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ABSTRACTS

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PLENARY SESSIONS

19 *Bacteriuria in the Premature Infant.* JOAN E. HODGMAN*, ALBERTO SCHWARTZ* and LAURI D. THRUPP*, Los Angeles County General Hosp., Univ. of So. Calif. Sch. of Med., and University of California College of Med., Los Angeles, Cal. (introduced by Paul F. Wehrle).

Asymptomatic bacteriuria may precede symptomatic infection in full-term infants, children and adults, and in some patients may lead to chronic infection. In the premature infant the detection, incidence and significance of asymptomatic bacteriuria has not been defined. Infants in the Premature Center of Los Angeles County General Hospital with birth-weights of 1250 to 2250 g were randomly selected on the second day of life. Each infant was studied at days 2 and 3, on two successive days at the end of the first week and similarly at the end of the second week. Voided urine specimens for quantitative urinalysis and quantitative urine cultures were obtained following special cleansing techniques. Of 72 infants studied, 18 (25 %) developed bacteriuria (single species clearly predominant and $\geq 10^5$ per ml) in two or more consecutive specimens. Bacteriuria usually developed by the end of the first week and 13 of the 18 were males. Of the 18 infants with 'confirmed' bacteriuria (2 or more consecutive specimens positive), 7 or 39 % developed symptoms suggesting infection and subsequently required antibiotic treatment. The physician initiating therapy had no foreknowledge of the patients' bacteriuric status. Two additional patients developed symptoms requiring antibiotic therapy after only one positive urine culture. In 19 additional patients (26 %), positive urine cultures occurred in only single urines and were of unknown significance. These findings suggest (1) that the significance of single positive urine cultures will require careful definition, but that (2) confirmed bacteriuria occurs in a surprisingly high proportion of premature infants and often precedes symptoms of infection.

Discussion

R.H. USHER (Royal Victoria Hospital, Montreal): I wonder what clinical signs the house staff were using which led to the treatment with antibiotics; in what way did the infants who were treated, and supposedly clinically septic, show this sepsis? Our own studies, presented by title elsewhere in the program, suggest that the proportion of systemic infections in full-term infants associated with pyuria or bacteriuria is about 40 %. It looks as though the same may be true for premature infants.

JOAN E. HODGMAN, Univ. of So. California, Los Angeles: The signs used to suspect clinical infection were lethargy, anorexia, vomiting, temperature change of greater than 1°F, abdominal distention, abnormal jaundice, the standard and not very specific clinical signs that are the early indications of possible infection.

E. SCHWALB (Hicksville, New York): In order to prevent contamination during the 45-minute interval between cleansing and collection, have you ever employed the Peres reflex, which involves stroking the paralumbosacral area of a newborn infant's back? If the bladder is full, the baby usually urinates immediately. This could prevent bacterial growth inherent in the possible 45-minute wait.

JOAN E. HODGMAN: JACK BOEHM has reported use of the Peres reflex for clean catch midstream cultures, with a low incidence of contamination. We did not use the Peres reflex in these infants. The small premature infant is perhaps not quite as suitable for this technique as the larger premature or term infant.

H.L. BARNETT (Albert Einstein College of Medicine, New York): This certainly is a vexing problem. Follow-up observations on both groups of children, those with bacteriuria and those with signs of sepsis, are especially important. I wanted to ask also whether the two children who were treated but who had no bacteriuria were the only two who had clinical signs of sepsis during this period; if not, how many infants had sepsis with no bacteriuria?

JOAN E. HODGMAN: The infants with confirmed bacteriuria along with controls matched for sex and birth weight are being followed. We do not have sufficient data as yet in the follow-up study. The majority of these patients have remained well and free of bacteriuria for periods up to nine months at the present time. The two patients without bacteriuria who were treated were the only infants in the negative group who developed symptoms suggesting infection. One developed symptoms suggesting infection within twelve hours of the completion of an exchange transfusion performed on the first day because of hemolytic disease of the newborn. The other baby developed abdominal distention and lethargy on the second day of life, which is early in our experience for these symptoms to appear. This baby had a positive blood culture for Proteus and the urine was negative before onset of therapy. There were no deaths in any of the infants selected for study.

J. ALPERT (Harvard Medical School, Boston): Were any of the male infants studied and found to have major

* By Invitation

renal anomalies? One of the explanations given for the increased incidence of infection in newborn males compared with females is circumcision. I assume, because these were premature, they were not circumcised; but I would like to be sure.

JOAN E. HODGMAN: Radiologic studies of the urinary tract were not done in any of these infants. None of them had any evidence of urinary tract abnormalities nor were they circumcised.

L. STERN (Children's Hospital, Montreal): I wonder, Dr. HODGMAN, are you in fact satisfied with this kind of data using cultures from below in newborn infants? I ask this question because our own experience has been that since we introduced the suprapubic technique recommended by Dr. PRYLES, our incidence of confirmed urinary tract infections has dropped from about 25 to 30 a year, using the so-called clean catch, to about 2 or 3, using bladder punctures. In many instances when one does simultaneous specimens, you get repeated cultures of a single organism of 100,000 below and absolutely nothing in the bladder, over and over again. Is it fair, therefore, to possibly commit a child to long-term treatment, as you should if you think that he has a urinary tract infection, on this kind of information?

JOAN E. HODGMAN: In answer to Dr. STERN, I think we are not satisfied with this technique. There are serious questions as to whether cultures collected from below are reliable, and I believe that we will have to confirm our results with cystotomy in order to know what they really mean.

I. KOOTA (Bellevue Hospital, New York): I was wondering if any of these babies happened to have positive blood cultures? How many of the babies with positive urine cultures also had positive blood cultures?

JOAN E. HODGMAN: There were five positive blood cultures in the entire group of babies, one in a baby who did not have bacteriuria (referred to above) and four infants who did. We routinely collect a blood culture before an infant is treated with antibiotics in our Center, and the other cultures on the treated babies were negative before treatment was instituted.

B.R. GIRDANY (Children's Hospital, Pittsburgh): If one does cystography on female infants, one observes that some may enter the vagina when the infant voids. This is a normal phenomenon. 'Vaginal voiding' has to be taken into account in evaluating 'clean catch and midstream' urine cultures in female infants.

JOAN E. HODGMAN: The question of the effect of vaginal urination I can't really answer. I can see no reason why this wouldn't be true and be a normal phenomenon. The single positives were as common in males as in females, but confirmed bacteriuria, as you could see from the data, occurred primarily in males. This would not be an explanation for the cofirmed bacteriuria, but perhaps could be for a single positive specimen.

C.V. PRYLES (Brooklyn): I think this is a very interesting paper, and more data on premature babies are needed. Several of the commentators have already discussed some aspects that I wanted to call attention to. I was particularly interested in the possibility of blood cultures being done at the same time that you were finding a significant bacteriuria in these children. There was a recent report showing that children may have a persistent bacteremia with gram negative organisms and yet be asymptomatic. I think we need to have more data correlating blood and urine cultures. We have right now a premature baby who on day one showed over 100,000 gram-negative bacteria in a clean voided specimen, who on day two had a suprapubic bladder aspiration done that was sterile. At the same time as the percutaneous bladder aspiration the clean voided sample obtained showed a significant bacteriuria. On day three the suprapubic bladder tap showed over 100,000 bacteria, as did the clean void. On day four both were negative. Now, this, to us, indicates an intermittent bacteriuria, and what it means we don't know. The baby is supposedly still doing well and is not being treated, and we're following this baby with repeated blood cultures and suprapubic bladder taps. Three blood cultures have all been negative. I think that, as far as the urine cultures go, one of the questions I wanted to ask was: In those patients over (one-half of your patients) who had a bacteriuria, how many of the six cultures per infant were positive?

JOAN E. HODGMAN: To answer Dr. PRYLES question about the number of positive urine specimens: If the contaminated urines are excluded, there were 83 urines containing a predominant organism of 10^5 or greater. In 3 of the 20 infants with a single positive culture not confirmed by a consecutive positive, a later urine was again positive.

20 *Pseudo-Phlorizin Diabetes.* FRANCIS X. FELLERS, VILMA PIEDRAHITA* and ENRIQUE M. GALAN*, Children's Hosp. Med. Ctr. and Harvard Med. Sch., Boston, Mass.

The renal clearance of glucose equal to the renal clearance of inulin (GFR) would suggest a new but not unexpected transport defect in renal tubular function. Similar isolated defects exist such as the renal handling of dibasic amino acids in cystinuria and the neutral amino acids in Hartnup disease, both with associated defects in the gastrointestinal uptake of the same amino acids. The additional demonstration of an oral carbohydrate intolerance in an 11-year-old girl with massive glycosuria and low normal blood glucose (G) level defines this previously unrecognized metabolic disorder. Repeated observations over 10 years in one patient, derived and calculated data from a 2nd patient from the literature, and a 3rd patient incompletely evaluated would confirm the identity of this new disorder. All 3 present a failure to thrive syndrome with normal intelligence, massive glucosuria with low normal blood G levels, acetonuria, massive hepatomegaly with some increase in normal glycogen, diabetic type of G tolerance studies, and a normal G increase with epinephrine. An associated bone disease is characterized by fragile under-calcified skeletal structure with little or no evidence of true rickets, and a low serum phosphorus (P) level. The P tubular maximum (Tm) was high normal. Generalized amino acid clearances were increased only slightly. During G loading, both P and amino acid clearance increased markedly. GTm on 2 occasions showed the addition of G to the glomerular filtrate. It is suggested that this newly described defect in a G transport system results in marked gluconeogenesis in both the liver and the kidney, that continuing G loss limits the essentials for growth and skeletal development, and that there must exist another transport system for G absorption at low levels.

Discussion

F.A. OSKI (University of Pennsylvania, Philadelphia): I'd just like to ask Dr. FELLERS if he noted any hematologic manifestations or had an opportunity to measure glucose transport into the red cells of these

patients. Phlorizin is known to affect red cell glucose transport and in terms of your analogy an abnormality might be demonstrated in a readily available tissue.

F.X. FELLERS: Dr. OSKI, we have not completed the glucose transport studies on the red cells.

J. METCOFF (Michael Reese Hospital, Chicago): I am interested in the observation of enhanced glucose excretion above the level of filtration. I wonder if Dr. FELLERS would care to dilate on this point a bit; perhaps his implication being that renal gluconeogenesis proceeds as rapidly as hepatic gluconeogenesis.

A. SASS-KORTSAK (Hospital for Sick Children, Toronoto): I am most fascinated by Dr. FELLER's description, and I believe we have a patient similar to this—or had one, because the patient died. We were impressed by the glycosuria, but we didn't really think that this was a primary or the most important parameter, the reabsorbing capacity for glucose, which was there in our patient. What I would really like to ask Dr. FELLERS is, whether he thinks he will ever find the phlorizin that causes the defect. There are obviously multiple tubular defects here; and I would also like to hypothesize, if I may, that we may find patients similar to this in whom the primary or the most important manifestation may not be glucose, but other tubular defects, and whether he has any idea of what this disease eventually will be.

F.X. FELLERS: Dr. SASS-KORTSAK, I do agree there are multiple tubular defects, but we would like to stress that this could be a specific transport defect, such as the syndrome of lysinuria-argininuria-cystinuria, with *the defect* involving glucose reabsorption. We described briefly the addition or secretion of glucose into the urine to try to stimulate interest, and I think this is what Dr. METCOFF is implying and also Dr. BARNETT—whether, indeed, one has demonstrated true addition of glucose to the urine; significant gluconeogenesis does occur in the kidney. There has been a great deal of work on this in the past year, some presented at the meetings in Chicago, where there were discussions on both sides. Some of the evidence suggests that glucose may indeed be added to the distal tubular urine by stop-flow techniques.

EDNA H. SOBEL (Albert Einstein College of Medicine, New York): How do you interpet the observation that vitamin D markedly enhanced nitrogen retention?

F.X. FELLERS: The response to vitamin D, Dr. SOBEL, I think perhaps all one could say was that the child felt better. There was some evidence that vitamin D in large dosage, certainly in refractory rickets, may actually increase the glucose reabsorption. Whether this is a nonspecific effect on glucose transport, I don't know. It might perhaps allow a better use of protein nitrogen, which then does not have to go for caloric and carbohydrate metabolism.

G.B. STICKLER (Mayo Clinic, Rochester): I would like to hear a little bit more about the tubular handling of hydrogen ions and phosphorus in these patients. It seems to me that there is also a defect as far as hydrogen ion excretion is concerned, as well as phosphorus reabsorption.

F.X. FELLERS: Dr. STICKLER, phosphorus clearance was increased, but our attempt to carry out a phosphorus Tm resulted in an increased Tm with serum phosphorus levels rising to 23 mg/100 ml.

J.A. GRUNT (Yale University School of Medicine, New Haven): How would you relate the current syndrome that you have described so well to the syndrome of Mauriac, that has many similarities and overtones?

F.X. FELLERS: Dr. GRUNT, the Mauriac syndrome—we haven't seen one of these youngsters. I do not know what the correlation might be, but the serum glucose levels are elevated in that syndrome.

C.R. SCRIVER (Montreal Children's Hospital, Montreal): FRANK, this was a very interesting paper. I would like to ask you whether the glucose Tm was established using arterialized blood? And, by way of comment, if you have a Tm, even if it is a low one, you are dealing with a saturable phenomenon, and not with transport by simple diffusion.

F.X. FELLERS: The Tm glucose procedures, Dr. SCRIVER, are certainly difficult to measure. One is always concerned because these children have a continued osmotic diuresis, and when one tries to add further glucose, the problems are compounded. I think the problem of interpreting glucose secretion is very difficult. Most of the sugar analyses were made on capillary blood.

A.E. LORINCZ (University of Florida, Gainesville): Do you have any ultrastructure data on the liver or muscle of these youngsters?

F.X. FELLERS: Electron microscopy studies—no, Dr. LORINCZ, we do not have that type of evaluation yet. Light microscopy showed large glycogen filled cells in both liver and kidney.

H.L. BARNETT (Albert Einstein College of Medicine, New York): In line with Dr. STICKLER's comment, I think this patient could very well have one type of the tubular dysfunction Dr. EDELMANN described yesterday where the urinary pH is not appropriately low at a concentration of serum bicarbonate of 20 or so, but where by reducing serum bicarbonate still further, a bicarbonate leak might have been demonstrated. Secondly, the demonstration of a glucose clearance in excess of inulin clearance is really quite unexpected, and before concluding that glucose is secreted, it would be necessary to determine whether glucose or inulin is measuring glomerular filtration rate. Finally, I have a little concern about the term given to this condition, pseudo-phlorizin diabetes. In the same way, renal tubular acidosis could have been called pseudo-diamox disease, which would have been confusing and might have concealed or retarded understanding some of the underlying mechanisms in this abnormality.

F.X. FELLERS: Again to Dr. BARNETT's always searching questions, there is certainly no question that there may be defects of hydrogen ion transport which would be recognized by doing, specifically, studies on bicarbonate clearance, even though the pH of the urine is reduced. The term 'pseudo-phlorizin', I think, was our effort to try to attach importance to a mechanism of glucose reabsorption or a defect in glucose transport, and to try to bring together observations of years ago with those of today. One often feels that there is nothing new in the world; and if we can only put one and one together, we can perhaps get the answer. This term was to stimulate further interest in this syndrome.

M.A. HOLLIDAY (University of California, San Francisco): I just wanted to ask if you had any experience feeding a glucose-free diet, with fructose or galactose.

F.X. FELLERS: Dr. HOLLIDAY, the substitution of a glucose-free diet was not done. We have given fructose, galactose or lactose separately, and found that fructose caused very severe diarrhea, although there was increased blood fructose and fructosuria. The same occurred with galactose and lactose, but without as much diarrheal disease.

H. GHADIMI (State University of New York, Brooklyn): What is the relation between the condition you

described and that described by LUDER and SHELDON? Short stature and gluco-phospho-aminoaciduria were also reported in Luder-Sheldon disease.

F.X. FELLERS: Finally, Dr. GHADIMI, I don't think this is related to Dr. LUDER's patients, although we do not have sufficient information on the glycosuria of that patient. Thank you. There are, of course, many studies still to be carried out, as hinted at by this show of interesting questions. I want to put one more slide on to give credit to others whose patients we had the opportunity to study. Patients 1 and 2: FELLERS, PIEDRAHITA and GALAN, Boston; patient 3: FANCONI and BICKEL, Zurich; patient 4; ROTTHAUWE, FICHSEL, HELDT, KIRSTEN, REIM, SCHMIDT, SCHMIDT and WESEMANN, Bonn; patient 5: ODIEVRE, Paris; patient 6: LAMPERT and MAYER, Nürnberg; patient 7: BRODEHL and HUNGERLAND, Bonn; patient 8: BAUER, Frieberg.

21 *Thiamine-Responsive Megaloblastic Anemia.* LON E. ROGERS*, F. STANLEY PORTER and JAMES B. SIDBURY, Duke Univ. Med. Center, Durham, N.C.

Thiamine deficiency results in classical beriberi with anemia occurring only after severe manifestations of generalized disease. The present report is of a heretofore undescribed thiamine-responsive megaloblastic anemia in a child without evidence of thiamine deprivation. This 11-year-old Caucasian female presented with a megaloblastic anemia of several months duration refractory to vitamin B_{12} and folic acid but responsive to a parenteral multiple vitamin preparation. Medication was withdrawn permitting a relapse over a six-month period. Initial studies of erythrocyte serine hydroxymethylase activity and trypophan metabolism were directed toward a presumed pyridoxine-responsive disorder. The anemia failed to improve with pyridoxine therapy, but the administration of parenteral thiamine hydrochloride was followed by a prompt hematologic response. When thiamine became implicated, erythrocyte transketolase and leukocyte pyruvic decarboxylase (thiamine-dependent enzymes) were assayed and found to be normal. Associated abnormalities included achlorhydria, aminoaciduria, grossly abnormal EEG, bilateral nerve deafness and diabetes. The results are of particular interest because this represents the first demonstration of an essential role of thiamine in nucleic acid synthesis.

Discussion

N.T. SHAHIDI (University of Wisconsin, Madison): Dr. ROGERS, did you attempt to titrate the response of the patient to thiamine and did you give this patient thiamine orally to evaluate the gastrointestinal absorption of this vitamin?

L.E. ROGERS: The answer to the first question is: No, we have not titrated this patient's response to thiamine. We in fact did not anticipate this response. We intend to give thiamine orally to this patient at a time when she shows signs of beginning hematologic relapse.

G. NIGRIN (Brooklyn Medical Center, Brooklyn): Do you advise to call this syndrome thiamine dependency?

L.E. ROGERS: Yes, we do think this is a thiamine dependency, rather than a thiamine deficiency, as I mentioned earlier.

T. YOSHIDA (Michael Reese Hospital, Chicago): There is another important thiamine-dependent enzyme to be considered. That is α-ketoglutaric dehydrogenase. This enzyme catalyzes synthesis of succinyl-CoA from α-ketoglutarate and coenzyme A. Succinyl-CoA is a primary precursor of heme. Therefore impairment of succinyl-CoA formation may inhibit heme synthesis and in turn causes anemia. I wonder if Dr. ROGERS has studied the enzyme activity of α-ketoglutaric dehydrogenase in granulocytes of this patient.

L.E. ROGERS: α-ketoglutaric dehydrogenase is a thiamine-dependent enzyme to which our attention has now been turned, but we do not as yet have data regarding levels of the patient's enzyme at this particular point.

R.C. NEERHOUT (UCLA, Los Angeles): On your chart showing the refeeding of the different vitamins it looked as if the reticulocyte response was starting at the time the thiamine was given. Are you sure that it was not a 72-hour response to the previous two vitamins?

AUDREY K. BROWN (Medical College of Georgia, Augusta): Was I right in noting that the reticulocyte count was high (5 %), even before vitamins were started and that there was a rise in hemoglobin after thiamine which occurred too early to be attributable to that vitamin?

L.E. ROGERS: The fact that she ran a reticulocyte count of about 5 % prior to treatment is quite correct, but when the total red cell count is taken into account this still represents quite a low reticulocyte level. The rise in hemoglobin did follow the onset of reticulocyte response, which was approximately 48 hours after the administration of thiamine. However, I do think it is impossible to say that the superphysiologic amounts of vitamins given prior to thiamine administration had no effect whatever; and I think to make such a statement would require that we give thiamine alone at a later date.

R.A. ULSTROM (University of Minnesota, Minneapolis): Several years ago there was a report of a couple of small epidemics of thiamine dependency in Japan. The apparent cause was a bacteria that came from shellfish. It is now designated as B. thiaminolyticus. After ingestion with the shellfish, it inhabited the gut in large numbers. The dietary thiamine was utilized by the bacteria, depriving the patient even though his oral thiamine intake was normal. The condition was cured by eliminating this organism from the gut. Have you looked at gut organisms as a possible cause of the dependency in your patient?

L.E. ROGERS: The epidemic which occurred in Japan, if Iam not mistaken, was due to a bacterial thiaminase and a low absorption of thiamine from the intestinal tract as a result of the vitamin having been destroyed in the gut. If this were the case in this patient, I should expect to find the general signs of beriberi, rather than a specific disorder such as the one that she has.

22 *Chronic Granulomatous Disease: an X-Linked Deficiency of Leucocyte NADH Oxidase.* ROBERT L. BAEHNER* and DAVID G. NATHAN*, Children's Hosp. Boston, Mass. (introduced by Louis K. Diamond).

Chronic granulomatous disease (CGD) is an X-linked inherited defect in the killing of bacteria by granulocytes associated with deficiency of leucocyte NADH oxidase, a cell sap enzyme. The diagnosis is established by a simple dye reduction test which may be quantitated with precision sufficient to establish that the mothers of afflicted male children are carriers. We have now demonstrated the carrier state in 3 such

mothers one of whom is also hypersusceptible to infection. The gene does not segregate with the genes for color blindness or blood group XgA.

It has been demonstrated by HOLMES *et al.* that staphylococci are ingested but not killed by CGD cells. Our present studies reveal that the granules of CGD leucocytes do not rupture during phagocytosis. This defect in granule rupture is demonstrated both histochemically and by direct assay of released granule enzymes (acid and alkaline phosphatase and β-glucuronidase). Of great importance in the killing of staphylococci is the release from the granules during phagocytosis of a protamine-like material described by SKARNES and by SPITZNAGEL. As is true of granule hydrolases the protamine is in the granules of CGD leucocytes. Since the hydrolases are not released from CGD granules during phagocytosis we postulate that the bacteriocidal protamine is also not released and that this failure of bacteriocidal protamine release is related to the basic deficiency of cell sap NADH oxidase.

Discussion

P.G. QUIE (University of Minnesota, Minneapolis): This presentation gave good evidence for a lack of NADH oxidase activity in the leucocytes of patients with chronic granulomatous disease but do you have evidence for a deficiency of this enzyme in the cells? Would not these be the expected results if lack of activation of this enzyme was secondary to diminished degranulation? Our observations on a number of these patients in Minneapolis are similar to those you presented today. In the polymorphonuclear leucocyte extracts Dr. ARTHUR PAGE and Dr. HOLMES have found similar oxidase activity in patients and controls. Since in your patients and in ours diminished degranulation of the cytoplasm was present, the lack of bacterial killing and NADH oxidase activity may both have been secondary to abnormal granule stability. The mothers of the boys with this disease which we have studied have shown a partial defect in killing of staphylococci and an intermediate abnormality in the metabolic events which were diminished in their affected sons. These results would parallel observations you presented using nitro-blue-tetrazolium. The abstract of a paper to be presented tomorrow by Dr. KAHLE from Cincinnati suggests that there may be a spectrum of abnormalities of function of leucocytes. Their patient also demonstrated decreased bactericidal activity by PMNs but the leucocytes apparently degranulate normally and there is diminished activity of one of the bactericidal factors. An interesting observation in our laboratory this last year was that polymorphonuclear leucocytes from children with chronic granulomatous disease were incapable of killing staphylococci and *Serratia* and certain other gram negative species. However, the PMNs from at least 4 children with granulomatous disease have normal bactericidal capacity when challenged with a number of streptococcal strains. This work will be presented next week at the Society for Microbiology but is included in this discussion to indicate the possibility that there may be deficiency of fairly specific bactericidal factors in certain of these patients as well as other abnormal metabolic responses to engulfment of bacteria.

R.L. BAEHNER: Regarding the deficiency of oxidase in these cells, the primary point we were trying to get across is that there is no deficiency of the oxidase within the CGD leukocytes at rest. The defect seems to be in the lack of stimulation of the oxidase during the event of phagocytosis. As you could see from the previously projected slides normal leukocytes showed a marked increase in oxidase activity during phagocytosis but, in contrast, the patient's leukocyte oxidase activity decreased. This would go along with the biochemical findings of alterations of oxygen consumption, hydrogen peroxide formation and hexose shunt activity during the event of phagocytosis. Since the oxidase activity of the CGD leukocyte is not stimulated during the event of phagocytosis, we believe that this is the reason why the nitro-blue tetrazolium test is effective in this situation; for if this test depended only on resting cells, then there would be no difference. The initially reduced dye, then, must stimulate phagocytosis and act as the stimulus itself for a further increase in the oxidase activity and subsequent dye reduction to the degree that it becomes grossly visible within normal leukocytes. Since the CGD leukocyte does not increase oxidase activity after phagocytosis, it is incapable of normal dye reduction.

H.N. KIRKMAN (University of North Carolina School of Medicine, Chapel Hill): I would like to ask two questions. You call this an NADH oxidase deficiency, but it seems that you have a number of enzyme deficiencies. How do you conclude that it's primarily NADH oxidase deficiency? Do I understand from your closing remarks that the behavior of the leukocytes of the heterozygous female is that which would be seen, say, in a mixture of affected males and normal males? In other words, is the appearance compatible with the Lyon hypothesis?

R.L. BAEHNER: Dr. KIRKMAN's question about NADH oxidase specificity is a good question and one which we have not completely resolved. Until this oxidase enzyme has been isolated, and its biochemical characteristics defined, all that we can say at this point is that the enzyme has a greater substrate specificity for NADH than NADPH. It also has an optimum pH of 5.0 and it is cyanide insensitive. The leukocytes of the female carriers exposed to the NBT dye does conform to the Lyon hypothesis since the dye can be identified in a percentage of leukocytes approximately one-half that of normal controls.

A.F. ABT (Veterans Administration Center, Martinsburg, West Virginia): I would like to ask: Have you ever come across any cases of erythrophagocytosis? I remember that I reported one about 30-odd years ago in a newborn baby. The article was entitled 'Mononuclear Erythrophagocytosis in the Blood of a New-Born Infant', which appeared in The American Journal of Diseases of Children *42:* 1364–1371 (December 1931), complete with a colored plate. The case history and clinical description was published in a subsequent article entitled 'Anemia of the New-Born' (Amer. J. Dis. Child. *43:* 337–349 [February 1932]).

R.L. BAEHNER: As far as Dr. ABT's statement about erythrophagocytosis, we do see erythrophagocytosis by the fixed tissue histiocytes, of course, during hemolytic episodes. There is an interesting report by KAKIUMA (Jap. J. exp. Med. *36:* 363 [1966]) regarding phagocytosis by guinea pig granulocytes of sheep red cells which have been coated with antigen, antibody and complement. It would appear from this report that the ingestion of these red cells took place without an increase in lactate production or ATP production by these granulocytes. Perhaps immune phagocytosis does not require energy expenditure.

23 *Plasma Luteinizing Hormone Levels in Normal Children and in Subjects with Pituitary and Gonadal Dysfunction: Determination by Radioimmunoassay.* DON S. SCHALCH* and MICHAEL F. BRYSON*, Univ. Rochester Sch. Med., Rochester, N.Y. (introduced by Gilbert B. Forbes).

A sensitive radioimmunoassay for luteinizing hormone (LH) has been developed utilizing a highly purified preparation of human pituitary LH (PARLOW). In 12 pre-pubertal boys the mean plasma LH level was 0.3 ± 0.1 mμg/ml ($\pm$S.E.) and in 11 pre-pubertal girls, 0.4 ± 0.1 mμg/ml. Pubescent children had significantly higher LH levels: boys, 0.9 ± 0.2 mμg/ml ($p < 0.05$) and girls, 0.9 ± 0.3 mμg/ml ($p < 0.02$) although considerable overlap with pre-pubescent values existed. These levels do not differ significantly from those of normal adult males and females (except at midcycle). Three girls with precocious puberty and 4 with premature pubarche or thelarche had normal values for their age. Low levels were found in 5 boys with delayed puberty and in 2 children with panhypopituitarism. Two girls with Turner's syndrome (age 15 and 26 years) not previously treated with estrogen had elevated LH levels (3.1 and 7.1 mμg/ml) while a third (age 6 years) had a normal level for her age. Plasma LH level was normal in an immature 13-year-old boy with XXYY chromosome pattern, but elevated (3.0 mμg/ml) in an anorchic 13-year-old. The plasma LH was elevated in an 18-year-old patient with feminizing testes both pre- and post-orchidectomy (4.9 and 5.1 mμg/ml). Estrogen therapy postoperatively suppressed plasma LH to a level of 1.3 mμg/ml while testosterone had no effect, a finding not unexpected in view of previous data indicating an androgen insensitivity in these patients.

Discussion

A.W. ROOT (Children's Hospital of Philadelphia): I should like to ask two questions: 1. What is the potency of the human LH standard used in this assay in terms of the Second International Reference Preparation? 2. What is the volume of plasma that is assayed in the method reported?

R.M. BLIZZARD (Johns Hopkins Hospital, Baltimore): My question is the same as Dr. ROOT's except that I would like to add that I think it's important that people using these assays not express their results as mμg/ml, because they will vary according to the standards that are used. It is important to refer to a common standard, either the NIH LH S-1 or, preferably, the Second International Reference Standard for LH.

M.F. BRYSON: Dr. ROOT and Dr. BLIZZARD, the potency of the purified LH that we used as our standard is approximatly 1 mg to 2000 units of the Second International Standard. Or, one mμg is equivalent to 0.002 units of this standard. The volume of the plasma utilized in the assay was 50 lambda per reaction tube.

S.D. FRASIER (UCLA, Los Angeles): What is the effect of TSH on this assay system? Can the detectable levels of LH found in prepubertal children be accounted for by cross-reaction with TSH? Is there any nonspecific interference in this system by plasma from hypophysectomized individuals?

M.F. BRYSON: Dr. FRASIER, we have found by running the LH antibody against radioactive TSH that there is a very small cross-reaction, which we feel is negligible in terms of the assay. In addition, the values that we obtained in the children, particularly the nondetectable values, would indicate that there is not any cross-reaction with TSH. All of these children were, to the best of our knowledge, clinically euthyroid. We have not as yet studied any children one would suspect as having high TSH levels, such as athyroidic cretins. We have assayed the plasma of just one patient with a hypophysectomy for LH. He had a non-detectable level.

W.W. CLEVELAND (University of Miami School of Medicine, Miami): I wonder if you would speculate about the findings in the feminizing testis.

M.F. BRYSON: Dr. CLEVELAND, the subject with feminizing testis is an intriguing problem. The high value that we found perhaps represents another failure of the end organ response to testosterone seen in this syndrome. In other words, the hypothalamic area controlling the secretion of LH did not normally supress in response to the high levels of testosterone which these people secrete. The fact that this subject did not respond to one injection of testosterone is not entirely certain for we were able to obtain only one specimen after the injection. Unfortunately she would not come back for any further studies with the testosterone. I think it is of interest that she did show a response to the estrogen-progesterone therapy. In this respect, the progesterone may well have played the dominant role, since there was clinical evidence of adequate estogenitation prior to therapy at a time when her LH level was high.

T.H. SHEPARD (School of Medicine, University of Washington, Seattle): Dr. S.E. LEVINA from Moscow has reported that human fetuses of the male sex have no LH in the pituitary gland. Have you measured any fetal pituitaries?

M.F. BRYSON: Dr. SHEPARD, we have not studied any fetal pituitaries. The cross-reaction with HCG would make this difficult.

24 *Growth Hormone Secretion in the Human Fetus and in Anencephaly.* SELNA L. KAPLAN and MELVIN M. GRUMBACH, Univ. of Calif. Med. Center, San Francisco, Cal.

The capacity of the human fetal hypophysis to synthesize and store growth hormone (HGH) by the 15th week of gestation was reported previously from this laboratory. To obtain information on the ontogenesis of HGH secretion, the concentration of plasma HGH was determined in 15 aborted fetuses from 70–162 days estimated gestational age. Levels in the acromegalic range were found in all samples and greatly in excess of the concentration we have found in pregnant women at comparable stages of gestation. The concentration of serum HGH was 20 mμg/ml in the 70-day fetus and exceedingly high values, some exceeding 150 mμg/ml (40–206) were found in older fetuses. In early 3rd trimester fetuses the concentration of serum HGH was 6 times the mean concentration in 30 cord samples obtained from fullterm fetuses. Serum chorionic growth hormone-prolactin (CGP) was low compared to maternal values and spurious elevation of serum HGH attributable to cross-reaction with CGP was excluded. The HGH content of pituitary glands obtained from 10 of the fetuses was 0.2–8 μg/mg and correlated with fetal age and pituitary weight. The serum concentration of HGH in 3 anencephalic fetuses was 12–16 mμg/ml (low normal range) compared to the maternal level of 2–6 ßg/ml. These data indicate that: 1. the fetal hypophysis secretes as well as stores HGH as early as 71 days gestational age; 2. the anencephalic fetus despite disruption of hypothalamic-pituitary connections secretes HGH; 3. provide further support for the lack

of exchange of HGH and CGP between fetal and maternal circulation. The role remains to be determined of the immaturity of the hypothalamic-pituitary regulatory mechanism, of alterations in rate of disposal, and of fetal metabolism in the elevated levels of serum HGH.

Discussion

R.L. BRENT (Jefferson Medical College, Philadelphia): In suggesting hormonal control of fetal growth during pregnancy, I wonder whether you would like to comment on the relative importance of all types of hormonal control with regard to the eventual size of the fetus, especially in view of the fact that, at least in experimental animals where we can control fetal growth, there is such great variability in the eventual fetal size? I am sure you are aware of the work of JOST and others, who decapitated fetuses and yet the fetuses grew normally. But even more important, investigators who have worked with polytocous animals and have eliminated all but one fetus in a litter have demonstrated that the one fetus can grow to double the usual fetal weight. In view of the fact that there is this tremendous variability and potential in fetal size just in relationship to maternal nutritional supply, I wonder how important hormonal feed-back mechanisms are in controlling fetal growth. My own bias is that fetal growth is primarily in the 'hands' of the fetus.

SELNA L. KAPLAN: In our presentation we emphasized that fetal and maternal pituitary hormones have if any effect only a limited one on fetal growth. The animal evidence in support of this concept has been reviewed by JOST (The pituitary gland, vol. 2, ed. G.W. HARRIS and R.T. DONOVAN [1966]) and the evidence in man by DUCHARME and GRUMBACH (J. clin. Invest. *40:* 243 [1961]). In the light of these observations, the data presented on the elevated concentration of circulating pituitary growth hormone has generated a good deal of discussion within our group. The role of the placental hormone, chorionic 'growth hormoneprolactin' is another matter since it may indirectly affect growth by its action on maternal metabolism and possibly the placenta itself. We have no evidence that it has a direct effect on the fetus. We have wondered whether some instances of intrauterine growth retardation associated with a small placenta may not be related in part to impaired or diminished production of CGP. Secretion of CGP seems to be related to placental mass and not to the homeostatic mechanisms regulating the secretion of pituitary growth hormone. Aside from CGP, which may affect the nutrition and hence growth of the fetus, many other factors may play a role in the growth of the fetus including the number of fetuses, fetal infection, the fetal genotype, and the presence of fetal hyperinsulinism.

25 *Mycoplasma Pneumoniae Infection in Families.* NERON BALASSANIAN* and FREDERICK C. ROBBINS, Dept. of Ped., Western Reserve Univ. Sch. of Med. and Cleveland Metropolitan Gen. Hosp., Cleveland, O.

Epidemiological and serological studies were conducted for 5 months on 8 families of 46 members, after infection with *M. pneumoniae* was confirmed in a member. On the initial visit 24 members of 48 were already infected. *M. pneumoniae* was isolated from 21, while all had serological evidence of infection. By 8 weeks, 12 more acquired infection and only two members with no detectable antibodies remained uninfected for at least 16 weeks. Of 41 members, clinical pneumonia occurred in 14, respiratory infection without pneumonia in 18 and asymptomatic infection in 6 while 3 were not infected. *M. pneumoniae* was isolated from 82 % of cases with serological evidence of infection. Highest isolation rate of 93 % was from the pneumonia patients. All 31 members with positive cultures for *M. pneumoniae* developed high titers of complement fixing and fluorescent stainable antibodies. Highest cold agglutinin titers were found in the pneumonia group. Incidence of infection below and above 10 years was the same. However, respiratory infection with pneumonia occurred more frequently in the older age group while respiratory infection without pneumonia and asymptomatic infection occurred more frequently below the age of 10 years. *M. pneumoniae* persisted in the respiratory tract at least 4–6 weeks in 50 % of the infected members regardless of circulating antibodies. Therapeutic doses of tetracycline in 4 children and 5 adults did not improve the clinical picture or eradicate the organism. *M. pneumoniae* isolates from 4 patients before and after therapy had similar sensitivities to tetracycline *in vitro*.

Discussion

H.A. WENNER (Merriam, Kansas): This study beautifully complements those of Dr. GRAYSTON of Seattle and even exceeds in frequency isolates obtained in families he studied. How often did you recover *M. pneumoniae* from infants under two years of age? Do you plan to continue observations on these families to see whether there is any recurrent infection with *Mycoplasma* after, let's say, a year's interval of time, or two years?

N. BALASSANIAN: *M. pneumoniae* was isolated from 4 of the 6 children below the age of 2 years and the youngest child was 6 months old. A 3-month-old child with otitis media, not included in this study, is the youngest child from whom we have isolated *M. pneumoniae*. We are planning to continue observation on these families for recurrence of infection and persistence of the various circulating antibodies.

A.J. STEIGMAN (Mount Sinai School of Medicine, New York): This is a beautiful presentation, fruitfully combining epidemiologic, clinical and laboratory findings. I have one question concerning the patients who had clear evidence of infection with *M. pneumoniae*, but without pneumonia. In those patients, was there any significant difference in the clinical appearance between the children and the adults? In particular, was non-bacterial exudative pharyngitis or tonsillitis observed? It will be recalled that the Respiratory Disease Commission in World War II inoculated young adult volunteers with washings from patients with 'primary atypical pneumonia'; a significant number of volunteers developed non-bacterial exudative pharyngitis without pneumonia.

N. BALASSANIAN: The number of patients in this study is not adequate for a final conclusion; however, except for the duration of symptoms, particularly cough, respiratory symptoms in the group of patients without pneumonia is not very different from symptoms manifested by infection of the upper respiratory tract by viral agents. Except for postnasal drip and nasal discharge lasting for weeks in children younger than 3–4 years, no significant difference could be noted in the clinical appearance of children and adults who had respiratory disease without pneumonia.

B.R. GIRDANY (Children's Hospital of Pittsburgh, Pittsburgh): I think my question is related to Dr. STEIGMAN's. What are the criteria for pneumonia? Are

they radiographic? Are they auscultatory? Is there a difference in the clinical condition between those children who had radiographic signs of pneumonia and those who did not? And did you not have any children with radiologic signs of pneumonia who were not clinically ill?

N. BALASSANIAN: Chest X-rays were taken on all symptomatic members irrespective of the auscultatory findings. Radiographic evidence of pulmonary infiltrates was used as the criteria for pneumonia. In this study we had only 4 children 10 years of age or younger with pneumonia and all required hospitalization, while none of the symptomatic children without pneumonia were hospitalized. Though very likely, however, we have not so far seen a child who may have radiographic evidence of pneumonia but not clinically ill.

S. PLOTKIN (The Wistar Institute and the University of Pennsylvania, Philadelphia): Could you tell us briefly which media you used to perform the mycoplasmal isolations? Were they commercially prepared? Secondly, can you tell us something about the epidemiology of this disease within your families? Which age groups introduced the infection into the families: adolescents, school-age children or younger children?

N. BALASSANIAN: PPLO agar plates prepared in our laboratory were used for this study. All specimens were obtained by a throat swab and were streaked directly and immediately onto the plates. Since 50 % of the members were already infected and in view of the variability of the clinical picture, I have no answer as to who may have introduced the infection into the families. It could have been any of the members or a frequent visitor. In this study we were rather surprised to learn of the rather slow dissemination of the infection in the face of the numerous carriers. This suggests that the person with the organism present in his nasopharynx is not equally infectious at all times. It would thus be of importance to set up studies to investigate the ability of infected persons with different clinical syndromes, to infect susceptibles. And lastly, to see if a carrier is able to spread infection among a susceptible group. In a recent epidemic of *M. pneumoniae* infection in a boys' school, we have had the opportunity to study the ability of an infected person to spread *M. pneumoniae* by coughing. Of the 200 cough plates obtained from infected members, the only positive plates were obtained from persons with productive cough or those with pneumonia and productive cough. Infected asymptomatic members and those with dry cough failed to yield positive cough plates.

S.L. KATZ (Children's Hospital Medical Center, Boston): Do you have any data to indicate the viral and bacterial flora of the respiratory tract of these family members at the same time as the mycoplasma cultures were obtained? One wonders about any possible synergism or antagonism which might influence the intrafamilial spread of mycoplasma.

N. BALASSANIAN: Viral cultures were taken on all members on the initial visit, while bacterial cultures were done on the initial and follow up visits. No viruses were isolated. As for bacteria, untypable *H. influenzae* was the only bacteria isolated from 85 % of the infected members; its significance as a synergistic agent no doubt is debatable.

F.W. DENNY, Jr. (University of North Carolina School of Medicine, Chapel Hill): You have pointed out very beautifully some of the aspects of the epidemiology of infection with *M. pneumoniae*. Your studies were done over a relatively short period of time, however, and I wish you would comment on the prevalence of infection with this particular organism in other periods of the year or other years. The infection rates that you report are very high and I would be curious to hear if these rates persisted for longer periods in the populations in Cleveland. I would also be interested if *M. pneumoniae* infections occur with regularity in your populations from year to year.

N. BALASSANIAN: These studies were done over a period of 15 months from 1964 to April, 1965, and since April, 1965, we have studied a few more families who have had about the same incidence of infection among the members. Epidemiologic and diagnostic studies with *M. pneumoniae* infection has and is playing a major role in respiratory infections of the Cleveland population, at least for the past 3 years. Whether this was different in the past or will change in the future is yet to be shown.

26 Role of 'Inhibitors' in Maintaining Normal Bacterial Flora. KATHERINE SPRUNT, WINIFRED REDMAN* and GRACE LEIDY*, Columbia University, New York. N.Y.

Our experience suggests that within fairly wide limits a man's nasopharyngeal bacterial flora is characteristic of him as an individual. *In vivo* and *in vitro* data indicated that interrelationships between the organisms comprising the flora play a significant role in maintaining the proportion of one type of bacterium to another. The interrelationship to be discussed is interbacterial inhibition. The flora of all normal individuals studied contains many organisms ('inhibitors') which *in vitro* inhibit the growth of other types of organisms. When inhibitors are decreased by antibiotic therapy gross changes occur in the floral pattern, resulting, in some instances, in potentially serious overgrowth of hitherto undetected types. The shifts which occur in pharyngeal flora of patients given massive doses of penicillin, dimocillin and streptomycin while undergoing open heart surgery will be shown. All detectable inhibitors were removed by this regimen and in most patients massive overgrowth of coliforms resulted. Viridans streptococci of several varieties are apparently the 'key inhibitors' in this situation. As the streptococci were reduced by antibiotics, gram negative bacilli increased until they made up 85–100 % of the patient's pharnygeal flora. After therapy was discontinued, streptococci reappeared and simultaneously the bacilli disappeared. In the few patients carrying penicillin resistant streptococci no such shift occurred. This interrelationship between α-streptococci and gram negative bacilli (coliforms, aerogenes, proteus, pseudomonas) has been demonstrated repeatedly *in vitro* as well as *in vivo*. Available evidence suggests that a complex interplay between organisms is one of the important mechanisms maintaining the bacterial status quo.

Discussion

C.A. JANEWAY (Children's Hospital, Boston): I am terribly glad to see that Dr. SPRUNT has put this problem in ecological terms. I would like to ask her two questions: 1. How often are the coliforms the source of difficulty in their patients? 2. Has she thought of implanting antibiotic-resistant streptococcal strains in order to prevent this phenomenon, if they are?

KATHERINE SPRUNT: It is difficult to discuss how often the overgrowing coliforms caused clinical infection. Most of the patients in the study were open heart surgery patients who were having varying degrees of

trouble as a result of their procedures. There were vigorous complaints of esophagitis, for instance, in, I should guess, more than half the patients, but they had all been intubated throughout their surgery. Coliforms grew from tracheotomy tubes in patients having difficulties within their thoracic cavities. However, all these complications were of questionable relationship to the presence of the organisms. None of the patients discussed today had serious trouble which was clearly related to his overgrowing coliforms. However, one of the four we were watching for various reasons just after the study was completed developed serious complications including septicemia, pneumonia and empyema apparently due to Serratia and Klebsiella type organisms. Trouble does develop, but I do not have the data to say how often. We have thought of implanting 'resistant' inhibitors, of course, but that is all. Implantation may prove to be feasible, however, because of the low degree of resistance which seems to be required for an organism to persist and apparently maintain its inhibitory function in the pharynx despite massive doses of penicillin given intravenously. Some of the organisms persisting and apparently protecting from overgrowth the patient whose data were shown in slide 3, were resistant to 0.1 and sensitive to 0.5 units of penicillin per ml. One would not be unduly horrified at the idea of such organisms as a cause of endocarditis.

M. Grossman (University of California Medical Center, San Francisco): Dr. Sprunt, you made a comment that you thought these coliforms came from the patient's own flora. I wonder if you have any evidence for this; or could they have come from the environment?

Katherine Sprunt: We have no conclusive evidence that the overgrowing coliforms come from the patient's own flora. They could come from the environment. Our impression of their endogenous source comes from little things like rapidity of appearance and reproducibility of the picture from patient to patient including those from different environments (i.e. having no connection with the open heart surgery regime). Occasionally, too, we see cultures which grow out some coliforms on agar containing penicillin, but show no coliforms from aliquots of the same culture materials plated on plain agar.

J. J. Quilligan, Jr. (Loma Lina University Medical Center, Loma Linda): The first slide that you showed, with the zones of inhibition around it—do you have any thoughts about the mechanism involved in this inhibition?

Katherine Sprunt: The zone of inhibition shown in the first slide was caused by a slow growing gram positive diplococcus or short rod which is not an α-streptococcus. We do not know anything about its mechanism of action beyond the fact that it is apparently not a hydrogen peroxide effect. Its inhibitory activity is not diminished by catalase.

H. Shinefeld (University of California, San Francisco): My question is also related to the mechanism of 'inhibition' or 'interference'. Were all of the inhibiting streptococci producers of some sort of antibacterial substance?

Katherine Sprunt: The streptococci are a mixed lot, some inhibiting many of our test organisms, some a few and some none. Their mechanisms of action are varied too and we still know very little about them. It is probably fairly safe to say that all inhibitors studied produce something which is antibacterial, i.e. that inhibition is something more than just competition for essential metabolites. One of the inhibiting substances is hydrogen peroxide (inactivated by catalase and peroxidase) but other substances are apparently produced as well.

F.W. Denny, Jr. (University of North Carolina School of Medicine, Chapel Hill): I am concerned by the development of this rather fantastic degree of resistance of *Streptococcus viridans*. Those of us who are trying to prevent the implantation of resistant organisms on heart valves would be more afraid of resistant streptococci than gram negative bacilli. Can you tell us if these resistant streptococci are metabolically healthy streptococci, and are they capable of causing subacute bacterial endocarditis?

Katherine Sprunt: I hope it is as unusual as I think it is for an infant to grow out in quantity streptococci resistant to more than 20 units of penicillin per ml. Do you suppose he managed to develop them in his own pharynx or did he pick them up? Either way they are as you say, rather terrifying when one thinks of them on heart valves. This particular baby was lost to follow up so I don't know how long the organisms persisted. We have followed another child who had some streptococci resistant to more than 20 units/ml at the time of heart surgery. A year later, 0.05 % of his streptococci were resistant to more than 5 units of penicillin/ml—the top quantity tested. In short, while the organisms may have persisted in this individual they make up a very small part of his streptococcal population.

27 *Assisted Ventilation in Hyaline Membrane Disease (HMD).* Atties F. Malan*, Frank M. Shepard*, Willard J. Blankenship*, James Gray*, William C. Young* and Mildred T. Stahlman, Dept. of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tenn.

The outcome of 71 infants with HMD who received assisted ventilation has been evaluated. All received 100 % oxygen and vigorous therapy with intravenous buffers and glucose. Sixty-one infants had prolonged apnea while 10 had aortic PO_2 levels of ≤ 35 mm Hg with or without $PaCO_2 > 75$ mm Hg as their indication for artificial ventilation. Negative and positive pressure were both used, in combination if necessary. Twenty-five infants (35 %) survived, while 46 (65 %) died. Survivors were the larger infants (mean weight 2066 g ± 520 versus 1666 g ± 632) and accepted for treatment later (mean 11.5 hours versus 6.6 hours). The initial pH, blood gases and lactic acid were less abnormal than in those who died. Survivors required respiratory assistance from 12 to 90 hours with no increased survival beyond this time. More severe respiratory and metabolic acidosis characterized the course of infants who died. They required ventilatory assistance earlier and only 3 of 33 infants less than 2000 g and ventilated before 30 hours of age survived. The PaO_2 just before (mean 37 mm Hg) and after ventilation (mean 149 mm Hg) were similar in both groups but infants who died deteriorated after variable periods. