higher concentration of cholesterol will alter the membrane permeability of the arterial wall is not entirely clear.

DONOUGH O'BRIEN (University of Colorado Medical Center): I would be interested to know if you have any information on the fatty acid composition of the triglycerides and the phospholipids in the plaques and in the other areas.

FELIX P. HEALD: We did not measure the fatty acid content of the phospholipids.

DAVID W. SMITH (University of Washington Medical School): Would you comment on the regional localization of the fatty streaks? Were they found anywhere, or were there particular locations? And if there were particular locations, do these coincide with the area where you most commonly find serious atheromatous lesion in the older adult?

Dr. HEALD: Very early in life around areas of turbulence you can find beginning intimal thickening.

What is the relation between the fatty streak that is found in the first 2 decades of life to clinical disease?

Certainly in the coronary arteries there is a very clear correlation between the fatty streak and the adult morbidity and mortality from coronary artery disease. The presence of the fatty streak in the human aorta does not precisely predict coronary artery disease.

However, the similarity chemically and histologically between the two lesions at least justified, for the purpose of this study, the use of the more readily available aorta.

LEWIS A. BARNESS (Hospital of the University of Pennsylvania, Philadelphia): I'd like to know whether these children were breast fed or artificially fed.

Dr. HEALD: We made no attempt at clinical correlations in this particular portion of the study.

SYDNEY SEGAL (University of British Columbia, Vancouver, Canada): Dr. PETER HAHN and his group have shown—and this relates to your previous question—that newborn rats, when fed a low fat diet, were less effective in controlling their serum cholesterol levels if they had been subjected in their neonatal period to early weaning onto a milk feeding which was low in fat content.

I wonder whether Dr. HEALD would like to comment on the possible effect of a low fat formula for routine use in the management of human infants, thinking particularly of his observations on both the formation of the fatty streak and the subsequent atheromatous changes in man.

Dr. HEALD: I can only give you my personal bias. Since the fatty streak is universal, and since it has been demonstrated in populations which have been subjected to famine conditions, the intimal thickening may not be primarily a cholesterol phenomenon, that is, there may be another agent which produces this intimal thickening, or, a reaction to injury.

The cholesterol story, at least in the first 2 decades of life, as far as I'm concerned, is a very cloudy one in its relation to this lesion.

ANDREW E. LORINCZ (Birmingham, Alabama): I'm a little bit confused and concerned about the techniques that are used and statements made in the abstract and presentation concerning the presence or absence of glycoproteins and localization of acid mucopolysaccharides. We are all well aware that a nitrogen-to-hexosamine ratio is a pretty grisly way of identifying or localizing acid mucopolysaccharides. Similarly, the colloidal iron histochemical techniques have their limitations. I am concerned as to the method of fixation used prior to the staining for polysaccharides. By virtue of the fact that these compounds are extremely water soluble, frequently the method of fixation in aqueous solvents might not permit histochemical identification or warrant drawing conclusions concerning mucopolysaccharide localization.

I just wanted to point out that formalin fixation is absolutely the worst way to fix tissues for localization of acid mucopolysaccharides.

Dr. HEALD: For histology, these sections were fixed in formalin, and I agree with your comments. This is a murky area. There is considerable conflict between the chemistry that has been reported on acid mucopolysaccharides and what has been demonstrated histologically.

JAMES R. HUGHES (Hitchcock Clinic, Hanover, New Hampshire): Although the predictive value of the fatty streaking in the aortas with respect to future coronary heart disease may not be absolutely established, we do have very firm correlation—of predictive value—of *cigarette smoking habits* with future coronary heart disease. The latter is an aspect of objective data upon which we have not yet taken very firm action as a pediatric body.

3 *Transfer of Fetal Lymphocytes to the Mother: Application to Prenatal Sex Determination and Immunologic Considerations.* F. CONTE*, J. WALKNOWSKA*, and M. M. GRUMBACH, Univ. of California Medical Center, San Francisco, California.

Lymphocyte cultures were prepared from the venous blood of 30 pregnant subjects between 14–37 weeks of gestation. In 21 women, one or more euploid metaphase figures with 5 small acrocentric chromosomes, interpreted as '46/XY' were found. Nineteen of the 21 women gave birth to male infants and 2 delivered female infants. Artifact or less likely, persistent chimerism for fetal 46/XY cells may account for the two false-positive results found. In the other 9 subjects no XY cells were found and 6 of 9 patients delivered female infants. Cells with 46/XY karyotype were detected as early as the 14th week of gestation (the earliest stage studied). The data suggest that the fetomaternal transfer of lymphocytes is common, occurs as early as the 14th week of gestation, and may occur by migration of circulating fetal lymphoid cells as well as leakage of fetal blood. The antenatal diagnosis of a male fetus can be made by karyotype analysis of lymphocytes in maternal blood. Similarly, fetal chromosome abnormalities could be detected. Mixed lymphocyte cultures (in the absence of PHA) of maternal venous and umbilical cord blood lymphocytes failed to show a response as assessed by mitotic rate. It is suggested that the transfer of fetal lymphocytes to the mother contributes to the acceptance of the fetus as a homograft.

Discussion

GORDON B. AVERY (George Washington University): I think that it may be a question of dosage, as to whether the mother is sensitized to fetal antigens during pregnancy. In erythroblastosis small doses of incompatible red cells ordinarily don't sensitize, but a more massive exposure may cause sensitization. The same thing may be true for transplantation antigens. We have done experiments in mice in which we surgically increased the exposure of the mother to fetal antigens, and it resulted in strong sensitization of the mother to skin grafts of fetal genotype. Thus, maternal tolerance of the fetus is not a case of the mother being overwhelmed by high doses of fetal antigens. However, occasionally very low antigenic doses also induce tolerance, and the low rates of emigration of lymphocytes demonstrated by the present authors may be crucial to the nonsensitization of the mother.

FELIX CONTE: The development of tolerance or immunity in response to a specific antigen is in part a function of the dose administered. But other factors such as the strength of the antigen, its route of administration, and its physical form are important. The intravenous administration of low doses of antigens has been found to be especially conducive to the induction of tolerance [BILLINGHAM, R. E.; BRENT, L.; MEDAWAR, P. B., Phil. Trans. Roy. Soc., Lond. Ser. B *239*, 357, 1956].

AARON MEISLIN (New York University): Were simultaneous measures of fetal erythrocytes in maternal circulation taken during the course of pregnancy to indicate how much transplacental bleeding occurred?

AUDREY K. BROWN (Medical College of Georgia, Augusta): My question is very much like the last one, but I'd like to pursue it even further to see it there is a relation to the fetal-maternal bleed.

For example, what happened immediately *post partum*? Was there an increase in the number of lymphocytes?

Concerning the relation between the studies that you did and chimera production, the survival of lymphocytes is, of course, distinctly different from the survival of erythrocytes, approaching several years and I wonder, therefore, whether you have more data than you presented concerning studies of second pregnancies following the birth of a male infant. You indicated that one of your false positives was such a situation. Would that be routine in such a situation?

What was the ratio of lymphocyte to erythrocyte leakage, or passage into the mother?

Dr. CONTE: Simultaneous measurements of fetal erythrocytes and lymphocytes in the maternal circulation were not carried out in our study. Fetal lymphocytes were identified by karyotypic analysis of PHA stimulated cells. Thus, the proportion of fetal to maternal metaphase that figures in the peripheral cultures cannot be assumed to reflect that existing *in vivo*. A variety of unevaluated factors could affect the proportion of fetal lymphocytes found *in vitro*. They are 1. the mode of duration of transplacental passage of fetal lymphocytes; 2. the relative sensitivity of fetal and maternal lymphocytes to the mitotic effect of PHA *in vitro;* 3. the generation time of fetal and maternal cells in culture; 4. the length of survival of fetal cells in the maternal circulation; 5. the relative proportion of fetal and maternal lymphocytes in the blood sample which survive 72-hour-culture; and 6. the question of chimerism of the mother with the implantation and multiplication of fetal lymphocytes in maternal tissue. Only one patient was studied both *pre-* and *post partum*. Fetal cells were identified up to 2 months *post partum* and were not found subsequently. This patient was not studied in the immediate *post partum* period. At 1 and 2 months *post partum*, the proportion of lymphocytes found was not different from that found antepartum in this patient. In one patient, whose first pregnancy 8 years previously had resulted in a male, a cell was identified with 46 chromosomes, including 5 small acrocentrics. This patient delivered a female infant. Whether this cell is artifactural or is evidence for persistent chimerism is not known. Further studies on *post partum* women and second pregnancies should help to resolve this question.

