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ABSTRACTS

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PLENARY SESSIONS

1 *Developmental Changes in the Physiology of the Placental Circulation.* ABRAHAM M. RUDOLPH and MICHAEL A. HEYMANN*, Cardiovascular Research Institute and Department of Pediatrics, Univ. of Calif., San Francisco, Cal.

The five-fold increase in fetal weight in the second half of gestation in the ungulate is associated with no increase in placental weight. We have examined the physiological changes necessary to provide the fetus with adequate oxygen supply in these circumstances. Twenty-two fetal sheep and goats, 85 to 147 days pregnant, were studied in utero during low spinal analgesia. Umbilical arterial and venous pressures were monitored. Fetal placental blood flows were measured by the antipyrine method and fetal cardiac output was derived from calculation of the distribution of nuclide-labelled microspheres injected into the fore- and hindlimbs, as previously described. Placental vascular resistance was derived from umbilical pressures and placental flow. Total fetal cardiac output increases with gestational age, but decreases relative to fetal weight. The percentage of total cardiac output distributed to the placenta increases with gestational age. Whereas total placental flow also increases with gestational age, it parallels fetal weight. Placental vascular resistance falls rapidly from 90–110 days and more slowly after this to term. These studies indicate that there is either a marked increase in the number of vessels in the placenta, or a dilatation of the placental vascular bed, with fetal development, allowing placental blood flow to meet fetal requirements without further placental growth. The change in placental vascular resistance also results in a redistribution of blood flow in the fetal-placental circulation. In early pregnancy although total cardiac output is high relative to fetal weight, the proportion distributed to the placenta is low, indicating a lower systemic vascular resistance. Based on these findings, possible changes in diffusional characteristics of the placenta with growth will be discussed. (Supported by NIH Grants HE 06285 and HD 02805.)

Discussion

F.H. ADAMS (UCLA Medical Center, Los Angeles): As usual, Dr. RUDOLPH has presented us with some new thoughts and ideas. However, I think more work needs to be done in this report before it is published. In this connection, I would like to make several points: First, I am concerned that the number of observations made on the fetuses varied for the different parameters studied. I would like to make a plea that, before final conclusions are drawn, the number of experiments for each of the parameters be the same, and that the gestational ages be the same. Some of the data included fetuses under 100 days' gestation, and other data did not. Second, as I understand the conclusions of the authors, it is postulated that the lamb fetus meets his nutritional requirements late in pregnancy by an increase in placental blood flow due to a reduction in placental vascular resistance. Yet the material presented showed virtually no change in placental vascular resistance after 110 days' gestation, a period when the fetus doubles his body weight prior to delivery. Third, the sheep is a good model to use in studying fetal physiology for several reasons: it is placid; the fetuses are large enough to perform surgical manipulations and to remove serial blood samples; the umbilical cord is long; the placenta does not separate immediately from the uterus upon removal of the fetus from the uterus; and a large amount of data already exists regarding the physiology of the lamb fetus. In spite of these favorable points, however, it must be remembered that the sheep is quite different from the human, and therefore conclusions drawn from sheep experiments cannot necessarily be applied to the human. The human uterus and placental relationships are quite dissimilar. Regional blood flows in the human fetus are not likely to be the same due to the relative differences in organ size such as the brain. Fourth, it must be recognized that the studies reported by the authors were performed under anesthesia, and this can have a profound effect on many vascular phenomena. It is well known that anesthesia can significantly affect the circulation to the uterus and to the lung. The circulation to the fetal lung is particularly sensitive to various drugs and chemicals, and thus these would affect the results.

A.M. RUDOLPH: Well, firstly, Dr. ADAMS, I think that we also can tell the difference between a sheep and a baby; (laughter) and I don't think we really meant to imply that this work carries over completely to human infants. I think the only mention I made of the infant was in reference to the fact that a baby with a very large cardiac output due to a ventricular septal defect may go into failure. I think it is fair to say a lamb may behave in a similar manner. I do think, however, that in spite of species difference limitations, the sheep is a useful model for fetal studies. As for some of your other comments, I agree that we have not presented the data on all the animals in every slide, and the reason for this is that we did not have every observation on

* By invitation

every animal. As I mentioned, we had cardiac output studies in only twelve animals. I do think, however, that this podium is a place where one should present information which is perhaps not finally completed work, for thought and consideration. Regarding the comment about the effect of spinal anesthesia on the fetus, I did not have time to go into all the details of our technique, but these animals were studied either with spinal anesthesia or in most situations where the animals had already recovered from their anesthesia. I would, however, like to make a point about spinal analgesia. Dr. ASSALI's findings that spinal anesthesia markedly interferred with uterine and fetal circulations, relate to rather extensive high spinal anesthesia. We have made some observations on sheep in which catheters have been chronically inserted into the fetus, and when we administer low spinal analgesia as we usually do, there was no interference whatsoever with the fetal placental flow or other parameters in the fetus. I again hope that this presentation will stimulate thought and more extensive work on the changes in fetal circulation and its distribution with changes in gestational age.

F.C. BATTAGLIA (University of Colorado Medical Center, Denver): I'd like to ask how you interpret the wide range in oxygen consumption of the fetus, from 3 cc/kg/min to 10 cc/kg/min, and at roughly the same gestational age, if I remember that slide properly. I gather that, in this study, the data obtained on an individual animal represents one point on the graph; and then another animal is studied at a different gestational age. Could the wide range in oxygen consumption for different fetuses at the same gestational age mean that it would be somewhat hasty to conclude that oxygen consumption per kilogram measured throughout gestation in the same fetus would not change?

A.M. RUDOLPH: I think this is a valid point. These were in most cases individual observations on individual fetuses, but I'm sure that there must be some variation in the same fetus at different periods of study. We have not got observations on the same fetus, except in the one fetus over a two-week period, where the oxygen consumption remained about the same.

F.C. BATTAGLIA: What I'm really asking—and I think you have answered this point—is whether it is not possible that a fetus who has a consumption of 3 cc/kg/min, say, at 100 days, remains at that level for many weeks of gestation, while another fetus starting out at 7 cc/kg/min at 100 days remains at its level—a different, fixed level peculiar to each fetal-maternal unit. I think that it's hard from a study carried out on different animals at each different gestational age, to decide on changes in consumption with gestation.

A.M. RUDOLPH: I don't know the answer to that question. Do you?

F.C. BATTAGLIA: No, I don't. It seems to me, it is still an open question.

A.M. RUDOLPH: Well, I don't know. We obviously need more information on animals in which the same fetus is followed over a large time period.

F.C. BATTAGLIA: I would agree. I would like to make one more comment. You are using the flow method of meschia, which relies upon the establishment of a steady state. I do believe this method has definite advantages in studies on fetal metabolism over other methods of measuring umbilical blood flow. I think he did show some time ago that in the sheep placenta the diffusing capacity to urea increased in the latter part of gestation, and paralleled the increase in fetal weight very nicely, in spite of the fact that placental weight was reasonably constant. So we do have one index, that placental function may continue to change at a rate parallel to the increase in fetal weight, but not parallel to the increase in organ weight of the placenta.

A.M. RUDOLPH: This would certainly be so. We have not looked at the diffusion problem.

W.B. WEIL, Jr. (University of Florida, Gainesville): I make a plea to consider this kind of information, such as oxygen consumption and flow, as the directly measured variable rather than expressing it as related to body weight. Unless it can be demonstrated that the measured variable is directly proportional to body weight erroneous conclusions can be drawn from the relation. Only if there is data to show that oxygen consumption per kilogram of body weight does remain constant over the weight range under study, is it appropriate to express this variable in this manner. But if the oxygen consumption changes in some way with weight, then it is incorrect to use the ratio, because it can distort the data. I think that we are frequently tempted to assume that these variables do vary directly with weight when, in fact, this may not have been conclusively demonstrated. It is easier if we can break away from tradition and evaluate variables such as oxygen consumption or flow in liters per minute—by themselves, and then relate that variable to weight or whatever other variables one wants to use.

A.M. RUDOLPH: We did in fact, in one of our slides, show that there was a roughly parallel relationship between oxygen consumption and fetal weight. Perhaps you missed it.

2 *Hyperviscous Blood and Perinatal Pathology.* RICHARD S. BAUM*, Harvard Medical School, Boston, Mass. (introduced by Clement A. Smith).

In newborn infants with neurological and/or cardiorespiratory dysfunction, we have noted extreme elevations of hematocrit with surprising frequency. Fifteen such neonates have been found with cutaneous hematocrits between 77 and 93 %, corresponding venous values ranging to 79 %, and viscosity increased to as high as 24.9 centipoise at presumed arteriolar-venular shear (11.5 sec^{-1}). The majority of these term and post-term infants presented in common a dysmature appearance (dry skin, sparse subcutaneous tissue, meconium staining) coupled with at least two of the following transient findings: 1. CNS disturbances (e.g. seizures, undue lethargy, protracted vomiting); 2. Cardiorespiratory symptoms (from mild dyspnea to profound cardiac decompensation); 3. Color changes (erythrocyanosis or, paradoxically, pallor). Rarer associated findings have included hyperfibrinogenemia, hypoglycemia and azotemia. Reduction of blood viscosity by partial exchange transfusion with plasma appeared in general to hasten cessation of the acute symptoms. Nonetheless, at least three of these infants have evidenced residual brain damage. Impaired perfusion of both placenta and baby by hyperviscous blood, it is suggested, should be added to the known causes of unsuccessful perinatal adaptation.

Discussion

H.L. BARNETT (Albert Einstein College of Medicine, New York): Before Dr. BAUM mentioned the high BUN, I was going to suggest that the fourth 'C' might be the cortex of the kidney, and I hope that he will—as we will—look into the effect of hyperviscosity on renal function in more detail.

FLOSSIE COHEN (Child Research Center of Michigan, Detroit): We have been quite interested in this problem and have studied a few babies, but have taken a different tack. Our interest started with the alleged reports of polycythemia being the result of maternal-fetal transfusion. One of the simplest ways of demonstrating this fact is by the hemoglobin content of the individual red cells of these babies; namely, by showing with the acid elution test whether there is a large percentage of adult hemoglobin-containing cells in these infants. Using this procedure we were startled to find that, quite to the contrary, there was not only an absence of an increased number of adult cells, but that there was not even the normal number of adult hemoglobin-containing cells seen in normal full-term infants, and that these babies behaved like prematures—with regard to both the percentage of cells with fetal hemoglobin and percent determined chemically by the alkali resistant hemoglobin which was in the 80% range. The exact mechanism for this is not clear to us though the possible role of intrauterine hypoxia delaying the 'switchover' mechanism seemed quite attractive. Do you have any comments?

R.S. BAUM: Early in this series we didn't think of doing acid-elution stains. In the latter portion we made both acid-elution stains and Singer determinations of fetal hemoglobin, and based upon these two different types of hemoglobin assay, our observations are a bit different from yours. If intrauterine stress did evoke an excessive outpouring of red cells, it did not favor to any clear degree the production of one type of hemoglobin over the other.

S. KARELITZ (The Long Island Jewish Hospital, New Hyde Park): I merely want to report that I have seen some newborn infants with hemoglobins of 22, 23, 24 gm who in the first few days of life put out urine of a very low specific gravity—at around 1.004–1.005. The hemoglobin began to drop after a week to levels of 15–17 g and at about this time they started to put out large amounts of urine. After several days they died even though fluid replacement seemed to have been satisfied, and at postmortem we found nothing. Postmortem revealed no explanation for the condition. The kidney revealed no pathologic changes.

R.S. BAUM: The only baby that after birth had any symptom related to the urinary tract was a child who failed to pass urine for forty-eight hours. In intrauterine life several babies had associated disturbances of amniotic fluid, either oligohydramnios or polyhydramnios. None of the babies has died, so we can't tell you what their kidneys look like.

M. HOLLIDAY (San Francisco General Hospital, San Francisco): Do you think the high hematocrit was due to a loss of plasma, which would be rather extraordinary or due to an increase in red cell mass? Second, in the nature of a comment: the low blood sugar, or high blood sugar or high NPN may be partly a reflection of the fact that you have blood of different red cell/plasma ratios, which might alter your results. Particularly of interest is the observation that the cutaneous hematocrit was so much higher than the central venous hematocrit, which would suggest that somewhere there is blood circulating that is of a lower hematocrit. I wonder if this might be to more critical organs, and whether this may be a protective device.

R.S. BAUM: We simply don't know whether these babies have a reduced plasma volume, or an increased plasma volume with a still more increased red cell mass, or what: and in the absence of blood volume measurements with a red cell label I don't think we can tell. Dr BILL ANDREWS in Louisville, however, has measured blood volumes with Evans blue in some infants like these, and found that often the plasma volumes are surprisingly low. The important thing, I think, is this. Whether there is an increased red cell mass, or whether there is a diminished plasma volume, wherever you have a relatively high proportion of cells to plasma you have relatively high viscosity, and viscosity is an independent variable which may have rather considerable effects on the circulation.

JOAN E. HODGMAN (University of Southern California, Los Angeles): We have had the same experience in our nurseries with babies with very high hematocrits, central nervous system symptoms and unpleasant clinical results. I assume from the birth weights you are talking primarily of term born infants, but I would be interested in the gestational ages.

R.C. PHIBBS (University of California Medical Center, San Francisco): Your hypothesis that the polycythemia becomes a selfworsening process above a particular level of blood viscosity and hematocrit is an attractive one and is similar to what has been considered with respect to the severe polycythemia in some of the people living at very high altitudes in the Andes. If this sort of a positive feed back phenomenon really is occurring in these infants then a central question would be: What is the critical hematocrit above which this vicious cycle begins? There may be an easy way of answering this question from data you already may have. Once an infant exceeded this critical hematocrit by just a little bit then, by the positive feed back phenomenon you have suggested, his hematocrit should rapidly rise to a level very much higher than the critical hematocrit. If you looked at the distribution of hematocrits in normal and polycythemic babies there should be a large group of babies with hematocrits at or below the critical level and a second group with hematocrits very much above this level but very few infants with hematocrits just at or slightly above the critical level. The clinical importance of defining a hematocrit above which one could regularly expect complications of polycythemia is that it raises the possibility of prophylacticly lowering the hematocrit in selected infants before these complications occur.

T.K. OLIVER, Jr. (University of Washington School of Medicine, Seattle): I wonder, Dr. BAUM, if you could answer three questions. 1. What did the placentas of these babies look like? Did the appearance suggest that oxygen transport was disturbed and therefore the stimulus for an increase in red cell mass? 2. Did any of the infants have Down's syndrome, since polycythemia at birth has been observed by ourselves and others, for reasons that are still totally obscure? 3. Could you comment on the lack of hyperbilirubinemia in these patients, since one would have thought that hyperbilirubinemia might well have occurred if the life span of the red cells in these babies was similar to normal infants. Have you studied red cell kinetics?

A.O. ALWAN (George Washington University Hospital, Washington, D.C.): In our nursery we had an interesting case of a newborn infant who was thirty-four weeks gestation with single umbilical artery and a hemoglobin level of 22 g percent. Have you observed in your study any infant with single umbilical artery?

W. OH (Michael Reese Hospital, Chicago): We have studied the effects of placental blood transfusion on the

renal functions of newborn infants during the first day of life. We found that infants having higher blood volume resulting from delayed cord clamping, have a higher effective renal plasma flow, glomerular filtration rate and urine flow than those infants who have smaller blood volume as a result of early cord clamping. How these data relate to low urine output in the infant with hyperviscous blood is not clear. My question is: Would your findings be an argument in favor of early cord clamping?

AUDREY BROWN (Medical College of Georgia, Augusta): I wonder, lest we think that this is a uniform population of babies, whether you have studied the hematologic natural history of these infants after the first few days. 1. When did the hemoglobin levels decrease? 2. What were the reticulocyte counts like at the end of the first week? We have been interested in the high percentage of cells in these babies. This would suggest a prenatal stimulus, would it not?

H.H. GORDON (Albert Einstein College of Medicine, New York): I am interested in your findings in relation to two clinical findings particularly in the babies of diabetic mothers. In some infants with tachypnea and plethoric facies, at about day 4 or 5 the plethoric facies clears and the respiratory rate comes down at about the same time. The second has to do with the patients that were reported by Avery and Oppenheimer sometime ago. These infants had renal vein thrombosis without sepsis or shock. One baby who had renal vein thrombosis was a still birth.

R.S. BAUM: Dr. HODGMAN, aside from one twin which may have been born at thirty-five weeks, these babies ranged from 37 ½ to nearly 45 weeks gestation. The bulk of them were at forty weeks or beyond. Dr. PHIBBS, I don't think it is possible to give a simple 'critical hematocrit', from the standpoint of a physiologist, anyway, for that hematocrit would depend to some extent upon blood volume. The relationship between systemic oxygen transport and hematocrit does vary according to the absolute size of the circulating volume of blood. For example, if an organism has hypervolemic polycythemia, then probably because of his ability to increase diastolic filling, he should be able to compensate for hyperviscosity somewhat better than if he is normovolemic or has a reduced blood volume. Therefore, blood volume is an essential consideration here. But as a practical clinical guide, I would think that if one found a symptomatic neonate with a venous hematocrit in the 70's, or perhaps even in the high 60's, then that hematocrit would be 'critical enough' to contribute to the baby's difficulties. Dr. OLIVER, most—but not all—of the placentae we could put our hands on did show pertinent pathology. Infarction, normoblastemia and longstanding meconium staining were among the abnormalities seen. All babies in this series were without congenital abnormalities (including umbilical artery abnormalities), but I have seen elsewhere two Down's syndromes and one 13–15 trisomy with heel hematocrits in the 80's and venous hematocrits in the 70's. The infrequency of hyperbilirubinemia I would relate to the fact these were largely term infants and had mature livers, but that's speculation, and I can't prove it. To Dr. OH's comment I would add only that many other effects of placental transfusion—which ordinarily are interpreted as due primarily to an increased volume of blood being on board—also might relate to the elevated viscosity accompanying increases in hematocrit.

3 *A Biochemical Basis for the Respiratory Distress Syndrome.* LOUIS GLUCK, MARIE V. KULOVICH*, ETSURO K. MOTOYAMA*, HELEN L. SMITS*, ARTHUR EIDELMAN*, AIDA KHAZIN* and CHARLES D. COOK, Yale Univ. Sch. of Med. New Haven, Conn.

Studies on the rabbit show that surface active lecithin, which can be separated from total lecithin by acetone precipitation, is stored in lung parenchyma long before it appears, late in gestation, in alveolar wash. At term only about 11 % of alveolar lecithin is surface active, increasing by one hour after breathing to 50 %, both in the rabbit and human. Two pathways for the *de novo* biosynthesis of lecithin in fetal and newborn lung were found:

1. CDP-choline + D-α, β-diglyceride → LECITHIN
2. Phosphatidyl ethanolamine (PE) + CH_3 → P-methyl-E + CH_3 → P-dimethyl-E + CH_3 → LECITHIN

Simultaneous injection of ^{3}H-choline and ($^{14}CH_3$)-methionine have shown extremely rapid *de novo* synthesis of lecithin at one hour by the premature rabbit with RDS, 90 % being formed by pathway (1) with predominant palmitic acid esters on α- and β-carbons of lecithin. However, the total amount of surface active lecithin is much smaller than in the breathing term animal. The human is markedly different and depends to a greater extent upon the pH sensitive pathway (2), during his respiratory adjustment. Studies on human tracheal aspirates using the surface active intermediary, phosphatidyl dimethylethanolamine as a marker (with its predominant α-palmitic/β-myristic acid ester pattern) indicate that with RDS the lecithin appears to be synthesized by pathway (1) and recovery in RDS coincides with markedly increased synthesis by pathway (2) with the α-palmitic/β-myristic lecithin, as well as with an increased amount of lecithin. There is evidence that pathway (2) acts to regulate the synthesis of the alveolar layer lecithin.

Discussion

M.H. KLAUS (Stanford Medical Center, Palo Alto): It is inviting to think that by assaying an alveolar wash or secretions from the pharynx that it is possible to determine chemical changes that are occurring on the very large internal surface of the lung. Could you defend your use of pharyngeal secretions and tracheal aspirate as a proper sample of the alveolar surface? Also, a problem with using pharyngeal secretions is the ability of the saliva of some individuals to reduce the surface tension of a clean saline surface to below 12 dyn/cm. A second question: In the sick infants were all the phospholipids reduced as well as phosphotidyl dimethylethanolamine?

L. GLUCK: I think, Dr. KLAUS, I should ask you to defend your own statements about the surface. The measurement of alveolar surface—traditionally—has been done by washing out the lung. We find no reason to change this. The aspirate and the surface measured by this washout match each other exactly. This we have shown again and again as though there wasn't time to go into detail—in the surface active fraction, and in the lipids that we are particularly interested in, the lecithin and the PDME, which do reflect a metabolic change. Now, either you accept that surface active phospholipids are important, or don't. If you do, then you have to accept the fact that the aspirates and the wash are identical in composition. Radioactivity comes through in the lecithin in exactly the same proportion

injected parenterally in the aspirate as in the wash. The fatty acid components are identical. I don't know how one can state anything else.

D.M. LANE (University of Oklahoma, Oklahoma City): Dr. GLUCK, I noticed that on your chromatograms there is a decrease in the eighteen carbon fatty acids with decreasing severity of the respiratory distress. Is there significance in this change, or do you think this is just quantitative in that you are dealing with a smaller specimen?

L. GLUCK: One of the problems of this is that we may be dealing with as small as 10^{-9} concentration, which is on a pretty ultra-ultra micro scale; but, as a matter of fact, the changes in the eighteen series are quite variable, and there is not any consistent pattern associated with them. The only consistent pattern has been with the sixteen and the fourteen carbon.

L. STERN (Children's Hospital, Montreal): I wonder, Dr. GLUCK, would you also extend your vote toward the early correction of the acidosis, in an effort to improve this synthesis?

L. GLUCK: Yes.

D.B. CHEEK (Johns Hopkins Hospital, Baltimore): This role of methylation in the formation of surface active material is of great interest to us. For some years we have emphasized the role of adrenalin in the respiratory distress syndrome where high concentrations of catecholamine are found in the blood of these premature infants (Pediatrics *31:* 374 [1963]). Adrenalin can diminish blood volume and increase the hematocrit and, as we heard in Dr. BAUM's paper, increase the viscosity of blood. In premature infants we have felt that enzymes responsible for the degradation of catecholamine—such as methyl transferase might be lacking hence there may be a situation of nonadaptation to stress with circulatory collapse from persistent and high levels of adrenalin or noradrenalin and failure of methylation or inability to move down to metanephrine. We suggested last year at these meetings that measurement of methyl transferase in the tissues might be most informative regarding this disease. Indeed most of the physiopathology can be explained on the basis of deranged catecholamine metabolism (CHEEK and ROWE, Pediat. Clin. N. Amer. *13:* 863 [1966]).

L. GLUCK: Furthermore, there may even be competition for the same methyl groups between the formation of the surface-active lecithin and the metabolism of the catecholamines in the O-methylation by the same mechanism.

4 *Fasting Hyperlipogenesis. An Inborn Error of Energy Metabolism in Prader-Willi Syndrome.* STANLEY JOHNSEN*, JOHN D. CRAWFORD, and HERBERT A. HAESSLER*, Harvard Med. School, Boston, Mass.

Seven mentally retarded patients aged 4 to 19 years with the clinical features of Prader-Willi syndrome have been studied. All showed poverty of fetal movement and extreme infantile hypotonia. With improvement in muscle tone, feeding difficulties abated and were replaced by uncontrollable hyperphagia. Plethoric obesity, retarded psychomotor development and diminutive hands and feet were noted. All teenagers were under 5 feet tall due to slowing of growth at the start of the second decade. Pubertal changes were delayed with oligomenorrhea in females and genital hypoplasia, with small pseudo-cryptorchid testes in males being characteristic of the affected teenagers. None of our patients was diabetic. Karyotypes, PBI, cortisol and Su 4885 responses were normal. Urinary gonadotropin was undetectable, plasma testosterone low, and biopsy in a 19-year-old showed immature testicular tissue. Serum cholesterol, NEFA, triglyceride and phospholipids were normal. Subcutaneous fat biopsies showed elevated palmitoleic acid levels suggestive of hyperlipogenesis. Fat synthesis from acetate during fasting was ten-fold greater than in tissue from unaffected sibs and hormone stimulated lipolysis was depressed. It is concluded that, as in the genetic obese-hyperglycemic mouse, patients with Prader-Willi syndrome have a defect in regulation of lipogenesis and lipolysis such that, during fasting, substrate continues to be utilized for formation of new fat rather than to satisfy energy needs. In the presence of deficient lipolysis, survival depends upon a continuous supply of exogenous calories.

Discussion

M. KOGUT (Children's Hospital, Los Angeles): Since these patients often develop diabetes later on, I wonder whether insulin has been assayed in your patients, and might not your data suggest increased insulin activity, rather than a defect in fat metabolism at the tissue level.

S. JOHNSEN: We have measured plasma insulin in all patients, but at this time have the results in only a few. Some of the insulin levels have been quite high. In one patient fasting levels of 60 microunits/ml were measured with a rise to over 320 microunits/ml in response to an intravenous infusion of glucose. The role that increased insulin activity, if indeed uniformly present, might have on the genesis of the obesity in this syndrome is not clear. One might be much more inclined to think it secondary as in simple obesity.

H. ZELLWEGER (University of Iowa, Iowa City): Fourteen cases of this syndrome, called Prader-Willi or H_3O syndrome (H for hypotonia, H for hypomentia, H for hypogonadism, and O for obesity) have been studied in the Pediatric Department of the University of Iowa. The uniformity of the clinical picture of our cases and cases observed elsewhere is striking. These children are almost atonic in the neonatal period and convey the impression of a severely compromised central nervous system. Tendon reflexes, as well as sucking, swallowing, Moro, withdrawal and Galant's tail reflex are absent. Improvement is slow and feeding by nasogastric tube may be necessary for weeks or even months. The combination of these findings with the impressively hypoplastic scrotum (often not more than a piece of corregated skin) may suggest the possibility of H_3O syndrome; however, several such cases—among them a case of balanced 14/18 translocation—did not develop hyperphagia and obesity. Thus it is prudent to postpone diagnosis until the latter manifestations have appeared. We have, together with Dr. H. SCHNEIDER and Dr. V. ROWLEY, reexamined our cases and made a few interesting observations which I would like to mention here. 1. These children lack control of their emotions and are in this respect somewhat similar to mongoloids. Both mongoloid and H_3O children can be extremely stubborn and fully inaccessible to any kind of exhortation. If they do not want to be examined there is little possibility in persuading them to comply with any test procedures. Temper tantrums—rare in mongoloids—are not unusual in these children. On the other hand they may show exuberant feelings of joy and pleasure and go almost into rapture when their nurse or doctor appears. We wonder whether or not the hyperphagia

and lack of emotional control are not functionally related, the former being possibly due to a defective control of hunger and satiety. 2. Mental subnormality is an almost constant finding and combines with delayed speech development and a consistent articulation defect. These functions tend to improve. The Wechsler Intelligence Score for Children (WISC) of one of our boys rose from 64 to 85 within a few years. 3. Most of these patients have a considerable delay of bone age which cannot be explained by growth retardation alone. PBI was normal in all our cases. 4. We lost one child whose obesity had reached Pickwickian dimensions (hypercapnia may have had a deleterious effect). The postmortem examination of the brain did not reveal any pathological findings. This case, however, taught us that efforts to limit food intake are mandatory. Reduction of calories alone will never be successful because of the incoercible urge to eat. Amphetamines (Dexedrine sulfate 5–15 mg/day) seemingly curbed the hyperphagia and allowed to limit food intake successfully in some cases, yet not all cases responded similarly to this treatment.

S. JOHNSEN: In only two patients has the bone age been as much as a year behind the Greulich and Pyle standards. There was one among six patients over twelve and one among the three under twelve. A nineteen-year-old patient had a bone age of seventeen and a four-year-old one had a bone age of two and one-half.

R. KAYE (The Children's Hospital of Philadelphia): I would like to comment on the relationship between obesity and hyperglucosemia and hyperinsulinemia. JOHN BUTTERFIELD has shown that in obesity insulin in the blood tends to bypass muscle. This shifts glucose disposal away from muscle to adipose tissue. As more insulin per unit of glucose is necessary for glucose uptake by adipose tissue, hyperinsulinemia results. Restoration of body weight to normal reverses the above process. I would expect that this relationship could be demonstrated in your patients.

G. HOLMAN (Medical College of Georgia, Augusta): We have been very fortunate to study some of these patients while we were at Kansas City, and also more recently at Augusta, and can confirm many of the findings that you have mentioned, although we have not your elegant studies in fat metabolism. One of the problems of comparing this interesting group of patients is that they change with age. Our patients when studied before three years of age did show insulin hypersensitivity. They were able to increase urinary catecholamine excretion during induced hypoglycemia. Unfortunately, we did not measure growth hormone levels at that time. Their clinical course would suggest, perhaps, some failure in growth hormone during the first few years of life, and then they appear abruptly to change around 2 ½ to 3 years of age, when the hyperphagia becomes manifest. We have indeed found markedly reduced bone ages in all of our younger patients. They tend to catch up as they get older. I wondered if you had a chance to study some of the younger children from the point of view of growth hormone secretion. One other comment: We have had, unfortunately, an opportunity to study two of these children at autopsy, and extensive evaluation of the hypothalamus has shown no anatomically demonstrable lesion. Also, one of these children died from a hypoventilatory syndrome from the extreme obesity, and I think this must be remembered as a possible sequela these patients may have.

S. JOHNSEN: The growth hormone assay was normal in a four-year-old. This is the youngest patient we have studied.

W.W. MCCRORY (Cornell Medical Center, New York): It was not clear when you showed your slide of the *in vitro* response to decreased lipolysis what your controls were. I would be interested in knowing whether your controls were other children of comparable age and sex with obesity, or were they nonobese normal children?

S. JOHNSEN: Our controls varied. Three were normal siblings, three were children undergoing surgery, and the remainder were adults. Only one control could be considered obese. His response was similar to the others. At this time, I cannot state how the fat tissue from a patient with simple obesity would respond to in vitro hormone-induced lipolysis. This has not been done in our laboratory.

5 *Physiologic Mechanisms in Infantile Hypoglycemia.* R.E. GREENBERG*, R.O. CHRISTIANSEN*, N. REMEN*, A.F. KOHRMAN* and G. REAVEN*, Depts. of Ped. and Med., Stanford Univ. School of Medicine, Palo Alto, Cal. (introduced by R. Alway).

A basic problem in evaluating infants with severe fasting hypoglycemia is the distinction between reduction in hepatic/renal glucose output and increased peripheral glucose utilization. The metabolism of glucose-U-C^{14} in three affected infants was used as a means of delineating physiologic mechanisms. Glucose-U-C^{14} was given IV in the fasting state, during constant glucose infusion or simultaneously with cessation of glucose infusion. Glucose was isolated from serial blood samples by extraction and thin layer chromatography and specific activity determined. Rates of glucose utilization were calculated by planimetry and compared to some studies wherein constant infusion of glucose-U-C^{14}, at a steady state of blood glucose concentration, provides a direct means of determining glucose utilization. Insulin turnover was determined following insulin I^{131} injection by measuring immunoreactive insulin and specific radioactivity.

The results of these studies indicated the following patterns. *Case 1:* marked reduction in the rate of hepatic gluconeogenesis (supported by *in vitro* studies); *Case 2:* persistence of enhanced peripheral glucose utilization independent of insulin secretion; *Case 3:* increased insulin secretion without a marked increase in glucose turnover.

These initial studies suggest that the use of these and similar techniques may facilitate comprehension of basic physiologic mechanisms in children with severe hypoglycemia.

Discussion

J.F. CRIGIER, Jr. (Boston): Have you tested the gluconeogenic ability of your first infant after the use of steroids or ACTH, using fructose tolerance test? It is difficult to understand why his hypoglycemia did respond to these hormones. Was the baby who died very young the smallest infant?

R.E. GREENBERG: No studies were done to evaluate the capacity for gluconeogenesis. Further, we have no means of evaluating the substrate pool. That baby was the small infant.

W.H. BERGSTROM (Syracuse): In the case of the third child, or a similar child where you suspected an inappropriate rate of insulin secretion—that is, not necessarily high, but disproportionate to the blood glu-

cose level—would it be apropos to try inhibiting insulin secretion with diazoxide.

R.E. GREENBERG: The baby was treated with diazoxide and epinephrine, individually, for significant time periods, without demonstrable effect. Steroids, ACTH and zinc glucogon were similarly ineffective. All therapeutic efforts preceded the isotopic studies.

D. SANDBERG (University of Miami School of Medicine, Miami): The third patient you described has a resemblance to patients with protein-losing enteropathy who have an abnormal rate of loss of radioactive level from the blood. We have had three or four infants who have had a glucose concentration in gastric juice that was 300 or 400 mg % at a time when the blood glucose level was around 20 mg % or less, and I wondered if you had had any experience like this.

M. CORNBLATH (University of Illinois College of Medicine, Chicago): Was the first patient a large or small baby? There is some recent evidence that free fatty acids are necessary in the liver to have effective gluconeogenesis. Did you measure free fatty acid levels in this particular baby? Were there any unusual findings in this infant's pancreas at autopsy? We had a similar infant with intractable hypoglycemia who did not respond to diazoxide and much to our surprise, she had an islet cell tumor at operation at 6 weeks of age.

R.E. GREENBERG: The baby was not a large baby. Free fatty acids were not measured; however, the concentration of glycerol was determined during periods of fasting and glucose infusion. Presuming the concentration of glycerol to be a reflection of rate of lipolysis, there was an appropriate increase in the hypoglycemic phase and decrease with glucose infusion. The pancreas was normal at autopsy.

D. FRASIER (University of California, Los Angeles): What is the evidence that the disappearance rate of labeled insulin reflects the turnover rate of metabolically active insulin?

R.E. GREENBERG: We have no data that would give assurance that insulin I^{131} is degraded at the same rate as endogenous insulin.

6 *A Possible Adrenal Medullary Defect in Hypoglycemic Fetally Malnourished Infants.* LEO STERN*, THEODORE L. SOURKES* and NIELS RAIHA*, MCGILL University, Montreal, Quebec, Canada, and the University of Helsinki, Finland (introduced by Alan Ross).

In the normal newborn infant insulin induced hypoglycemia activates the adrenal medulla with a resultant striking increase in urinary adrenaline excretion. Five foetally malnourished infants with severe symptomatic hypoglycemia in the first week of life (convulsions associated with blood sugars below 10 mgm %) were studied between the 2nd and 8th week following apparent recovery from the acute neonatal episode. Hypoglycemia was induced with insulin 0.1 u/kg, with resultant blood sugar levels from 6 to 18 mg %. Although resting urinary adrenaline excretion rates were normal, no increases were obtained following the hypoglycemic stress. Concomitantly there was a failure of hypoglycemic responsiveness with blood sugars not recovering spontaneously after the initial fall. By contrast control infants, both full term and truly premature, showed a 4 and 8 fold increase in urinary adrenaline excretion under insulin stimulation simultaneous with a normal post-insulin spontaneous glucose recovery curve. It is proposed that this defective adrenal medullary response may both contribute to, and intensify the degree of, the neonatal hypoglycemia seen in these infants. Its persistance beyond 6 months of age in at least one of the cases studied suggests a possible link between this condition as it presents in the neonatal period and the so-called 'idiopathic hypoglycemia' seen in older children.

Discussion

E. ROSSI (Kinderspital, Berne, Switzerland): I would like to know if this picture in newborns resembles the picture that was described by BROBERGER, the later stage, in which you have exactly the same situation. Have you done any studies to determine the level of insulin in these cases? And, secondly, have you taken leucin loading tests? It might be that the insulinemia is normal by the induced hyperglycemia.

L. STERN: Thank you, Professor Rossi. These children were studied, in fact, in the identical manner to those in ZETTERSTROM and BROBERGER's original studies. They had eight infants who were older and had so-called idiopathic hypoglycemia, and they were able to demonstrate this kind of defect in four of the eight. The suggestion is, that if one goes back in the history of these infants, a high proportion of them turn out in fact to be fetally malnourished at birth, and it may be that their symptoms are suppressed, because as young infants they are fed rather frequently. It is only when they go on regular meals with longer intervals that hypoglycemia appears. We did not do any insulin levels, but we did do casein (leucine) tolerance tests in two of them, and they were normal. They were done long after the babies appeared to have recovered from the episode.

M. CORNBLATH (University of Illinois College of Medicine): Did all of your babies have recurrent hypoglycemia? If so, your experience is unique. In a few babies that we have studied, all showed an increase in catecholamine excretion after tolbutamide given a week or two after recovery from their transient neonatal hypoglycemia. How did you collect the urines? What was the period of time? What did you measure? In one of our babies, for example, Dr. IRA M. ROSENTHAL, who did the determinations, found that the metanephrine content was thirty times that of epinephrine in the urine. Therefore, did you measure 3 methoxy-4 hydroxy mandelic acid (VMA), metanephrine, normetanephrine as well as epinephrine and norepinephrine? Does the data change if you express the excretion per milligram of creatinine rather than total catecholamine?

L. STERN: Thank you. The only baby who has an apparently permanent defect is the fifth one of these children. The others are now apparently well, although the defect appeared to persist on induction with insulin for some period of time. Regarding the technique, the urines were collected over a three-hour period. We did a control day and then a test day and then a second control day to compare the two controls to each other, as a reference standard. We measured adrenalin, noradrenalin and dopamine. I haven't put the figures for noradrenalin or dopamine on, but there was no change in these. They are shown on the last slide, where again only adrenalin is increased. We also assayed the catecholamine results per milligram of creatinine, and there was the same kind of increase as determined per kilogram of body weight.

T. YOSHIDA (Michael Reese Hospital and Medical Center, Chicago): Recently we studied the effects of adrenalectomy and/or different diets on renal gluconeogenesis in rats. Adrenalectomy caused a decrease of renal gluconeogenesis in rats fed on diets which contained a normal fat content. While a carbohydrate-free died per se did not increase renal gluconeogenesis, carbohydrate-free diet containing high (21 %) fat increased gluconeogenesis markedly. With such high fat diet, adrenalectomy showed no effect to alter the enhanced gluconeogenic response. It seems, therefore, that lack of adrenal hormones cannot promptly be related to the cause of hypoglycemia. Limitation of available substrates, particularly fatty acids, by various aetiologies should also be taken into consideration with regard to hypoglycemia.

L. STERN: I don't know the answer to that. I do know that there is a fair bit of evidence to suggest that the increase in free fatty acid is, however, itself mediated through either adrenalin or noradrenalin, in response to a stimulus. It is by no means certain that the defect is due to the poor adrenalin excretion. It may simply be that the adrenalin excretion is a reflection of the fact that the problem has been going on for some time.

R.E. GREENBERG (Stanford University School of Medicine, Palo Alto): The basic problem in considering the relationship between epinephrine secretion and hypoglycemia, is whether epinephrine exerts an obligatory role in the maintenance of the concentration of blood glucose under normal conditions. Following adrenalectomy of a mammal or human, hypoglycemia does not occur even under conditions of prolonged fasting if the individual is maintained on corticosteroids. If one compares the dose-response curve of epinephrine and glucogon in terms of their effect on glycogenolysis or enhancement of gluconeogenesis, it is clear that epinephrine is far less effective. The question remains: Of what physiologic meaning is the demonstration of the absence or diminution of epinephrine secretion in response to induced hypoglycemia?

L. STERN: I think that's quite correct. It's because of this that we have not gone so far as to suggest that this is the cause of the problem. We have found similar findings, by the way, in infants of diabetic mothers. The only difference in those infants is that not only do they have abysmally low levels when they are hypoglycemic, but they have disastrously low levels even when they are supposed to be normoglycemic.

7 *Monkey Pox: Studies on Pathogenesis and Immunity.* HERBERT A. WENNER, PAUL KAMITSUKA*, FRANCISCO MACASAET* and PATRICIA KIDD*, University of Kansas Medical School, Kansas City, Kan.

Monkey pox virus (MPV) produces cytopathogenic effect typical of the vaccinia subgroup of pox viruses. Thirteen cynomolgus monkeys were inoculated with MPV either intradermally, intravenously or intramuscularly; all developed monkey pox. Concentrations of virus in dermal lesions were 5.0 $\log_{10}$ $TCID_{50}$/g, and 2.8 $\log_{10}$ $TCID_{50}$ in regional lymph nodes. Viremia was not demonstrated in these monkeys. One of 3 sentinal controls developed fever and converted serologically. Dermal lesions were not observed. In another test, 22 monkeys, 11 rhesus and 11 cynomolgus monkeys, were inoculated intramuscularly with MPV. The clinical course in cynomolgi paralleled those noted above. The disease in rhesus monkeys was less severe. Low levels of viremia were revealed in 5 monkeys. MPV was recovered readily from local lesions of skin, mucous membrane and lymph nodes; MPV was rarely recovered from spleen, lung, testes and central nervous system tissues. Seroconversion was 100 %. These monkeys, in contrast to controls resisted infection with vaccinia virus. One of 3 controls developed generalized vaccinia. Monkeys surviving challenge with vaccinia virus were subsequently challenged with Yaba (an oncogenic pox virus producing histiocytomas) virus; all challenged monkeys and inoculated controls developed tumors. The pathogenesis of MPV infection should provide basic data for work in experimental chemotherapeusis of pox virus diseases.

Discussion

H. KEMPE (University of Colorado Medical Center, Denver): Until one of your monkeys had a brain virus titer of 2.4, did this particular animal show signs of illness? Have you recovered the monkey pox virus from the mouth or the throat of the infected animals, since you are able to get inapparent infections in contact monkeys? Have you tried intranasal or aerosol infection of monkeys with monkey pox virus?

H.A. WENNER: The monkey yielding virus in the brain tissues was the only one to do so among the 9 tested. This monkey was no sicker than any of the other animals; this finding may be related to the viremic phase rather than any multiplication in situ. I can only answer your question about in the mouth indirectly. In the pre-eruptive state we removed the soft tissues in the posterior pharynx and separated them from the tonsils; pharyngeal and tonsillar tissues were tested separately. Whether the virus recovered is on the surface or deep, I just can't answer that. We have not yet performed aerosol infection. We are priming up for this. We took the most convenient route first.

J.W. ST. GEME, Jr. (University of California at Los Angeles Medical School, Torrance): Dr. WENNER, you made a comment about experimental chemotherapeusis in your abstract and in your presentation. Have you attempted to modify this systemic infection with systemic chemotherapy using pyrimidine analogs?

H.A. WENNER: We have not.

8 *Infectious Hepatitis: Evidence for Two Distinctive Clinical, Epidemiological and Immunological Types of Infection.* SAUL KRUGMAN, JOAN P. GILES* and JACK HAMMOND*, New York Univ. Sch. of Med., New York, N.Y.

Studies in progress since 1964 have provided evidence for the presence of two distinctive clinical, epidemiological and immunological types of infectious hepatitis. The two viruses have been designated MS-1 (derived from the serum of a patient during the first attack) and MS-2 (derived from the serum of the same patient 6 months later during the second attack). The following distinctive characteristics of the two types of infectious hepatitis have been observed: Incubation period—31 to 53 days following MS-1 infection and 41 to 108 days following MS-2; abnormal serum transaminase activity—relatively short following MS-1 (3 to 15 days) and very long following MS-2 (35 to 200 days); thymol turbidity activity—consistently abnormal after MS-1 and frequently normal following MS-2 infection; contagion—MS-1 infection was highly contagious and MS-2 was less contagious; immunity—evidence of homologous immunity following MS-1 and no evidence of heterologous immunity following MS-2 hepatitis infection.

These studies suggest: 1. that Willowbrook MS-1 type infection is typical infectious hepatitis; 2. that Willowbrook MS-2 type infection is probably 'serum' hepatitis; 3. that both viruses are infective by oral route, although MS-2 contact infection is considerably less frequent than MS-1 contact infection.

Discussion

R. WARD (Los Angeles): If your MS-2 virus is indeed a strain of serum hepatitis, and if, as you have shown, it spreads to contacts and is infective by mouth, then it seems to me a mystery has been cleared up, and one does not have to invoke vertical spread—that is, from mother to fetus—to account for the persistence of serum hepatitis virus in nature. It makes much more sense to have it spread horizontally—that is, from person to person—and Dr. KRUGMAN's work provides additional evidence that this is the way it is.

S. KRUGMAN: I agree. The horizontal spread from person to person is a more logical explanation than the vertical spread from mother to fetus.

E.B. SHAW (San Francisco): 1. It is probably presumptuous to comment on the basis of purely clinical opinions with carefully controlled observations. However, in our County Hospital, we have encountered numerous cases of hepatitis among our 'Hippie' population. Many of these have shared needles with others and are definite cases of serum hepatitis. Some others are probably simply infectious hepatitis. In still others, however, some of those who have not taken drug injections themselves and have had intimate exposure to patients with serum hepatitis have had hepatitis. I have been encouraged to believe that patients with serum hepatitis may transmit infection, doubtless by fecal contamination, to close contacts who have not had parenteral injections. The evidence you present reinforces this opinion. 2. You reported early rise in transaminase in contact infection. Is this not evidence of an even shorter incubation period than is evidenced by clinical symptoms? 3. I am a little uncertain if MS-1 and MS-2 correspond to the viruses of IH and SH so that my further speculations may not be relevant. Most prolific sources of serum hepatitis contain not only the virus but also antibody. A plasma pool which is responsible for serum hepatitis also contains large amounts of antibodies—precisely the same plasma pool which may be highly infectious being a source of gamma globulin preparations which are effective for prophylaxis. Antibody and pathogen exist simultaneously and, as we have learned in rubella etc., the specific antibody does not destroy the virus. Even a very small inoculum of infected blood or plasma not only introduces virus but also introduces antibody, and the latter is highly protective in minuscule amounts. Is it not likely that prolonged incubation is not evidence of a slowly-acting virus but is simply accounted for by the presence of antibody which is introduced along with the virus so that symptoms appear only after complete loss of coincidentally-introduced gamma globulin. Most viruses which are introduced parenterally have a short period of incubation, less than that which follows natural exposure, but with infectious hepatitis simultaneous injection of very small amounts of immune globulin along with the infective virus may prevent symptoms until the complete disappearance of antibody occurs.

S. KRUGMAN: In our experience the serum transaminase usually becomes abnormal before onset of clinical symptoms and jaundice. It is obvious, therefore, that infection has begun before clinical manifestations are apparent. After reviewing our data I find no evidence that the viruses were modified by human passage. They seem to be unchanged. The high incidence of anicteric hepatitis is probably related to the age factor; hepatitis with jaundice is more common in children. There is no evidence to support the hypothesis that the presence of gamma globulin in infected plasma is responsible for the long incubation period. Our studies suggest that there may be biologic differences between the MS-1 and MS-2 strains. It is likely that similar differences exist between IH and SH hepatitis viruses.

H. SHRAND (Tufts University, Boston): Does bed rest make any difference at all in the treatment of a child with infectious hepatitis?

S. KRUGMAN: Most children with infectious hepatitis are anicteric and asymptomatic. Under these circumstances ambulatory patients have not been kept at bed rest. It is usually unnecessary to restrict the activity of children during the convalescent phase of infectious hepatitis.

J. STOKES, Jr. (Henry Phipps Institute, University of Pennsylvania, Philadelphia): Dr. KRUGMAN and his co-workers have added much to the knowledge concerning viral hepatitis. I should suppose that there are probably several strains of virus, not only the serum hepatitis such as Dr. KRUGMAN has described, but perhaps others, some of which can be prevented by large doses of gamma globulin, and some of which cannot be so prevented. Although there is suggestive evidence that the epidemic virus is a single strain or type, there is a good deal of evidence suggesting that the serum hepatitis virus may have several strains or types. Sufficient studies over many years were conducted by MACCALLUM in England, HAVENS and PAUL at Yale, MURRAY in Bethesda and our group in Philadelphia to indicate that several strains of serum hepatitis (SH) virus were not infective (even no anicteric hepatitis) when given by mouth. In such studies it was usually not difficult to determine the presence of anicteric hepatitis. The volunteers in these studies were young adults.

S. KRUGMAN: Thank you for your comments, Dr. STOKES. The type of serum hepatitis described by MIRICK and SHANK had several features in common with our MS-2 type. The two diseases were spread by contact and the incubation periods were essentially the same.

AUDREY K. BROWN (Medical College of Georgia, Augusta): I'd like to pursue a point to which Dr. WARD alluded. Ordinarily we associate infectious hepatitis in the mother with no sequelae in the infant. If serum hepatitis is a form of infectious hepatitis, will our thinking on this point have to change? How long were the children with either form of hepatitis infective?

S. KRUGMAN: Sequelae in newborn infants are uncommon following maternal serum hepatitis as well as infectious hepatitis. Children with infectious hepatitis have been shown to be infective before onset of jaundice and within 8 days after onset of jaundice; they were not infective 19 or more days post-jaundice. The period of infectivity of serum hepatitis is unknown.

9 *The Physiological Function of Gamma-Globulin.* BERNARDO V. FIDALGO* and VICTOR A. NAJJAR, Dept. of Microbiology, Vanderbilt Univ. Sch. of Med., Nashville, Tenn.

A physiological function for γ-globulin has recently been proposed (NAJJAR, V.A.: Physiol. Rev. *43:* 243

[1963]). Investigations along this line showed that specific γ-globulin fractions bind to autologous blood cells in isotonic sucrose in man, rabbit and dog. These fractions can be isolated by salt elution of cells washed in such media. They represent fractions III and IV of whole γ-globulin separated on phosphocellulose columns. Fraction III binds primarily to erythrocytes while fraction IV binds to leucocytes. Both fractions are manufactured primarily by the spleen. Splenectomized dogs show almost complete disappearance of III and marked reduction of IV after 6–8 weeks. Concomitantly, the half life of the erythrocytes is reduced by 40–50 % and severe anemia finally develops. These can be prevented by injection of III and IV. Four to seven months later some animals develop bartonella infection. Fraction IV is essential for maximum phagocytosis of *staphylococcus aureus*. Dog leucocytes isolated by washing with Hank's medium (naked cells) show 0–9 % phagocytosis. Those isolated by washing with isotonic sucrose retain bound IV (coated cells) and yield 40–50 %. Fractions I, II, III and serum albumin, bovine or canine added to naked cells show a basal nonspecific effect of 15–18 % phagocytosis, whereas IV results in 45–55 %. All protein components are heated at 56° for 1 hour and absorbed 3× with the microorganisms. Staphylococci used for phagocytosis are pre-exposed to heated autologous serum for 2 hours (opsonized).

Discussion

E.R. Stiehm (University of Wisconsin, Madison): Is there any correlation between the fractions you get off the column and gamma A, gamma G and gamma M immune globulins? Has immunoelectrophoresis been performed on the four fractions to determine if they consist only of gamma-globulin? Can you be certain that splenectomy, instead of altering the gamma-globulin, has not altered the red and white cells?

V.A. Najjar: The four fractions have been very well characterized. Fraction 1, the bigger one, has gamma A and gamma G. Fraction 2 has gamma M and gamma G. The third and fourth fractions which we have dealt with, which coat the red cells and the white cells, are all pure gamma G. This has been done by immunodiffusion and immunoelectrophoresis. The reason we know that we have not altered the cells is that after splenectomy—which I did not have a chance to tell you about—the phagocytic rate some six weeks later becomes basal. In other words, there is no stimulation at all beyond that obtained with bovine serum albumin. Now, that can indicate that either the cells were damaged after splenectomy or the reduction in that fraction was responsible. When these cells were either added to serum before splenectomy on that same dog or to Fraction 4 obtained from the same serum before splenectomy, full phagocytic rate was maintained.

D.N. Meadearis, Jr. (Pittsburgh): Did any one of a variety of bacteria adsorb your Fraction 4? One considers the possibility that there is some relationship between your fraction and substances in serum that have promoted phagocytosis, some of which have been adsorbed preferentially by the less virulent bacteria. The question remains despite the fact that you used optimum opsonization in your experiments.

V.A. Najjar: We did that with the dog only. There was no adsorption from dog Fraction 4 on the staphylococcus we have used for phagocytosis.

A.J. Nahmias (Emory University School of Medicine, Atlanta): I wonder if you studied the coating of lymphoid cells with this gamma-globulin. This raises the question in studies using the indirect fluorescent technique to detect human globulins in lymphoid cells as to whether the lymphoid cells are actually synthesizing the gamma-globulins or just coating them.

V.A. Najjar: The lymphoid cells show definite but minimal amounts of gamma-globulin on them as revealed by immunofluorescence. Most of the gamma-globulins that we isolate come from the polymorphonuclear leukocytes. One can elute it, isolate and fractionate it as I have shown you. It's about 1 mg/cc of packed polymorphonuclear leukocytes.

10 Statistics: Their Use and Misuse. A. Ashley Weech, Children's Hospital, Cincinnati, O.

On May 3, 1935, in Cleveland, at the Annual Meeting of the American Pediatric Society the author presented a paper, entitled 'The Meaning of an Average'. In it he tried to introduce his colleagues to the mathematics of probability and to show how a rapidly developing sister science could be used to evaluate the significance of observational data. During ensuing years this science has invaded the domain of medicine so effectively that misuse through fear of nonuse has been an inevitable consequence. Civilization is entering a new era, the age of the electronic computer. This marvelous contrivance is already performing miracles of computation which a generation ago were virtually impossible. But, the machine can never do more than respond to the commands of a human brain. If the instructions are wrong it can make mistakes ten thousand times faster than was ever possible before. The author has assembled an impressive list of medical papers published within recent years where the mathematics of probability has been improperly applied in the evaluation of data. The errors are chiefly concerned with lack of judgment in picking a measure of central tendency, with the presentation of standard deviations that have absolutely no meaning in describing the distribution of data, with the unwise selection of methods for determining the significance of differences, with failure to understand the quantitative aspects of the product-moment correlation coefficient. Most of the authors are members of one of the learned pediatric societies. Illustrative data from their papers will be shown on slides but, since there is no wish to embarrass, authorship will be withheld. It is anticipated that some of those in the audience will recognize their own handiwork.

Discussion

J. Yerushalmy (University of California, Berkeley): I want to mention the disease of the 'little fellow who isn't there.' I refer to results which never appear in print, which bias those which do. Assume several investigators studying the same phenomenon, each on a small number of animals. Even if no correlation exists, some of the experiments will yield positive results, others negative ones. There is a tendency on the part of the investigator, program committees of scientific societies and editors of scientific journals to publish the positive results and neglect the negative ones. The author may be very careful and state that because they are based on small numbers the results are not statistically significant. However, after some years the literature is reviewed and because the negative findings are not there to counterbalance, the accumulation of all the positive ones presents an impressive picture. The results get into the textbooks, and it takes years and

generations before they are finally dislodged. I want, therefore, to make a plea to investigators, program committee members, and editors to take a more positive attitude to negative results.

A.A.WEECH: I leave you with a little text from THOMAS HENRY HUXLEY: 'Science is simply common sense at its best; that is, rigidly accurate in observation and merciless to fallacy in logic.'

11 *The Care-by-Parent Unit.* VERNON L.JAMES, Jr.* and WARREN E.WHEELER, Department of Pediatrics, University of Kentucky, Lexington, Ken.

A pediatric 'in hospital' unit without registered nurses, where the parent provides complete care for the child is a functional and economical addition to pediatric care. High cost of hospitalization, shortage of nurses and desirability of keeping parent and child together prompted the design of this facility. It provides private room accommodations for parent and child regardless of economic status. It is adjacent to the OPD and is staffed by OPD students and house staff. Our 14-room unit had over 700 admissions in the first year. Patient variety has been striking. Admissions are selected according to severity of illness, adequacy of parent and treatment plan. Both parents sometimes stay. More than one child can be admitted with a parent. Admission can efficiently be multi-purpose involving many specialities. No restrictions are made by social class, culture or race. Advantages include: 1. Reduction of hospital costs due mainly to the absence of registered nurses; 2. Availability of more pediatric beds unrelated to personnel shortages; 3. Opportunity for observation and evaluation of adequacy of parents given full responsibility of care; 4. Application of newly learned skills by parents while still under supervision; 5. Opportunity for house staff and medical students to observe how well their instructions to mothers are carried out. The 24-hour presence of the parent emphasizes doctor/parent relationship to the house staff and medical students and provides excellent opportunity to develop these skills. The pediatric staff is actively demonstrating that a motel-like Care-by-Parent Unit can work effectively and efficiently.

12 *Gaps in Doctor-Patient Communication.* BARBARA M.KORSCH, ETHEL K.GOZZI* and MILTON S.DAVIS*, University of Southern California, School of Medicine, Children's Hospital of Los Angeles, Cal., and New York Hospital, New York, N.Y.

To explore whether the 'art of medicine' can be analyzed scientifically 800 pediatric out-patient visits at Children's Hospital of Los Angeles have been studied. The conversation between parent and pediatrician was tape-recorded and follow-up interviews with the mother in hospital and at home yielded information about the mother's own ideas and feeling about her child's illness, her perceptions and understanding of what the doctor told her, her attitudes about the visit and her response to the medical advice given. Records of the consultation between parent and pediatrician as well as the interview material were analyzed qualitatively and by quantitative computer data processing. Sampling, control groups, etc. were planned carefully. On preliminary analysis it appears that the content of the verbal interaction between physician and patient does have specific effect on the outcome of the visit. Some patterns of successful communication have been recognized. A number of gaps in communication between doctor and patient have also been identified: unmet expectations from the visit to the physician on the part of the patient, differences in language that is used and comprehended, lack of warmth and concern on the part of the physician, etc. In addition the patient's background, nature of the illness as well as the nature of the treatment prescribed do influence the parents' responses to a particular medical visit. The study is an interdisciplinary venture and emphasizes methodology per se as well as content.

Discussion

L.M.FRAAD (Bronx Municipal Hospital Center, Bronx): 1. What do you do about third party payments to pay for this unit? 2. Are you doing anything to evaluate what you are doing there?

V.L.JAMES, Jr.: In answer to your question about third party payments, you must remember that these are in-hospital admissions and the fact that the parent stays has nothing to do with third party payment. To date, we have had no objections from any of our third party payment agencies and I would suspect that the fact that this ward is much cheaper has been quite favorably accepted by them. In answer to your next question regarding evaluation of the ward, at the present time I am working intensively with the Department of Behavioral Science to develop an acceptable research design for careful evaluation of the unit itself. We have several research projects going in individual areas of evaluation, but at the present time do not have an overall evaluation project under way. We hope to have this ready by this summer. I am sure you recognize that it is extremely difficult to compare patients on a general active acute pediatric ward and patients on the Care-by-Parent ward. They are a very different group of patients and methods of evaluation must be carefully designed to try to take into account all of the variables.

R.B.SCOTT (Howard University, Washington, D.C.): Dr. JAMES, your presentation is reminiscent of a procedure that I saw in operation in Newcastle upon Tyne in England, where Sir JAMES SPENCE, I think, more or less routinely permitted the mothers of preschool children to remain with them during hospitalization and to assist with their general care. I am wondering if you could tell us a little more about how the children and their parents were selected for this particular hospital unit? I would particularly be interested in knowing if you feel that this type of procedure could be successfully employed in a municipal hospital, where one may be dealing with many mothers of low socio-economic and educational backgrounds.

V.L.JAMES, Jr.: Patients are chosen from those who have any type of illness or condition which the mother can care for while the child is in the hospital. The physician who requests the admission determines whether he feels the mother is capable to care for the child or not. After admission we have a full-time social worker assigned to the ward who can help us with our evaluation. I feel this ward could be used in a municipal hospital, though you would probably have to be a little more careful about selection of parents. All admissions to this ward are made through me or my representative and, therefore, I control all admissions. I feel that it is absolutely necessary to have admissions through one control point by someone who knows what is going on at the ward at all times.

RUTH C.HARRIS (New York): I think Dr. JAMES has an added bonus for this kind of program that he doesn't

realize. For many years in Europe, Asia and Africa parents have stayed with their children in hospitals. Three years ago I was fortunate – or unfortunate – to be visiting a hospital in the Philippines; five days after I arrived there was a fire which involved about eight city blocks and finally burned down the hospital. All the patients were removed from the hospital, and we had to spend time trying to get the newborns into a boat and across the river away from the fire and smoke hazard. I kept looking around to see where the children were—and there were no children. All the nurses had to do to clear the children's ward was to say to the parents: 'Take your children to the other hospitals'—and they vanished. In a catastrophe you have a responsible parent there to take command of the situation.

J. ALPERT (Harvard University, Boston): Dr. KORSCH, you have shown us in an elegant manner how it is possible to begin to measure and quantify the doctor-patient relationship. Were these visits with patients who had been seen previously by the doctor, or were they all new visits? We are currently evaluating the length of time that the doctor knows his patient and how this affects doctor-patient communication.

BARBARA M. KORSCH: We deliberately selected first visits which were concerned with a new illness to study. We did this because we were already beset by numerous variables and we did not want to complicate the situation. In an established doctor-patient relationship we would have had to account for the effect of the previous relationship as well as information about the illness and treatment that the patient had received on other visits. As a model a new situation seemed simpler to study.

13 *Functional Evaluation of Children with Renal Tubular Acidosis.* J. RODRIGUEZ SORIANO*, H. BOICHIS* and C.M. EDELMANN, Jr.*, Albert Einstein College of Medicine New York, N.Y. (introduced by H.L. Barnett).

We have divided renal tubular acidosis (RTA) into two forms: proximal, caused by a defect in HCO_3^- reabsorption, and distal, due to an inability to establish an adequate pH gradient between blood and urine. This classification is of importance because of major differences in clinical features, complications and therapy. Eight children with RTA have been evaluated on the basis of HCO_3^- reabsorption during continuous infusion and excretion of H^+ following administration of NH_4Cl. Two were shown to have distal RTA. HCO_3^- threshold and Tm were normal despite continuous presence of small amounts of HCO_3^- in urine, even at low levels of serum HCO_3^-, due to inability to depress urinary pH below 6.5 .Six children had proximal RTA, due to Fanconi syndrome in two, primary in the other four. All had a low HCO_3^- threshold (16–20 mmoles/l); four had a low Tm (1.8–2.2 mmoles/100 ml glomerular filtrate). Despite this, urinary pH and excretion of H^+ during metabolic acidosis were within normal limits in each subject when the serum bicarbonate was below threshold. In establishing the diagnosis of RTA, interpretation of urinary pH and excretion of H^+ during acidemia requires knowledge of the bicarbonate treshold. Normal acidification does not rule out RTA. Conversely, abnormal acidification may be due either to distal RTA or to proximal RTA with inadequate depression of serum bicarbonate. Separation of RTA into proximal and distal forms will remove much of the confusion now existing with regard to clinical features and response to therapy.

Discussion

H.E. HARRISON (Baltimore City Hospital, Baltimore): This has been a very nicely presented delineation of these two physiologic entities. A number of years ago we presented some data to indicate that in distal renal tubular acidosis, in which there is failure of development of a H^+ ion, there is also an abnormality of the renal handling of citrate. These patients did not have measurable amounts of citrate in the urine, and we also presented some experimental evidence suggesting that this might be the mechanism of nephrocalcinosis. In the patients with the Fanconi syndrome with acidosis, who are comparable to the ones you have studied, we did find normal or even increased amount of citrate in the urine. These patients did not have nephrocalcinosis. We have not studied any patients or have not recognized patients of the type which you described as proximal renal tubular acidosis without glycosuria, and I would be curious to know what their urine citrate excretion might be since the absence of renal calcinosis in those patients, according to our theory, would demand that they have measurable or appreciable amounts of citrate in the urine. We have assumed that citrate is an important factor in the solubilization of calcium and the prevention of renal calcinosis. Have you any measurements of urine citrate in the patients with primary, proximal RTA?

M.A. HOLLIDAY (San Francisco General Hospital, San Francisco): Two children that we have seen could be classified as having proximal tubular acidosis had medullary cystic disease. One of these was studied very extensively by Dr. CURTIS MORRIS. I wonder whether these children with proximal disease are related to each other or whether you detected a familial pattern, and whether they might have intrinsic renal disease that would conform with medullary cystic disease.

EDNA H. SOBEL (Albert Einstein College of Medicine, New York): We know that in distal renal tubular acidosis, despite all its complications, simple treatment with alkali will reduce the marked acidosis. Is that also true of proximal renal tubular acidosis?

C.R. SCRIVER (Children's Hospital, Montreal): Were there family histories? In this case, one would expect that a discrete transport defect, as described here, might have a hereditary basis.

W.W. MCCRORY (Cornell Medical Center, New York): I too would compliment the authors on tackling a rather complicated problem, and ask two questions. I believe that in addition to serum bicarbonate level, changes in serum pH has been shown to be accompanied by changes in threshold for bicarbonate reabsorption. I wondered if there was any difference in your subjects in the different classes in extent of changes in serum pH at start or during the bicarbonate infusion. Secondly, did you have an opportunity to detect or correct what might have been existing potassium deficiencies? If I remember, your distal group all shared hypokalemia in common. We have observed a patient with persistent acidosis in spite of the administration of large amounts of sodium lactate. No potassium had been provided and a potassium deficiency was present. We had the opportunity to observe a marked diminution of the renal tubular bicarbonate 'leak' in this child presenting with RTA after repletion of the potassium deficiency.

F.G. SMITH, Jr. (University of California School of Medicine, Los Angeles): One of the other important things that influence the Tm for bicarbonate is the CO_2

tension in the blood, and I was wondering if you had any data on this particular aspect.

C.M. EDELMANN: Dr. HARRISON, we agree with your concept of the contributory role of citrate in the development of nephrocalcinosis. Unfortunately, we have no data concerning citrate excretion in our patients. Dr. HOLLIDAY, we are aware of Dr. MORRIS' studies in patients with medullary cystic disease. Examination of our patients with primary proximal renal tubular acidosis by both intravenous urography and percutaneous renal biopsy revealed no abnormalities. Renal function as measured by the clearance of inulin and PAH, and concentrating capacity was normal. Dr. SOBEL, we have been gratified with the response of these patients to citrate or bicarbonate therapy. Their catch-up growth is comparable to that seen in patients with distal renal tubular acidosis. In addition, since they have normal renal function, they may have an even better growth potential. Dr. SCRIVER, it may be of significance that the mothers of two of our patients are sisters and that all the children that we have studied to date are males, suggesting the possibility of a sex linked disorder. To date we have insufficient data to draw firm conclusions. Dr. MCCRORY, to my knowledge pH does not directly affect the level of the bicarbonate threshold. However, in answer to your question and Dr. SMITH's, levels of pH and pCO_2 throughout each study were similar to those observed in normal infants. Our patients with primary proximal renal tubular acidosis were not cation wasters and therefore did not have hypokalemia. Those with distal renal tubular acidosis did not show a bicarbonate leak despite their hypokalemia. Clinical and experimental data suggest that potassium deficiency causes an elevation in the bicarbonate threshold and Tm. This may offer an explanation for the high level of Tm observed in one of our patients with distal renal tubular acidosis.

14 *The Response of Growth Hormone (GH), Insulin (INS) and Glucose (BG) to Arginine in Children.* A.W.ROOT*, C.SAENZ-RODRIGUEZ*, A.M.BONGIOVANNI and W.R. EBERLEIN, Children's Hosp. of Philadelphia, Pa.

The effect of l-arginine-HCL (0.5 g/kg) intravenously upon GH, INS and BG was determined in 19 normal children (12M, 7F; 1.5–17 yr) and in 10 with hypopituitarism (5M, 5F; 4.5–16.5 yr) in order to evaluate this stimulus as a test of pituitary function. Mean control and maximal responses to this stimulus were:

	BG (mg %)		GH (mμg/ml)	
	Cont.	Max.	Cont.	Max.
Normal	71.3	94.7	3.1	12.8
Hypopit.	58.3	94.9	<1	<1

	INS (μU/ml)			
	Female		Male	
	Cont.	Max.	Cont.	Max.
Normal	12.3	51	7	20.8
Hypopit.	9.4	37	6.9	14.8

The mean fasting BG of patients with hypopituitarism was significantly less ($p < 0.005$) than that of controls. GH concentration increased in 16/19 normal subjects. There was no increase in GH in any patient with hypopituitarism following this stimulus. The mean increment in INS after arginine was significantly greater ($p < 0.005$) in females irrespective of pituitary function. Paired insulin and arginine tests were performed in 12 controls and all patients with hypopituitarism. There was no increase in GH in response to either stimulus in the latter. The peak GH following arginine exceeded that induced by hypoglycemia in 6 controls and was less than that induced by hypoglycemia in 3. In 3 male children with normal GH response to hypoglycemia, no GH response to arginine was observed. Treatment of 2 of these with stilbestrol (20 mg) was followed by an increase in fasting GH without further response in one subject and by improved GH response to arginine in the second. It is concluded that although arginine is a stimulus to GH release in most subjects, an absent GH response to arginine is not diagnostic of hypopituitarism.

Discussion

F. KENNY (Children's Hospital of Pittsburgh, Pittsburgh): Is data available on serum growth hormone or serum insulin responsiveness to arginine in males with the syndrome of feminizing testes? Despite the laughter aroused by my question, this would be an interesting group to study in an attempt to determine whether the different responses which you have described in three normal males, compared with the females, are on a genetic or a hormonal basis. This is because these patients have peripheral unresponsiveness to androgen, despite a male XY chromosomal constitution.

A. DRASH (Children's Hospital of Pittsburgh, Pittsburgh): We have carried out very similar studies to those reported by Dr. ROOT. We are quite in agreement with the results he has presented in normal children. However, we have found significant differences from those of Dr. ROOT in the evaluation of children with diabetes mellitus. We have studied 36 children with new, previously untreated diabetes mellitus, using intravenous arginine infusion, intravenous tolbutamide, oral glucose tolerance, and subcutaneous glucagon techniques. Blood glucose, serum insulin and serum growth hormone response to these stimuli have been evaluated. We observed no increase in serum insulin concentration in any of the patients to any of these four modes of islet cell stimulation. We found an increased serum growth hormone release following arginine infusion in our diabetics as compared with our nondiabetic control population. The mean peak value was approximately twice as great in the diabetics. We did not observe hyperglycemia in association with arginine infusion in the diabetics. Quite the contrary, there was a gradual continuous decline in blood sugar during and after discontinuation of the arginine infusion. The rate of decline in blood glucose in these patients following arginine was quite similar to that observed in the same group of patients following intravenous tolbutamide administration. Finally, to our surprise, we observed a prompt increase in the concentration of growth hormone in serum following glucagon administration to our diabetic patients but no increase in our nondiabetic controls. As you do not report serum insulin response in your diabetics following arginine, I assume that these are old diabetics who have interfering insulin antibodies. I would think that the difference in glucose response to arginine in our two groups may be a reflection of the duration of diabetes mellitus in these patients.

D.A. HILLMAN (Children's Hospital, Montreal): We have also done a number of arginine infusion studies and have studied two normal control females that show no response. In addition, we have studied a group of

ten newborn infants in the first two days of life, when presumably they would have a fair amount of estrogen stimulation from the maternal source and found that of the ten there were four infants that showed no response. One other interesting feature of the newborns was that, of these babies, four had severe fetal malnutrition, and the HGH values in the fetally malnourished babies were among the highest we have had—values of 40 and 60 mμg/ml. I wondered if Dr. ROOT has studied arginine infusion tests in newborns.

S. RAITI (Johns Hopkins Hospital, Baltimore): I'd like to congratulate Dr. ROOT on his excellent presentation. In Dr. BLIZZARD's group we have also been able to evaluate the arginine and insulin tolerance tests. In twenty-two cases studied so far we have found that fourteen of these responded to both tests. However, in six patients we did not obtain a response to insulin, but did find a response to arginine; and in two cases there was no response to arginine but there was a response to insulin. We tend to agree with RABINOWITZ and his group, who suggest that the mechanism of action of these two stimuli is probably different. We also feelthat if patients do not respond to one stimulus, this is not in itself conclusive evidence of growth hormone deficiency, and that such patients ought to be subjected to the other stimulus before such conclusions could be drawn.

A.W. ROOT: Dr. KENNY, we have not performed any arginine tolerance tests in patients with male pseudohermaphroditism or in phenotypic females with Turner's syndrome and XO sex chromosome constitution. We plan to study both types of patients. Dr. DRASH, all of the patients with juvenile diabetes mellitus reported in this presentation had their primary disease one year or longer and had been treated with insulin. Endogenous plasma insulin levels were not measured in these patients. Dr. HILLMAN, we have not observed any normal female subject who failed to respond to arginine administration by increase in plasma growth hormone concentration. We have studied two newborns with trisomy 21 approximately one month of age, and found normal growth hormone responses to arginine administration. Dr. RAITI, we have had one patient who has had absent growth hormone response to insulin induced hypoglycemia on two occasions, but who does have an increase in growth hormone concentration following arginine administration. This patient has an abnormal SU4885 response, indicating an abnormality in the pituitary-adrenocortical axis, and he is growing at a rate compatible with hypopituitarism.

15 *Etiological Factors in Idiopathic Hypopituitary Dwarfism.* J.D. BAILEY*, H.W. BAIN, M.M. THOMPSON*, J.J. GAGLIARDINO* and J.M. MARTIN*, The Hospital for Sick Children, Toronto, Canada.

The etiology of hypopituitary dwarfism in the majority of children is designated as idiopathic since an organic lesion cannot be identified. In a series of 25 children with anterior pituitary deficiency producing dwarfism, 5 patients had craniopharyngiomas and the remaining 20 were considered to be of idiopathic etiology. Serum levels of growth hormone were low in all patients tested and failed to show a significant rise after insulin-induced hypoglycemia. Evidence of deficiency of other adenohypophysial hormones was demonstrated in all cases and there were no children with selective growth hormone deficiency in this series. Five of the children were members of 2 families. The parents of 3 of these children were first cousins. In the nonfamilial cases, 8 of 15 children had a history of complications at the time of delivery or in early neonatal life. Three of these patients represent one of twins. One of these was an identical twin with a birth weight slightly greater than his unaffected brother. One female in the series showed intrasellar calcification which has remained unchanged over 8 years and we believe represents hemorrhagic infarction of the pituitary at the time of birth. Two siblings with severe dwarfism, retardation of bone age and hypoglycemia showed excessively high serum levels of growth hormone. The parents of these children were first cousins. From this investigation we have concluded that idiopathic pituitary dwarfism may be caused by damage at birth to the hypothalamic-pituitary region or by a genetic defect of simple recessive type producing insufficiency or unresponsiveness to growth hormone.

Discussion

A.W. ROOT (Children's Hospital, Philadelphia): Hypopituitarism may be possibly transmitted as a sex-linked recessive characteristic in some instances. We have observed hypopituitarism in two male half-siblings with the same mother but with different fathers.

M.M. GRUMBACH (San Francisco Medical Center, San Francisco): I congratulate Dr. BAILEY on this excellent survey of etiologic factors in idiopathic hypopituitary dwarfism. My colleagues, Dr. SELNA KAPLAN and Dr. HARVEY GOODMAN, and I have had the opportunity to study 16 children with isolated growth hormone deficiency and 18 children with multiple pituitary tropic hormone deficiencies, all of the idiopathic group. In only one sibship was a second sibling affected. We have sought possible etiologic factors in these patients in terms of birth trauma, head trauma, central nervous system infections, associated congenital anomalies. In our group of patients we could not implicate these factors. It is necessary to emphasize that we do not know the site of the defect in most instances—whether it resides in the hypothalamic growth hormone-releasing area or in the pituitary gland itself. When hypothalamic growth hormone-releasing factor is available it should be possible to separate hypothalamic from pituitary defects. We observed an interesting difference in the sex ratio of the two groups of patients. There was the typical preponderance of males in the patients with multiple pituitary deficiencies whereas the sex ratio was close to one in the group with monotropic growth hormone deficiency. Have you noted a similar difference in your group of patients? Hypopituitary dwarfism unrelated to expanding lesions is a fascinating model of the heterogeneous etiology of an endocrine disorder. There is good evidence that some cases of monotropic growth hormone deficiency as well as multiple pituitary hormone deficiencies may be of genetic origin. The familial form of isolated growth hormone deficiency in which immunoreactive serum growth hormone is absent may be due to a mutation in the structural gene which determines the amino acid sequence of the hypothalamic growth hormone-releasing factor leading secondarily to growth hormone deficiency or to a mutation in a regulatory gene controlling the rate of synthesis of the growth hormone molecule itself. An example of a mutation in the structural gene which specifies the amino acid sequence of pituitary growth hormone is represented by the patients reported by Dr. BAILEY and by the familial cases of LURON who apparently secrete an immunologically reactive but biologically inactive growth hor-

mone—an abnormal growth hormone. Of course, some genic defects may lead to the synthesis of a 'growth hormone' which neither reacts with anti-HGH serum nor exhibits growth hormone activity. On the other hand, many of the familial cases of idiopathic multiple pituitary hormone deficiency, and quite possibly the nonfamilial cases as well, may be due to a progressive degenerative lesion of the hypothalamus and its median eminence involving the nuclei which produce releasing factors. Such a lesion may also be of importance in some patients with monotropic growth hormone deficiency, especially the nonfamilial type.

J.D. BAILEY: In our series there was a more equal sex distribution in the familial group than in the sporadic cases. One of the factors producing the male predominance is due to the fact that many females with hypopituitarism do not come to the attention of the physician until the mid-teens when delayed sexual development is of concern. These patients are more commonly seen in adult clinics, and, in fact, two of the three female patients in our series were referred by endocrinologists with an adult practice. Dr. ROOT and Dr. GRUMBACH have both pointed out that there are probably other etiological groups of patients than the ones we have reported comprising the idiopathic cases of hypopituitary growth failure. Other hereditary types of defect involving the releasing factors or the synthesis of growth hormone will no doubt be elucidated in the future. Sex-linked inheritance of some of these defects might explain the male predominance noted in this growth disorder.

16 *Rh Hemolytic Disease in ABO Incompatible Offspring. Observations on the Protective Effect of Heterospecific Pregnancy.* FLOSSIE COHEN*, WOLF W. ZUELZER, THERESA H.J. HSU* and JOSE TERUYA*, The Child Research Center of Michigan, Detroit, Mich.

The combination of Rh and ABO hemolytic disease (HD) was studied in 48 unselected cases, verified by elution of both antibodies. A significant majority (80 %) proved 'mild' and only 8 % were 'severe', judged by the initial hemoglobin, clinical condition, indications for exchange transfusion (half of all Rh HD) and distribution of maternal titers.

In investigating the protective effect of heterospecific pregnancy on the fetus, blocking due to coating of RBC's with ABO isoantibodies, seemed eliminated by a comparison with 69 cases of Rh HD in ABO incompatible infants with only Rh antibody; the profile of this group was similar to the doubly sensitized group.

The difference between the two groups with respect to the presence of anti-A or -B was found to correspond to the prevalence of group O mothers in the first, and group A or B mothers in the second, indicating that the presence of 7S isoantibodies determines whether or not such infants are doubly sensitized.

Part of the protection seemed related to quantitative differences in maternal Rh antibody production, resulting from the small number of ABO incompatible fetal cells surviving in the maternal circulation as antigenic stimuli. However, the disease was predominantly mild in heterospecific offspring even when the sensitizing pregnancy had involved a compatible fetus and irrespective of the maternal Rh antibody titer. The findings suggest that the protective effect of heterospecific pregnancy involved retention of Rh antibody at the placental barrier, perhaps related to the binding of anti-A or -B in the placenta.

Discussion

W.E. WHEELER (University of Kentucky Medical Center, Lexington): May I ask whether these babies from whom you could not elute anti-A had an A-1 antibody in their serums?

FLOSSIE COHEN: No.

17 *Response of Ectopic Supraventricular Tachycardias to Cardioversion.* RAMÓN RODRIGUEZ-TORRES*, VINOD KAVETY* and LLOYD REISER*, Pediatric Department, State University of New York, Downstate Medical Center, Brooklyn, N.Y. (introduced by Jonathan T. Lanman).

The present study analyzes the result of our experience with the use of cardioversion in the treatment of ectopic supraventricular tachycardias in infants and children. Ten patients with the following cardiac arrhythmias: paroxysmal supraventricular tachycardia (8 cases), auricular flutter (1 case) and auricular fibrillation (1 case), were admitted to the Pediatric Medical Intensive Care Unit of Kings County Hospital, Brooklyn, N.Y. Seven were males and 3 were females. Eight patients were under 2 years of age and 2 above 2 years. Five patients had congenital heart disease, 4 had normal hearts and 1 patient chronic rheumatic heart disease. At the time of admission to the Unit, 5 patients had received digitalis, 1 had received digitalis and quinidine, and 4 patients had not received any medication. After sedation with paraldehyde or phenobarbital, 8 patients received an electrical countershock (D/C) of 10 W/sec, and 2 patients 50 W/sec. Sinus rhythm was reestablished in all cases immediately after the electrical discharge and no recurrence of the abnormal rhythm occurred in 8 patients. Five electrical countershocks were given to 1 patient in less than 24 hours because of recurrence of the arrhythmia. In 1 patient recurrence of the ectopic rhythm occurred after 24 hours and disappeared spontaneously. No complications occurred during or after the electrical discharge. Our study indicates that cardioversion is a useful and safe procedure to restore sinus rhythm in ectopic supraventricular tachycardias in infants and children.

Discussion

R.L. FOWLER (L.S.U. School of Medicine, New Orleans): Although we have had satisfactory results with cardioversion of arrhythmias in children, we have continued to utilize digitalis for the management of paroxysmal supraventricular tachycardia. We have fortunately never had a case which did not respond to adequate digitalization and wonder whether the reported failures are not usually due to timidity in the use of the drug rather than to failure of the drug itself. An important action of digitalis in the management of this arrhythmia is reversal of the cardiac failure which may ensue from relatively long duration of the excessively rapid rate. Since it is well known that the failing heart is much more susceptible to the development of serious arrhythmias, including ventricular fibrillation, it would seem wise not to choose electrical conversion as the treatment of choice in the presence of this complication. I wonder if the authors have had any experiences with this type of situation?

R. RODRIGUEZ-TORRES: No, we don't feel that we would create a more serious arrhythmia by giving this method. We believe that digitalis is the drug of choice in the treatment of ectopic supraventricular tachycardias. However, we have had patients who did not

convert to sinus rhythm despite the use of high doses of this drug. As far as the creation of a more serious arrhythmia by cardioversion in a failing heart, we have not had this complication. It seems that the production of dangerous post-reversion arrhythmias occurs in patients who have received large doses of digitalis or quinidine before the electrical countershock. Since cardioversion has been found to be a simple and safe technique in restoring sinus rhythm we have adopted this method as our treatment of choice for this type of arrhythmias before using anti-arrhythmic drugs.

18 *Evidence of Cell Selection in Mosaicism.* IAN H. PORTER* and CHARLES D. BROWN*, Albany Medical College, Albany, N.Y. (introduced by PAUL R. PATTERSON).

It has been suspected that in cases of chromosomal mosaicism one stem line may proliferate at the expense of others. This could be demonstrated by comparing the ratios of the various stem lines at different ages in patients with mosaicism. The following is one of the first cases in which a proliferative advantage of one stem line has been demonstrated *in vivo*. A boy with the clinical signs of D_1 trisomy was found to be a mosaic for a D/G translocation chromosome and Group D trisomy. Chromosomes from 562 cells were examined from preparations obtained from peripheral blood on four different occasions and from fibroblasts obtained from skin biopsies on two different occasions. At birth four stem lines were present with different chromosomal constitutions. At each subsequent examination before death at ten months only one stem line remained: the karyotype was characteristic of that associated with D/G translocation Down's syndrome. Thus, selection probably worked against the stem lines which were trisomic for a Group D chromosome after they had irrevocably influenced embryonic development. Patients, therefore, in whom the clinical signs do not match the chromosomal findings may in some instances be mosaics in whom one stem line has proliferated at the expense of others after the latter have determined the course of subsequent development and hence the phenotype of the patient.

Discussion

L.I. GARDNER (Upstate Medical Center, Syracuse): I think this is a very important and interesting observation. We will all find it necessary to do repeated cytogenetic studies on children who have chromosomal mosaicism for this possibility of differential selection of cell lines in the organism as time elapses. In line with this observation, ATKINS, SCEERY and KEENAN (J.med. Genet. *3:* 134–138 [1966]) have reported a 14-month-old infant with muscular hypotonia, motor retardation and seizures who showed a 46/47 mosaicism with an extra ring chromosome. Right after birth 25 % of the leucocytes showed the ring chromosome; there was a progressive fall in the percentage so that by age 13 months only 8 % of the leucocytes showed the ring. The culture of skin cells showed at first a percentage of cells with the ring chromosome similar to the first leucocyte cultures, but subsequent harvesting of the same skin culture showed a gradual elimination of the ring *in vitro* over a period of 2 months. We have made somewhat similar observations to this latter *in vitro* one (NEU, KAJII and GARDNER: Lancet *i:* 51 (1967). We found a very large submetacentric marker chromosome and a missing no. 3 chromosome quite regularly in 48 hour cultures of tissue from a lymph node biopsy in a case of reticulum cell sarcoma. After 18 days in culture the cells gave normal karyotypes with no aberrations seen.

A. ROBINSON (University of Colorado Medical Center, Denver): I, too, think that we have all observed a somewhat similar phenomenon and these are very interesting observations. I might suggest that where we make observations like this, if possible, bone marrow preparations be obtained on direct smear, because, as Dr. PORTER mentioned, there is always a possibility of selection *in vitro*.

L. LUZZATTI (Stanford University School of Medicine, Palo Alto): I wonder if Dr. PORTER would like to speculate on the mechanism that produced the mosaic of four cell lines observed in the initial analysis. Assuming that the zygote started with trisomies of both chromosomes 21 and 18, how did the four different cell lines originate? In the initial analysis only 4 % of the cells had the double trisomy.

J.J. QUILLIGAN, Jr. (Loma Linda University Medical Center, Loma Linda): I would'nt know one chromosome from another, but I do know a little bit about tissue culture, and I wondered whether you had altered your medium in any way. We had thought quite some time ago of the possibility of altering amino acid concentrations, doubling the total amount, and so on, with the notion in mind that some of these abnormal cells might very well have different appetites for different portions of the medium.

I.H. PORTER: With regard to Dr. LUZZATTI's question about how the initial mosaic arose, I think one might postulate that the translocation chromosome arose de novo in one of the parent's gametes as neither they nor any of their other children had this abnormality. The mosaicism must have arisen by nondisjunction or by anaphase lag after fertilization. There is, as you know, some evidence from both work with animals and from observations in Man that structural anomalies predispose to numerical anomalies. There are several ways in which four stem lines might have arisen but exactly how is entirely speculative. The point about the possibility of a change in medium I think is very important. We did, of course, not realize that we might be dealing with cell selection until after we had examined the chromosomes from the second culture. When, however, we did realize with what we might be dealing we did check our own stock of tissue culture medium and we did check with the company from whom we get the medium to see if there had been any changes. As far as we can tell, no changes had been made. For the third and fourth cultures we were very careful to use medium from the same batch as on the first two occasions. I think the fact that there was a progression in the development of cell selection does favor that what we observed was due to in vivo cell selection. The point that you raised, Dr. QUILLIGAN, is indeed an interesting one, and I think it would be worthwhile looking for constituents or conditions of tissue culture media which might influence the relative rates of growth of various stem lines in mosaics.