JONATHAN T. LANMAN (Downstate Medical Center, Brooklyn, New York): We have just heard a comment regarding the effect of a small dose of lymphocytes as failing to sensitize. There may be another factor involved here which has received emphasis recently, and that is that, although very large doses of antigen may induce immunologic tolerance, it is less well known that very small doses of antigen likewise will produce tolerance.

The mechanism of this induction of tolerance is not known, but it suggests that there is a U-shaped curve such that both small doses and very large doses of antigen have similar effects.

ROBERT J. MCKAY (College of Medicine, University of Vermont): Great caution should be used in using this method to diagnose fetal chromosomal abnormalities. In studies that we did several years ago, we found that a significant percentage of women have chromosomal abnormalities in a small percent of their metaphase cells, and many of these abnormalities were trisomies.

Dr. CONTE: We have also noted a small percentage of trisomic cells in our pregnant patients. The significance of these cells is dependent upon whether the cells are fetal in origin, as for example by the demonstration of a Y-chromosome, their frequency in the normal pregnant female, and the statistical correlation between their presence and the fetal karyotype. At present, pending more data on the significance of these cells, we would recommend confirmation of a presumptive abnormality of fetal karyotype by culture of amniotic fluid cells.

JOHN H. GITHENS (University of Colorado Medical Center, Denver, Colorado): Did you have the opportunity to correlate your findings in the maternal blood with the presence or absence of cytotoxic antibody against foreign cells?

Dr. CONTE: We did not study maternal blood for cytotoxic antibodies.

SANFORD LEIKIN (Children's Hospital of the District of Columbia, Washington D.C.): We have been looking at the question of the passage of maternal cells into the fetus and have not found female metaphases in the cord blood of male infants. Did you make any observations along these lines?

My second point relates to the mixed cell cultures of maternal and umbilical cord blood. As there was no response in these cultures as assessed by examination of mitotic figures, one would presume that neither population of cells was capable of responding to the other. However, we do know that cord blood lymphocytes are stimulated by unrelated adult homologous leukocytes. Would you conclude then, from your observations, that the cord blood lymphocytes are unresponsive to maternal leukocytes?

Dr. CONTE: We did not study the maternal-fetal passage of cells. TURNER and co-workers [Amer. J. Obstet. Gynec., July, 1966] analyzed over 5000 lymphocytes from 183 newborn male infants. Although they did find 46/XX cells in two newborn males, they concluded that 'mitotically capable leukocytes do not cross the placenta from mother to fetus.' Further, BERNIRSHKE *et al.* (International Symposium on the Feto-Placental Unit, Milan) reported the presence of a few 46/XX cells in placental blood, but not in the newborn.

WILLIAM W. CLEVELAND (Univ. of Miami School of Medicine): Have you done *post partum* tests of histocompatibility between the infants and their mothers?

DOUGLAS C. HEINER (Royal Victoria Hospital, Montreal, Canada): In your mixed leukocyte cultures did you have an opportunity to use tritiated thymidine as a measure of blastogenesis, because I think it may be a little more reliable and sensitive than blast cell counts.

I think the point of whether or not tolerance is induced is crucial. It would be of interest to follow the patients to see whether the mixed leukocyte culture reactions changed and whether there really was chimerism. The mother's lymphocytes should react to the infant's mitomycin-treated lymphocytes after the passage of time if tolerance is lost.

Perhaps also a monkey model might be an interesting help to answer the question of whether there is temporary tolerance, because a fetal tissue graft should not be rejected by the mother. I think you could experimentally study this possibility in the monkey. If grafts were accepted from fetal or infant skin, one would be more certain that the mother was really tolerant of the fetus.

Dr. CONTE: The mixed lymphocyte cultures are an attempt to assess histocompatibility between mother and fetus. Studies utilizing tritiated thymidine are in progress.

4 *Inclusions in Terminal Air Spaces of Fetal and Neonatal Human Lung: Morphology and Chronology.* G. S. SPEAR*, O. VAEUSORN*, M. E. AVERY, R. NACHMAN*, J. WOLFSDORF*, R. A. BERGMAN*, Departments of Pathology, Pediatrics and Anatomy, Johns Hopkins School of Medicine, Baltimore, Md.

Using thin, toluidine blue stained sections of plastic embedded lung, we have found, by light microscopy (LM), different morphologic and chronologic characteristics for inclusions than previously has been reported. The inclusions we studied are, we feel, the osmiophilic lamellated bodies (OLB) of electron microscopy (EM) that are believed to be associated with pulmonary surfactant. The inclusions in our material generally were translucent and either colorless or lightly to moderately colored; often irregular in shape; varied in size from barely discernible to larger than a nucleus; sometimes had internal structural patterns; and even by LM often resembled the OLB of EM. These differences in characteristics, when compared with the results of others, may at least partially result from the fact that the primary fixative in our studies generally has been glutaraldehyde, but in investigations of others, osmic acid. We have observed inclusions, although irregularly, in fetuses as small as 310, 600 and 710 g, earlier than has been noted by others. Surfactant, as measured in saline extracts of minced lung on a Wilhelmy film balance, frequently was not detectable despite the presence of inclusions, but precedent for this disparity exists in others' animal studies. This is the first known study in the human fetus in which inclusions and surface activity have been investigated in the same specimen of lung.

Discussion

ANDREW E. LORINCZ (Birmingham, Alabama): Would you speculate about the plastic embedded light microscope sections, about the metachromatic nature of these inclusions?

Are you convinced that what you see in the EM and light preparations are the same? The inclusions relative to the size of the nucleus were very much smaller in the EM material.

THOMAS K. OLIVER (University of Washington School of Medicine): I wonder if you noted any differences between stillborn infants and those that either breathed spontaneously or had been resuscitated for any length of time. In short, does breathing air make a difference?

Dr. KLAUS (Babies and Childrens Hospital, Cleveland, Ohio): Were the inclusions you demonstrated in the very young fetus found on all of the sections, or was this a rather unusual section?

G. S. SPEAR (Closing discussion): The inclusions are not metachromatic; I grant that they looked so in the slide I selected for projection, but that was a freak

staining reaction. I used that particular slide merely because the inclusions that were endowed with the peculiar tint you noted showed up well upon projection.

The inclusions doubtless contain significant amounts of lipid. Droplets that contain lipid vary greatly in appearance in plastic embedded tissue stained with toluidine blue, but the reasons for this variability are simply not known. Some are blue (and many shades of blue can be seen), but many are green, gray or are colorless.

I am, of course, not certain that the inclusions I have described today are the osmiophilic lamellated structures we see by electron microscopy. However, I think that this is very likely. As to their size proportionate to the nucleus, this is, I think, generally comparable to that of osmiophilic bodies in relation to nuclei when a specimen is examined by electron microscopy.

With reference to Dr. OLIVER's question, we did not study variation in appearance as a function of variation in physiologic state. Of course, all specimens in our *chronologic* study were from *stillborn* fetuses; we chose stillborns deliberately in order to avoid factors that would complicate our study of chronology, factors such as respiration and resuscitation.

When inclusions were seen, they were identifiable in many sections. Some sections might not have them, but in general there was little problem in sampling.

5 *Acid-Base Response to Hypercapnea in Premature Infants.* HELMUTH MENTZEL*, NICHOLAS M. NELSON*, RUTH B. CHERRY* and CLEMENT A. SMITH, Department of Pediatrics, Harvard Medical School and Boston Hospital for Women, Boston, Mass.

The rapid recovery from birth acidosis and hypercapnea in normal full-term and premature newborns is well documented as being largely complete within an hour or so of birth. Little information is available concerning compensation by premature infants for hypercapnea persisting some hours after birth. The present study was performed to find answers to the questions: 1. to what extent can hypercapnea be compensated for after birth? 2. What is the acid-base response to different grades of hypercapnea? In 23 premature newborns (not receiving parenteral fluids and not in respiratory distress) arterial pH, P_{CO_2} and P_{O_2} were measured at 10–30 min intervals after birth; bicarbonate was calculated. While P_{CO_2} ranged from 31–81 mm Hg in the first 5 hours of life, the mean difference between successive samples was 3.1 mm Hg (range 0–13 mm Hg); of the total of 147 such differences, only 6 were greater than 10 mm Hg and 11 were greater than 7 mm Hg. H^+ concentration ranged from 37–71 nanomoles/L. There was a highly significant relation between P_{CO_2} and H^+ concentration at all times and a surprisingly good compensation for respiratory acidosis by bicarbonate regeneration. These data define an acute *in vivo* whole body CO_2-titration curve within which postnatal respiratory acidosis seems well tolerated. Bicarbonate regeneration by the acidotic newborn should not be neglected in assessing the 'success' of exogenous alkali therapy for respiratory acidosis.

Discussion

RALPH DELL (Columbia University): Three years ago I presented to this Society a model which related the expected acid-base changes which would be seen in infants with varying types of body composition who are acutely hypercapneic. The predictions of the model are somewhat different than the data that you have obtained. In particular, the slope that you obtained is considerably steeper than that which would have been predicted by the model.

Perhaps these differences are due to a nonsteady state, and in particular to changes in cardiac output which may be taking place during the first 5 hours of life, and to changes in tissue perfusion.

Since we presented this model, we have completed a series of experiments in dogs which tend to confirm the predictions of the model, the dog preparation being a little bit easier to interpret in that we are assured in the dog of a steady state.

JUNE BRADY (University of California Medical Center, San Francisco): You pointed out the importance of hemoglobin as a buffer. As there is a hemoconcentration, with loss of fluid from the intravascular into the extravascular space during the first 4 hours of life, I wonder how the hemoglobins changed in your babies, and how this would affect your results.

HELMUTH MENTZEL (Closing discussion): Our statement concerning successive sample differences in carbon dioxide tension (PCO_2) was in support of the idea that these infants were, for periods of at least 10 minutes, in a reasonably steady state of carbon dioxide balance during acute hypercapnea. We must concede that we do not have the same assurances concerning a steady state in infants as would you in a laboratory animal. Nevertheless, we thought it better to attempt study of the problem of acute hypercapnea in a clinically pertinent setting than not to do it at all. I think our results clearly show that a high correlation between hydrogen ion and PCO_2 exists during these early hours, that significant hydrogen ion disappears in the first hour after birth without therapy, that the steadiness of the stated relation between hydrogen ion and PCO_2 improves with time, as demonstrated in our significance bands, but that the buffering capacity does not change and appears to be greater than can be accounted for in theory. We can only presume that this discrepancy relates to the duration and tissue buffering of hypercapnea unless it indicates poor tissue perfusion. While the differences between your animal studies and our clinical observations indeed question the existence of a steady state, at the same time, these differences question the appropriateness of your model to the clinical situation we have observed.

Dr. BRADY—concerning hemoglobin concentration. Of course, this would very much affect the buffering capacity. However, hemoglobin concentration did not change significantly during the observation period in these babies.

6 *Long-Term Developmental Outcome of Infants Who Had Clinical Hyaline Membrane Disease.* MILDRED STAHLMAN, GUNNELL HEDVALL*, VIRGINIA KIRK*, Vanderbilt University School of Medicine, Nashville, Tenn.

Sequential evaluation of developmental and somatic growth has been made at yearly intervals on 133 infants who had respiratory distress and x-ray findings characteristic of clinical hyaline membrane disease. Twenty-eight infants had disease of such severity as to require assisted ventilation for more than 9 hours. Prior to discharge from the nursery all study infants had an AP and lateral x-ray of the chest, an EKG, and an

ophthalmologic examination. At 6 and 12 months and at yearly intervals thereafter interval histories were taken and a neurological evaluation and chest x-rays were made. Developmental quotients were done at yearly intervals. Bone age films have been done at 4 or 5 years. Of the 28 infants who received assisted ventilation, 1 has a developmental level less than 75, and none has gross neurological deficit. In the entire study group, birth weight was better correlated with developmental quotient than was severity of the disease ($\overline{\text{MD.Q.}}<2000$ g = 85.2 vs $\overline{\text{M}}$ D.Q.>2000 g = 98.0). Analysis of variance between 43 study infants and their own siblings shows no significant difference between the two groups. D.Q.'s have generally improved with age and no allowance has been made for prematurity. All infants were above the 10th percentile birth weight for gestational age, but many infants' somatic growth patterns have been retarded from birth onward. Over half of the children examined have delayed bone maturation. No retrolental fibroplasia has been identified. In spite of persistent lags in somatic growth in some, the generally good intellectual capacity and the absence of gross neurological deficit in this population is highly encouraging.

Discussion

BEA J. VAN DEN BERG (University of California, Berkeley): In the setting of the Child Health and Development Studies, we studied morbidity in infants in the first 5 years of life, that is, the number and type of diseases that were medically attended. In these studies, infants with hyaline membrane disease did not have any significantly higher morbidity in the first 5 years after the neonatal period than children of the same birth weight and gestation without hyaline membrane disease.

Also, the results that you showed of mental development were the same as those in our studies.

MARIA DELIVORIA-PAPADOPOULOS (University of Pennsylvania School of Medicine, Philadelphia): In those babies that were ventilated during the acute stage of their illness, do you have any follow-up studies on their pulmonary function, at least on those that are 3.5 years of age or more?

J. A. GRUNT (Yale University School of Medicine): Could you delineate any signs of hypoglycemia in any of these children subsequent to the neonatal period, particularly between 1 and 5 years of age?

LARRY TAFT (Albert Einstein College of Medicine): The authors report grossly normal intelligence and absence of gross neurological deficits in the children followed to 5 years of age. This should not be interpreted to mean that hyaline membrane disease can be ruled out as a 'high-risk' factor in the causation of brain damage. The CNS dysfunction may not become apparent until school age, when reading and learning difficulties become manifest. A 10-year follow-up study may be more revealing.

A. FREDERICK NORTH (George Washington University): Is it possible that retarded statural growth and bone age are related to the social class of these infants, rather than to anything special about having hyaline membrane disease? The same kind of comparisons with siblings that you used so well for your developmental testing might show whether these children are born into families where heredity or environment leads to slow growth or whether the apparent retardation of height and bone development is actually related to their low birth weight or their specific disease.

JOHN C. SINCLAIR (College of Physicians and Surgeons, Columbia University): I think there are animal studies to show that two early influences that could have a long-term effect on growth could be hypoxia and undernutrition, by causing a decrease in the rate of increase in cell number.

Do you have information as to the caloric intake in the first weeks of life and the level of oxygenation in these babies that could be correlated with their subsequent growth?

LEO STERN (The Montreal Children's Hospital, Quebec, Canada): There are a number of reports now of children who have had severe hyaline membrane disease, both on and off respirators, and who have gone home from nurseries apparently well, with radiographic recovery, and who have had severe, recurrent bronchiolytic type illnesses during the 1st year of life and later on.

MILDRED J. STAHLMAN (Closing discussion): I'd like to thank all of the discussants for their comments. Perhaps several of them can be answered together.

At the present time we are calling in these older children for pulmonary function studies, so that will be the subject of another communication at a later date.

As far as the clinical signs and symptoms of subsequent pulmonary disease, in this population it is extremely high. We have looked at 70 of these babies quite carefully, and 27 of these 70 children have had episodes characteristic of bronchiolitis in their first 2 years of life.

We have great difficulty in having a control population for this sort of phenomenon, since bronchiolitis was prevalent in our non follow-up babies' population at that particular time. In the children—particularly those who have been on respirator—lower respiratory tract disease does seem to be beyond that which is seen in the general population.

We have not detected any signs of hypoglycemia in the postnatal period. These babies, as I have indicated, are for the most part what we would classify as normally grown *in utero*.

We are well aware, and in my concluding remarks I indicated, that these children may show up with learning problems and perceptual difficulties which are only apparent after they reach school age. For this reason they are being followed into their school experience and, hopefully, at least through their 11th year.

We have not looked at their caloric intake in their first weeks after birth in their recovery phase, but I'm very suspicious, as I'm sure Dr. SINCLAIR would be, that we probably starve sick babies, and that this may well contribute to some of the things that happen to their postnatal growth pattern.

As far as the eventual stature of these babies is concerned, it may well be that this is more related to socioeconomic factors than disease. We have not tried to divide this population on a socioeconomic level. It represents a very broad span of socioeconomic and genetic backgrounds of patients.

I was encouraged that the babies with the lowest D.Q.'s were not necessarily the babies with the lowest statures.

7 *The Metabolic Defect in Familial Hyperlysinemia.* JOSEPH DANCIS, JOEL HUTZLER*, RODY COX* and NORMAN C. WOODY, New York University School of Medicine, New York, N.Y. and Tulane University School of Medicine, New Orleans, La.

Hyperlysinemia (10–23 mg %) has been demonstrated in three siblings and a first cousin. Investigations of two of the subjects with lysine-^{14}C revealed a reduction in the ability to degrade lysine to about 5 % of normal. A recent report that rat liver will convert lysine to saccharopine prompted us to explore the possibility that the children suffered from a defect in the responsible enzyme. Our initial studies concentrated on demonstrating, partially purifying and characterizing an active lysine-ketoglutarate reductase in human liver. The information was applied to a study of skin fibroblasts grown in tissue culture. The three siblings had a reduction in enzyme activity to 5–10 % normal. These observations identify the metabolic defect, and provide evidence that the saccharopine pathway is the major degradative pathway for lysine in the human.

Discussion

HOSSEIN GHADIMI (Methodist Hospital of Brooklyn): I wish to compliment Dr. WOODY and Dr. DANCIS for finally elucidating the block in the pathway of the lysine in hyperlysinemia.

Our concern with this problem stems from the fact that in Seattle 4 years ago we described two cases of hyperlysinemia simultaneously and independently of Dr. WOODY's case.

I would like to add a word of caution to those who use radioactive amino acids for elucidation of a metabolic pathway. The label on the vial of uniformly labeled ^{14}C-L-lysine generally asserts 99 % purity. By application of a conventional amount, namely, less than 0.1 μCi on paper or a short column, the impurities will be completely overlooked. There would be one radioactive spot, for instance, on the paper chromatogram. On the other hand, application of 0.5 μCi or more to a Moore and Stein column connected to a liquid flow scintillation counter would reveal 9 to 11 radioactive peaks. This has already been verified with lysine obtained from three different manufacturers. These impurities are presumably intermediary metabolites in the biosynthetic pathway of lysine, including saccharopine.

For obvious reasons, one must ascertain that the radioactive lysine used in this type of experiment does not contain saccharopine or other intermediary metabolites which may be converted to saccharopine. We have been interested in saccharopine formation for the last 3 years. In our laboratory, we have learned to use column-purified lysine for our experiments. The purification involves application of the commercially available lysine to a Moore and Stein column which is connected to a liquid flow scintillation counter. When the lysine peak appears on the radiogram (after some 17 h), we begin collecting the eluate.

I am confident that Dr. DANCIS and his colleagues have competently taken care of any impurities; nevertheless, it would be helpful to know what technique they used to ascertain that the sample of ^{14}C-lysine was completely free of saccharopine.

In Dr. DANCIS' paper, which was recently published in Biochim. biophys. Acta [*158:* 62, 1968], the skin was found to have the least amount of enzyme of seven tissues tested (liver, kidney, heart, adrenal, thyroid, brain and skin). 'Lysine-ketoglututarate reductase' activity of the skin was found to be only 1.5 % of that of the liver. With this in mind, I would like to ask by how many counts the saccharopine activity of the fibroblasts exceeded the background, in both the controls and the patients.

In the studies involving intravenous injection of ^{14}C-lysine and measurement of expired $^{14}CO_2$ in patients and controls, it is similarly imperative to ascertain the purity of lysine sample, since the expired $^{14}CO_2$ may well come from the intermediary metabolites contained in the sample.

Lastly, I wish to ask what percentage of lysine is converted to saccharopine *in vitro*? What is the capability of that route?

JOSEPH W. ST. GEME, Jr. (UCLA Medical School, Harbor General Hospital): The question that has been raised so many times involves the issue of the relevance of cells cultivated from human tissue to biologic and biochemical function of the intact individual.

There is increasing evidence accumulated in the last few years concerning many of these metabolic problems which supports the fact that cells do seem to breed true *in vitro*.

In your work, how long do these fibroblasts seem to retain their biochemical characteristics of abnormality? Did they undergo successful passage and retain their abnormal function or was this only a brief observation?

JOSEPH DANCIS: The radioactive lysine was checked for purity before use and, much in line with Dr. GHADIMI's experience, significant contamination was found. The contaminants were removed before being used in the experiments that we described.

However, 'purity' is always a relative term, and the results of experiments using radioactivity must be interpreted keeping this clearly in mind. A radioactive contaminant would probably have been metabolized equally well by the hyperlysinemic and the normal and could only have minimized the differences between the two groups. In fact, the differences were clearly defined.

It is true that skin has a very low level of lysine-ketoglutarate reductase activity. However, the skin fibroblast may exhibit performance that is superior to skin as it did in this case. This is a fascinating area for study. At times, enzymes that are not demonstrable in intact skin are active in the fibroblast, and sometimes the reverse happens.

The enzyme persists in tissue culture through many generations. The studies that we have described were performed after 15–20 transfers. The conversion of lysine to saccharopine was about 20 mμmoles/mg protein, making this a very active tissue.

The overall interpretation of these results is actually fairly clear. Here we have children with high blood lysines and *in vivo* evidence of an inability to degrade lysine. To that, we add the fact that in the skin fibroblast grown from these children there is a deficiency of an enzyme that is directly concerned with lysine degradation.

8 In Vitro *Demonstration of Intestinal Transport Defect in Hartnup Disease.* VIVIAN E. SHIH*, E. MAY BIXBY*, DAVID H. ALPERS*, CHRISTOS S. BARTSOCAS*, and SAMUEL O. THIER*, Massachusetts General Hospital, Boston, Mass. and Wrentham State School, Wrentham, Mass. (introduced by John D. Crawford).

Hartnup disease is a rare hereditary disorder characterized by severe renal neutral aminoaciduria; its clinical manifestations vary from mental retardation, intermittent cerebellar ataxia and pellagra-like rashes to none. The presence of an intestinal transport defect

was suggested by excessive and prolonged urinary excretion of indican and indolic acids following tryptophan loading [MILNE *et al.*, Quart. J. Med. *29:* 407, 1960] and by the presence of increased fecal amino acids [SCRIVER, New Engl. J. Med. *273:* 530, 1965, and SEAKINS and ERSSER, Arch. Dis. Child. *42:* 682, 1967]. We studied a family in which two mentally retarded patients, 9 and 16 years of age, manifested the characteristic aminoaciduria and demonstrated the deficient intestinal mucosal transport of neutral amino acids by means of *in vitro* uptake measurements. Uptakes of both tryptophan and methionine were less than 30 % of the controls. Feedings of tryptophan, phenylalanine and methionine at 100 mg/kg resulted in lesser increases in blood level in patients than in controls. No differences in lysine uptake by intestinal mucosa and its lading curve were observed. Stool amino acids were not increased at any time. The results of amino acid lading correlated well with those of the *in vitro* studies. This confirms the previous suggestions that both renal and intestinal neutral amino transport systems are defective in Hartnup disease.

Discussion

PAUL WONG (Chicago Medical School): It has been suggested that the subnormal physical growth in the young patients with Hartnup disease may be due to the excessive loss of amino acids, both in the urine and in the stool.

In 1964, DELAEY and his associates suggested that nicotinic acid would correct the biochemical defects in this disease in one patient. During nicotinic acid therapy, amino acid transport in the kidney and in the intestine approached normal.

We have studied the effects of nicotinic acid therapy in two patients with Hartnup disease. With prolonged nicotinic acid therapy, up to 300 mg/24 h, we could not demonstrate any improvement in the metabolic defects in our two patients.

I think it would be valuable for Dr. SHIH to repeat the *in vivo* transport experiments both with the addition of nicotinic acid in the incubation mixture and after the patients have received additional amounts of nicotinic acid therapeutically. Such observations may have direct implications in the treatment of these patients.

STANLEY W. WRIGHT (UCLA): Could you discuss how the clinical diagnosis of Hartnup disease was made on these patients? Were the clinical manifestations in these patients characteristic of Hartnup disease?

Secondly, indolic acids were present in the urine. These substances may be exogenous in origin. They occur very frequently in patients who are constipated, and this is a very common problem among the mentally retarded. They may come from substances in the diet. Many of the preservatives that are used in fruit juices will be absorbed and excreted in the urine as indolic substances. Were these patients placed on a modified diet which would eliminate exogenous substances, or were they put on neomycin to see if the indolic aciduria disappeared?

VIVIAN E. SHIH (Closing discussion): First, in response to Dr. WONG's question, we also tried nicotinic acid therapy on one of our patients and did not observe any change in urinary amino acid excretion. I think his suggestion of adding nicotinic acid to the *in vitro* study is a very good one, and we might try that.

Dr. WRIGHT, our criterion for diagnosing Hartnup disease is based purely on the presence of the characteristic aminoaciduria. As you know, the clinical manifestations vary quite a bit. They can be normal; they also may have the pellagra-like rash, intermittent cerebellar ataxia, psychotic behavior, etc. We therefore felt that clinical manifestations are not reliable enough in making a diagnosis.

In our patients, there is no history of any kind of rash or ataxia. Behavior and disposition are somewhat different from that of the other retarded siblings. They have a difficult temperament, but that's about all I can say about the clinical picture and, in view of the family history, I certainly would not relate their mental retardation to the biochemical finding.

Now, as far as the indolic acids in the urine are concerned, without loading they do not excrete excessive indolacetic acid or indican, so we have not given them neomycin and we have not controlled the diet. All these indoles appeared only after tryptophan oading.

9 *Ontogenesis of Growth Hormone, Follicle Stimulating Hormone, Thyroid Stimulating Hormone, Luteinizing Hormone, Chorionic Prolactin, Chorionic Gonadotropin and Thyroglobulin in the Human Conceptus.* DAVID GITLIN and ANITA BIASUCCI*, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

The synthesis of pituitary and placental protein hormones and thyroglobulin was investigated in the same conceptuses from 29 days' to 18 weeks' gestation by incubation of tissues in ^{14}C-labeled amino acids followed by radioimmunoelectrophoresis of the culture fluids. Synthesis of growth hormone was first evident at 9 weeks' gestation and enzymatic hydrolysis of the isolated hormone confirmed incorporation of the labeled amino acids into the primary structure of the protein. Synthesis of thyroid stimulating hormone was first observed at 14 weeks' gestation, by which time synthesis of follicle stimulating hormone was well established even in male fetuses. Tissues as early as 29 days' gestation synthesized a protein which was precipitable with antisera against luteinizing hormone, but was probably not the hormone. Synthesis of both chorionic prolactin and chorionic gonadotropin was evident as early as 29 days' gestation; chorionic prolactin synthesis was constant per unit weight of placenta from 29 days' to 18 weeks' gestation, the period studied, but synthesis of chorionic gonadotropin was greater in placentas of the first trimester, the decrease in synthesis coinciding in time with the increase in pituitary hormone synthesis. Thyroglobulin synthesis was already established by the 29th day of human development, when the thyroid is simply a bud of cells close to the pharyngeal floor, 6 weeks before protein iodination begins and 10 weeks before thyroid stimulating hormone is synthesized.

10 *Hereditary Lymphopenic Agammaglobulinemia Associated with a Distinctive Form of Short-limbed Dwarfism and Ectodermal Dysplasia.* R. A. GATTI*, N. PLATT*, H. H. POMERANCE*, R. HONG*, L. O. LANGER*, H. E. M. KAY*, and R. A. GOOD, Variety Club Heart Hospital, University of Minnesota, Minneapolis, Minn.; Long Island Jewish Hospital, New Hyde Park, New York and Royal Marsden Hospital and Institute of Cancer Research, London, England.

The etiology and pathogenesis of lymphopenic immunologic deficiency is unknown. In a family which we have studied, lymphopenic agammaglobulinemia

was associated with short-limbed dwarfism and ectodermal dysplasia in a brother and sister. Four similar cases have been reported as sporadic experiences and have involved both males and females. Our observation, together with these other cases, suggests an autosomal transmission and indicates a basic association between skin, bone and lymphoid system development. The dwarfism in these children is not, as was previously suggested, characteristic of achondroplasia. Absence of the trident sign, depressed nasal bridge and dominant inheritance, as well as a characteristic roentgenological picture, differentiate this form of dwarfism from achondroplasia. The immunological deficiency observed represents a broadly-based combined system defect probably reflective of a stem cell abnormality as has been previously suggested for other forms of lymphopenic agammaglobulinemia. The first of the two children died of a graft-versus-host reaction following transfusion, emphasizing the hazards of transfusion in lymphopenic immunologic disorders. Graft-versus-host disease can, we now believe, be identified as a specific clinical and pathologic entity. The second child died of overwhelming infection. Efforts to reconstitute the immunologic deficiency of the second sibling by implanting fetal liver cells were unsuccessful. The implications of these findings for understanding of the basic developmental anomaly of the lymphoid apparatus and immunologic function will be considered.

Discussion

THOMAS H. SHEPARD (University of Washington School of Medicine): One comment I would like to make is that the strain of rabbits with achondroplasia (ac/ac) described by BROWN and PEARCE in 1945 did have abnormally enlarged spleens and thymuses.

JOHN H. GITHENS (University of Colorado Medical Center): I was particularly interested in your attempt at transplantation with fetal tissue. We have also been attempting to transplant children with thymic alymphoplasia and dysplasia with fetal stem cells and/or with fetal thymus, with the same lack of success. One of our patients has documented evidence of a graft of lymphoid tissue, but the cells remained immunologically nonfunctional.

Would you speculate on the reason why fetal tissue apparently does not graft successfully in these children and does not reconstitute their immunologic system in the same way that it does in the d'George syndrome?

R. A. GATTI (Closing discussion): We used fetal liver rather than thymus in this case because we feel that the small thymus noted at *post mortem* examination in children with lymphopenic agammaglobulinemia is a secondary phenomenon and is not the basic defect in this disease. Such patients lack competent immunologic stem cells in their bone marrow which then fail to repopulate the thymus, resulting in the small thymuses usually seen.

As you are probably aware, we recently transplanted another infant with lymphopenic agammaglobulinemia with bone marrow and peripheral leukocytes from a sister who has matched with the patient across the HL-A major histocompatibility locus by both lymphocytotoxic assay and mixed lymphocyte culture. We were successful in restoring the immunologic competence of the child and later, when an iatrogenic aplastic anemia developed, a second bone marrow transplant from the same donor restored his hematopoietic capacities. This child actually developed a mass, evaluated by tomography, in the region of the thymus about 8 weeks post transplantation. He is presently doing well, 8 months following the first transplant.

The second infant described today did not have a living sibling as a potential donor. (There had been three siblings in this family. The first child, whom I did not mention in my presentation, died at 5 months of age, presumably with an intussusception and was also noted to be dwarfed at *post mortem* examination. So it is possible that there were three children in this family with this syndrome of lymphopenic agammaglobulinemia and dwarfism.) Because no sibling donor was available, we elected to try fetal liver cells as a source of competent stem cells.

We used fetal liver rather than unmatched bone marrow in hopes that 12-week-old stem cells, which are uncommitted cells and have not yet developed a significant concentration of surface antigens, would minimize any graft-versus-host reaction in the recipient. Unfortunately, the patient died of pneumonia before we were able to evaluate the results of these efforts.

11 *Automated Analysis of Human Chromosomes.* H. LUBS*, F. RUDDLE* and R. LEDLEY*, Yale University, New Haven, Conn. and National Biomedical Research Foundation, Silver Spring, Md. (introduced by C. D. Cook).

Major chromosomal variation occurs in 0.5–1.0 % of newborns. Only ¼ of these infants can be diagnosed clinically. In addition, minor variations in chromosomal length are present in more than 20 % of newborns. In order to characterize accurately and screen for chromosomal variation in man, both quantitiative and automated technics are needed. The results of computer analysis (FIDAC-FIDACSYS) of 50 cells were compared to values from an XY plotter, where the centromeres and end of arm placement were determined manually. Length and arm measurements were expressed as a faction of the total length of the ten longest chromosomes. Mean values from both technics were comparable although coefficients of variation were higher with FIDAC analysis. These were used to make a statistical comparison of variation between individuals. A plot of short arm against total length demonstrated that the group means ± 1 S.D. overlapped in only one instance (E 17–18 and F 19–20). These data represent the first production run of chromosomal measurements by an automated technic. (Research supported by NIH Contract No. 43-67-1463.)

Chromosome group	Total length XY Plotter	Total length FIDAC	Area FIDAC
A 1	0.118	0.123	0.121
A 2	0.111	0.113	0.116
A 3	0.095	0.098	0.097
B 4–5	0.087	0.090	0.091
C 6–12	0.074	0.073	0.070
D 13–15	0.056	0.053	0.050
E 16	0.052	0.050	0.043
E 17–18	0.048	0.041	0.040
F 19–20	0.042	0.040	0.031
G 21–22	0.037	0.031	0.023
Y	0.036	0.037	0.027

Discussion

JOEL J. ALPERT (Children's Hospital, Medical Center, Harvard Medical School): You certainly excited me. I've learned something.

My question is whether you have a normal population. The need to have this information about a normal

population is obvious. Do you have the data that let you say that Yale has a large unbiased population, simply because there is only one other hospital in town? In choosing a hospital, a significant bias may be introduced and your population may not be normally distributed.

H. LUBS: This gets to be complicated. The other hospital there doesn't have the same sort of statistics. We're in the process of getting them from the other hospital, so that we can compare them, but the preliminary information that we have shows that there are not great differences.

The other thing we can do when this material becomes public is to report in detail the precise nature of the population which we have described.

FREDERICK HECHT (University of Oregon Medical School): I'd just like to comment on several of the features that I think are embodied in the paper. One of these features is the population approach. It seems to me that a lot of answers are not going to be forthcoming until one has similar studies of significant size populations.

And the second is the concept that chromosomes may not fit into a procrustean bed, and that all chromosomes, even in their normal form, may come in various sizes and shapes, such that many if not all chromosomes in man may have polymorphisms as exist with serum proteins and red cell enzymes.

12 *Effective Light Treatment of Crigler-Najjar Syndrome.* MYRON KARON*, ALLEN SCHWARTZ*, DANIEL IMACH*, BERNARD SINGSEN*, and AIMY TANIGUCHI*, Childrens Hospital of Los Angeles and the University of Southern California, Los Angeles, Calif. (introduced by Denman Hammond).

The level of serum indirect bilirubin has been maintained below 8 mg % in a newborn infant with Crigler-Najjar syndrome by light therapy. Calculations indicate that 6–10 mg of bilirubin per day can be cleared from the serum by this mechanism.

At 3 days of age the bilirubin was reduced from 24 mg % to 8 mg % by 2 exchange transfusions of 450 ml each. The bilirubin then rose, 3 mg %/day (days 4–10) to 32 mg %. Two additional exchange transfusions (450 ml/exchange) reduced the bilirubin to 10 mg %. From days 16–24 and days 37–42, the bilirubin rose 1 mg %/day without light treatment. Continuous light reduced the bilirubin from 16 mg % to 4 mg % at the rate of 1.4 mg %/day (days 26–34), and 10 mg % to 4 mg % at the rate of 0.4 mg %/day (days 47–60) on 12 h of light/day. The indirect bilirubin has remained below 8 mg % on intermittent light therapy (10–12 h/day) at home. Direct bilirubin concentration has ranged from 0.4–2.2 mg %.

A defect in the conjugation of salicylate was demonstrated following sodium salicylate administration. The percentage of drug excreted as glucuronide was: patient, 1–2; mother, 10; father, 5; controls 8–10. 70 % was in the acyl form in all instances. 80–90 % was excreted as salicyluric acid (glycine conjugate), the most common metabolite of aspirin.

Growth and neurological development have remained normal. Sleep patterns have been unaffected.

(Supported by grants TOI-HD-00048 and CA-11050 from USPHS.)

Discussion

LAWRENCE M. GARTNER (Albert Einstein College of Medicine): We have also been studying the effect of artificial light on a 2.5-year-old child with chronic nonhemolytic unconjugated hyperbilirubinemia of the Type I variety and have found that the serum bilirubin concentration fell from 32 mg/100 ml to 16 mg/100 ml over a 4-day period of continuous exposure. During the following 14 days of light exposure, the serum bilirubin concentration remained at the same level. These observations are very similar to those of the authors.

Two forms of chronic nonhemolytic unconjugated hyperbilirubinemia with marked glucuronyl transferase deficiency can be distinguished on the basis of the response of the serum bilirubin concentration to phenobarbital administration. Type I is characterized by virtual absence of bilirubin in bile; no detectable glucuronide in bile; an autosomal recessive mode of inheritance; a high incidence of kernicterus; and serum bilirubin concentrations between 20 and 35 mg/100 ml. Type I appears to be identical with that found in the patients described originally by CRIGLER and NAJJAR. Type II chronic nonhemolytic unconjugated hyperbilirubinemia is characterized by a degree of glucuronyl transferase deficiency similar to that of Type I as estimated *in vitro*, but by a nearly normal amount of bilirubin in bile; by considerable amounts of glucuronide in bile; by an autosomal dominant mode of inheritance with variable penetrance; a very low incidence of kernicterus; and by serum bilirubin concentrations between 8 and 22 mg/100 ml. Administration of phenobarbital in doses of 5 mg/kg/24 h to Type II patients results in decrease of the serum bilirubin concentration to approximately 2.0 mg/100 ml within 2 weeks. Similar administration of phenobarbital to Type I patients results in no response of the serum bilirubin concentration.

Although the amount of phenobarbital and duration of administration to this patient may have been insufficient, the complete lack of response suggests that this patient is a Type I.

THOMAS SISSON (St. Christopher's Hospital for Children, Temple University School of Medicine, Philadelphia): Have you measured pigment excretion in either urine or feces or noted a difference in the character of the stools and urine?

I would like to know also if the lamps used either at home or in the hospital had been changed or whether after this long period of time the same fluorescent lamps were still in use. The apparent lack of effective photodecomposition later on in treatment may have been due, in fact, to decay in the energy output of the lamps in the wavelengths wherein bilirubin is photochemically reactive.

JEROLD F. LUCEY (De Goesbriand Hospital, University of Vermont College of Medicine): I'd just like to ask the authors why, living in sunny California, they chose to use artificial light, rather than natural sunlight screened through glass.

If we could figure out a way of exposing babies to natural light, which has 10,000 foot candles to it, we would be better off, I think, than using light machines which supply 400 to 500 foot candles. The quality of the light is pretty much the same, and there should be a greater effect if we could use natural light.

RONALD POLAND (U.S. Army Hospital, Fort Gordon, Georgia): Since bilirubin confined to the intravascular space is of no great clinical concern, was there any attempt in this case to show that the tissue levels of bilirubin fell? Were any parameters of bilirubin toxicity investigated such as the impairment of renal

concentrating ability in the Gunn rat as described in Amer. J. Physiol. *212:* 931 [1967]?

ROBERT J. MCKAY (College of Medicine, University of Vermont): Having watched Dr. LUCEY at work, I was interested that the picture of your baby under the light was taken with a shirt on and apparently part of a diaper. Is this the way your babies are exposed?

Dr. KARON: We wish in retrospect that we had used a higher dose of phenobarbital since the failure to see a significant response after 2 weeks could be the result of insufficient dosage. The effect of phenobarbital on liver is complex. It can induce glucuronal transferase, but it can also increase bile flow. Phenobarbital increases the production of endoplasmic reticulum and thus, increases the capacity of the liver to synthesize degradative enzymes. In animal systems, phenobarbital can increase heme synthesis as well as the 'early-labeled' peak. The effect of phenobarbital is really a very complex issue, and I certainly wouldn't want to make any conclusions now except to report data that we derived after 2 weeks of treatment at a relatively low dosage of phenobarbital (5 mg/24 h).

Pigment excretion was not measured in either feces or urine. The stool and urine color appeared normal for the most part.

We have regularly replaced our lamps after 200 hours use, so we can't attribute the fact that the bilirubin level could not be reduced below 10 mg/100 ml at the age of 5 months to bulb fatigue.

It would be nice to utilize California sunshine, but it is not as predictable as electricity and doesn't shine at night.

We have not measured tissue levels of bilirubin nor have we tested for renal toxicity.

The photographer dressed his subject up before taking his picture. Ordinarily, our patient was kept in the nude or sometimes with a small diaper.

13 *Studies of Immediate Hypersensitivity in Children by* in vitro *Measurement of Antigenic Release of Histamine from Leucocytes.* CHARLES D. MAY, MARGARET LYMAN* and ROSALINA ALBERTO*, New York University School of Medicine, New York, N.Y.

Antigenic release of histamine from leucocytes depends on interaction with antibody attached to cells of persons susceptible to immediate hypersensitivity reactions, and their sera contain specific antigen-neutralizing antibody. Antigenic histamine release (HR) and antigen-capacity of serum (ANC) can be measured by convenient quantitative *in vitro* procedures; examples of clinical and basic applications may serve to indicate advantages. In connection with an immunochemical evaluation of traditional long-term injection therapy of allergic disorders, an accelerated intensive dosage regimen was used to evoke maximal responses in a short time. Ten allergic subjects received amounts of ragweed, alternaria or house dust extracts in a few months that would require a year or more in customary regimens. Frequent measurement of HR and ANC revealed: specificity in responses to each allergen; greater though variable degrees of change in treated than untreated subjects; in no case were leucocytes completely 'desensitized'; reduction by ½ and up to 3-fold increase in sensitivity to HR and increases in ANC of 3- to 500-fold were observed ultimately. Thus immunologic efficacy of allergen extracts could be tested before extensive clinical trial. Usefulness of the *in vitro* procedures in elucidation of basic mechanisms in HR can be illustrated by a study of action of certain compounds on the process. Sufficient antigen for liberal release of histamine was allowed to react with sensitive leucocytes in the presence of graded amounts of the compounds to be tested. Concentration-dependent inhibition of HR occurred with nicotinamide, theophylline, cyclic AMP, and ethanol. This effect was found associated with another action shared by these compounds in the same concentrations—inhibition of glucose utilization by leucocytes, an essential source of energy for HR.

Discussion

WILLIAM T. KNIKER (University of Arkansas Medical Center): I have two questions. The first has to do with the stimulation of homocytotropic antibodies by the antigen. Most of these reaginic antibodies are IgE. With your intensive therapy, do you feel that you may be stimulating other kinds of homocytotropic antibodies, for example, IgG, whose biological characteristics would be expected to be different? The second question refers to the effect of various drugs upon the release of histamine in your test system. I wonder if you have tested disodium chromoglycate, which is supposed to inhibit the release of histamine from sensitized mast cells and basophils. The efficacy of this drug is still in doubt, and evidence obtained in your laboratory would be helpful.

ROLAND B. SCOTT (Howard University College of Medicine): Dr. MAY has modified this technique for measurement of histamine release so that smaller samples of blood are required. This makes the procedure more applicable to pediatric usage. As a result of your experience with this technique in studying various patients, I am wondering if you have made any observations which would have practical application regarding the ideal or optimum time interval that should elapse between injections of antigens for specific hyposensitization therapy for allergies such as hay fever.

LOUIS K. DIAMOND (University of California Medical Center, San Francisco): Is the percentage of basophils, either in the circulation or in the tissues, of any importance to the reaction of the individual or the test tube reaction?

CHARLES D. MAY (Closing discussion): As to which antibody may be affixed to the histamine-containing cell—the basophil—and interact with materials in the antigen, this, of course, could be accomplished by antibodies of other classes than immune globulin E, and homocytotropic antibody could be one.

The virtue of the procedure we have employed has seemed to us to be that we are in essence measuring the biologic reaction of the cell to the immune event so that the evaluation of the consequences of the interplay between the antigenic material and the sensitized cell are expressed in terms of release of a natural mediator of the immediate type of hypersensitivity.

We do not have additional data on whether the homocytotropic antibody may be involved.

Insofar as dichromoglycate is concerned, we have tested this in the *in vitro* systems, and it has no inhibitory effect upon release of histamine in this system in our hands.

A particular usefulness of such a procedure is in the determination of a proper dosage schedule for injection therapy. Indeed, we believe that the ascertainment of such a proper schedule should be a prelude to any serious effort to evaluate these materials clinically. Unless one knows what dosage regimen will maintain a state of immune response which one be-

lieves would have a promising clinical effect, there is little reasonableness in trying to evaluate a clinical response to an injection of unknown effect.

Thus far, our experience has been with a customary schedule type of injections and a more extreme type of intensive therapy; the chances are that, insofar as the dosage schedule required for sustained and promising effect is concerned, a proper regimen is probably somewhere in between.

Along with this, one cannot help but sound a note of caution: it may not be entirely desirable to see how high one can raise the titer of neutralizing antibody in the serum, because one may find himself also producing large amounts of soluble antigen-antibody complex, which may have more dire consequences than seasonal rhinitis or an occasional attack of asthma.

The amount of histamine in the circulating blood is indeed directly dependent upon the content of basophils in the circulating blood. However, the data presented are an expression of the percent of the total available histamine released by antigen and so are not dependent on whether the blood content is high or low. A low basophil level is a source of concern only when it is so low that the control values in the reaction are proportionately high and make the accuracy of the procedure somewhat questionable. This rarely occurs.

14 *Therapeutic Trials of L-Asparaginase in Children with Neoplastic Disease.* M. L. MURPHY, L. TALLAL*, C. TAN*, M. SCHWARTZ*. Memorial Hospital and Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Cells of some leukemias have a unique requirement for exogenous L-asparagine. Therefore they are inhibited by L-asparaginase. At Memorial Hospital 125 children were treated with L-asparaginase prepared from *E. coli*. Most of the patients were treated with daily intravenous injections; dosages ranged from 10 to 5000 I.U./kg/day. With the various preparations used the half-life in patients' plasma after a single i. v. injection was from 12–22 h. Plasma enzyme levels on maintenance therapy ranged from 0.4–57 I.U./ml. There has been a remission rate of 60 % in 90 children with acute lymphoblastic leukemia including some previously untreated and many resistant to conventional drugs. There were no remissions in 13 children with acute leukemia of the monoblastic and myeloblastic types, nor in one with chronic granulocytic leukemia. No response has been observed in 23 patients with tumors (Wilms, neuroblastoma, lymphoma, bone sarcoma, embryonal rhabdomyosarcoma, and adenocarcinomas). In children with leukemia, remissions were achieved with all dosages. Clinical improvement became evident as early as 24–48 h, but the usual time to establish marrow remission was 21–28 days. Remission duration in patients who relapsed was 1–8 months. Retreatment with a higher dosage achieved remission in 4 of 17. In contrast to conventional agents, depression of normal bone marrow elements has not been a problem at any dosage. Transient side effects, however, include fever, nausea, weight loss, abnormal liver function tests, hypoalbuminemia, hypofibrinogenemia, hypolipidemia, low grade anemia, and allergic reactions. (Supported by grants NCI CA-08748 and NCI CA-05826.)

Discussion

DONOUGH O'BRIEN (University of Colorado Medical Center): The use of enzymes to combat metabolic problems seems to be growing, and the use of fungal glucosidases in some types of glycogen storage disease is a nice example.

Because of this, I would be very interested to know whether you can tell us whether the half-life of the administered asparaginase changed when the children went into remission. This might provide helpful information as to how readily antibodies are formed to an intravenously administered enzyme.

AARON RAUSEN (Mount Sinai School of Medicine): At the clinical oncology meetings last month another complication which perhaps has more grave consequence was noted by the workers from the NCI. Of approximately 50 patients that they treated with L-asparaginase alone, they had two instances of hyperglycemia, one with ketosis and, at least in their abstract report, death, presumably due to fulminating diabetes.

This last month, using L-asparaginase in combination with other agents which admittedly could have potentiated the diabetic effect, we had the misfortune of having one child go into fulminating acute diabetic ketoacidosis, requiring insulin for 15 days. This was a temporary affair, and diabetes is perhaps another complication of treatment with L-asparaginase.

M. L. MURPHY (Closing discussion): In answer to the first question, we have observed when a patient is receiving a dose of 5000 units per kg that he may begin to develop hypersensitivity in about 10 to 14 days, and at that time, although the level of the enzyme has been maintained at the plateau, which was characteristic of that particular enzyme source, it will fall as the patient develops his allergic reaction. In one adult, Dr. SCHWARTZ determined that this fall in maintained enzyme level occurred several days before the onset of the clinical allergic manifestation.

We have not observed diabetes, as was described both in that patient whom I heard about over the telephone or in the ones that were reported from NCI. We may have missed it, but the patients who have had long maintenance of adrenal steroids seem to be more susceptible to the hypoproteinemia, and they might be expected to also become diabetic, but as yet we have not observed a diabetic state during therapy.

15 *Chronic Hemodialysis and Diet Therapy: Rehabilitative Measures for Children Awaiting Cadaver Renal Transplants.* C. D. LARSEN*, D. E. POTTER*, J. SIMMONS*, M. A. HOLLIDAY, Department of Pediatrics, University of California Medical Center and San Francisco General Hospital, San Francisco, Calif.

During the past 27 months we have treated 5 children, ages 2, 11, 14, 15, and 16 (at onset), on hemodialysis for 9, 10, 10, 18, and 16 months respectively. The 2- and 14-year-olds were dialyzed as outpatients; the others were dialyzed at home after center training. All were dialyzed 3 times/week on a Kiil kidney. The 11- and 14-year-olds received a successful cadaver renal transplant 6 and 17 months ago. Hypertension, hypervolemia, anemia, and hyperphosphatemia have been controlled by adequate dialysis, diet restrictions, and occasional transfusions. Three attend(ed) school during dialysis and a 4th has a home tutor but is physically active. All are keeping up with their classes. The 16-year-old girl is again having menses. None have grown on dialysis. All but the 2-year-old are stable in weight but wasted on a self-selected low salt diet. The 16-year-old averaged 1700 kcal/day. A 3 kcal/ml supplement

providing 800 extra kcal increased total intake to 2500 kcal/day. Over 6 weeks cell mass (TBW-ECW) increased. The 2-year-old was wasted after 6 months dialysis and poor food intake (300–500 kcal/day). Fed a complete formula by gastrostomy the last 3 months, to ensure adequate intake, she gained weight and improved. These experiences suggest that anorexia can lead to calorie deficiency which can be overcome by high density calorie supplements. Chronic hemodialysis, including home dialysis, has proven technically possible and clinically feasible as a means of rehabilitating children awaiting cadaver transplantation.

Discussion

CONRAD M. RILEY (2800 East Cedar Avenue, Denver, Colorado): First, a technical question: Were you able to use the Kiil kidney on the 2-year-olds without the aid of a pump, or was their arterial pressure enough to keep it going?

Secondly, in the younger patients on whom we have carried on dialysis for any length of time, their emotional status has been a great problem. In the ones that are old enough to realize that they are waiting for a kidney, it has been a good deal of a problem to carry them through until a kidney has become available. I'd be curious as to how your patients bore up emotionally.

HARRY MEDOVY (Children's Hospital, Winnipeg, Manitoba, Canada): I think this question must have occurred to several of us in the audience, and I wonder if we could have some further detail on the emotional problems presented by these children, and how these problems were managed.

C. D. LARSEN (Closing discussion): The first question: Priming solutions of either blood or normal saline are used for the first few dialyses. Thereafter, the children are able to prime the kidney with their own blood without any significant changes or alterations in blood pressure. A pump has been required only with very slow flows secondary to shunt complications.

As regards the second question, I would say that the overall emotional adjustments have been quite satisfactory. With the small children, the emotional status has correlated better with the success of dialysis and their physical well-being than any basic psychopathology. One 2-year-old ate very poorly, and this was a point of contention between her and her mother. Since she had had a subtotal gastrectomy, we are not sure how much of her resistance was emotional and how much physical. With gastrostomy feedings, which she readily takes, she has improved in all respects.

Of the older children, one is attending school regularly after 20 months on dialysis and seems to be fairly well adjusted, although she does have labile periods. At no time has any professional (psychiatric) help been required.

The teen-ager who has been on dialysis for 18 months has high anticipations for a transplant, and on occasion we have to discourage this, hoping that he will more realistically accept dialysis. We have not been fully satisfied with his overall emotional adjustment in the sense that he has dialyzed in the daytime and has had a home tutor throughout this period rather than attending school. A strong family dependency, especially between him and his mother, exists, whereas we would like him to express more independence.

The prepubertal and early adolescents have adjusted quite well, perhaps the best of the whole group.

BARBARA M. KORSCH (Children's Hospital, Los Angeles): We have studied 23 families, with children ranging from 2 to 18, and have made a special effort to follow up on their psychologic, emotional, and social rehabilitation. I well be presenting the results on Saturday and don't want to take too much time now.

Essentially, our results are very consistent with what the northern Californians have observed. We haven't had so many nutritional problems. I don't know whether our climate is more conducive to good food intake. Most of the children on dialysis have automatically, with increased well-being, increased their calorie intake, and we have also documented growth in the younger children.

Psychologically, we have seen a number of problems, but none of them, really, in the nature of psychopathology. We have seen what you would expect with any chronic illness and major medical treatment program.

We have given a great deal of support to these families all through the couple of years that we have been working with them, and we have achieved, as was described, school experience for all the children. We have seen no major family decompensations or breakdowns.

We have only one adolescent girl who I would say falls in the category of having had a major psychopathologic reaction, to the extent that she became very depressed, and there was question about her taking her medication, but this, interestingly, was after transplant. As a matter of fact, if there is one observation we have made which has been of special interest to us and came as a surprise, it is that the period of posttransplant, rather than the period of extended hemodialysis awaiting transplantation, with the various complications, surgical, medical, and psychologic, has been the one when these patients needed our support and help the most. It's during the months after transplant, when the health of the kidney is most in danger, that the health of the child also, in our experience, seems to be most vulnerable, and when we have had to give a great deal of support and attention to the family and the child, most especially the adolescent age group.

Dr. LARSEN: Our experience with the successful transplants is that the children seem to adjust and require less medical contact and support than those on chronic dialysis. However, the greatest number of our cases have been in the preadolescent age groups, which may explain the difference between our findings and those of the Los Angeles group.

16 *A Study of Children With Increased Lead Burden.* S. M. PUESCHELL*, L. KOPITO*, J. DEHLINGER* and H. SHWACHMAN, Children's Hospital Medical Center and Harvard Medical School, Boston, Mass.

The recognition of children with increased lead burden who would normally escape medical attention continues to be a major public health challenge. The present study applies a newly developed, simple and inexpensive screening procedure based on the analysis of lead in hair to find such affected children. During the summer of 1968 in a house-to-house survey of a run-down section of Boston, parents of 800 children between the ages of 1 to 6 years were interviewed and hair samples taken from the children. Of 705 determinations of lead in hair, 316 were elevated. Blood samples

were obtained from those children with high values (over 100 mcg/g) and a history of pica. When the blood lead concentration was more than 40 mcg % (in 114 children) and/or when the lead content in hair was markedly elevated, radiologic studies were performed and a test dose of a chelating agent was administered. Ninety-eight children were found with high blood lead levels (more than 50 mcg/g) and/or where chelation evoked a high urinary lead output (more than 500 mcg/24 h). Of these children with the increased lead burden, 60 had a detailed examination including neurological, motor function, psychometric and other laboratory studies. They are being followed in a long-range study. This survey points to the high incidence of the increased lead burden in one of the 'high risk' areas in Boston. Public Health measures are being instituted.

Discussion

J. JULIAN CHISOLM, Jr. (Baltimore City Hospitals): This is an obviously intriguing method of screening young children for early detection of plumbism. It is intriguing because it may be much more acceptable to the population at risk than is the drawing of large amounts of blood. On the other hand, a useful screening test must be both accurate and easy to interpret.

I would like to ask Dr. PUESCHELL three questions. How many children in this group who had normal levels of lead in hair (i.e., < 100 μg/g) also had both EDTA mobilization tests and blood lead determinations? In other words, if the concentration of lead in the segment of hair adjacent to the scalp is < 100 μg/g, can we be confident that the child has no evidence of an increased body burden of lead? As I understand it, the purpose of this simple screening test is to identify children with an increased body burden of lead. Such control data would be of great help in interpreting the material you have just presented.

Secondly, how are we to interpret an elevated level of lead in hair? Does it reflect recent exposure only without regard to body lead burden or must the body lead burden be increased before lead begins to accumulate in hair?

Thirdly, a number of people, including myself, are worried about the question of external contamination of the hair by lead. Thus, the amount of lead measured by your technique could include lead from two sources: namely, lead incorporated into hair by the hair follicle and lead from external atmospheric sources bound to hair, but not incorporated. Obviously, if lead in hair is to provide an index of lead absorbed into the body, external contamination of the sample must be minimized or excluded. To what extent have you been able to control this sampling problem?

FRANK A. OSKI (Hospital of the University of Pennsylvania): What other objective biochemical data do you have to indicate that the lead burden was associated with lead poisoning?

Specifically, did you measure delta-amino-levulinic acid in the urine, or some nonspecific urinary findings, such as glycosuria, proteinuria, coproporphyrinuria? What percent of these children were anemic or iron-deficient?

EDWARD B. SHAW (65 Arguello Boulevard, San Francisco, California): In your book you refer to the fact that the place to look for a lead line is around the mucocutaneous junction of the anus.

LEWIS A. BARNESS: That's when I was in psychiatry, Dr. SHAW.

Dr. SHAW: I've never seen it, but it occurs to me as an important ingredient of a physical examination, and I wonder if this has been observed at all in this series of yours and if it has some clinical value in investigating the incidence of lead poisoning in children with a heavy degree of exposure—I don't know. We don't see lead poisoning often in San Francisco. We don't burn battery boxes, and we use different paint, I think.

S. M. PUESCHEL: In reference to Dr. CHISHOLM's first question, asking for the number of 'false negatives', we found seven children with an increased lead burden whose hair-lead content was below 100 μg/g. However, since we obtained a relatively reliable history of all children under study with regard to their ingestion of lead-containing materials, we probably did not miss any of the acute cases where a very recent intake of paint or plaster was not detectable in hair at the time of survey. Therefore, it is of importance that historical data be obtained when hair analysis is employed as a screening device for lead poisoning.

Dr. CHISHOLM's second inquiry relates to the disease process in its acute or chronic stage. The majority of our children with increased lead burden presented with a mixed picture, since ingestion of lead-containing materials usually occurred both during the past year and also more recently. This is supported by the hair-lead analysis and radiological examination, as well as the nearly linear age distribution of the given children.

In reply to Dr. CHISHOLM's third question concerning external contamination of the hair by lead from the atmosphere, we indeed observed a gradual increase of the lead concentration in hair, examining segments from proximal toward distal. This important factor, however, can be controlled by subtraction of the known contamination in normal hair in the respective segment.

Referring to Dr. OSKI's question as to whether other laboratory studies had been performed, we examined urine for delta-amino-levulinic acid and corproporphyrin in some of the involved children. Since these data are incomplete and inconclusive, they have not been reported. Furthermore, a urinalysis and urine amino acid chromatogram were done on all children with an increased lead burden. Both of these tests proved to be within normal limits for 95 % of the children. In addition, we found 7 % of the children with an increased lead burden to be anemic, and in 65 %, basophilic stippling was observed.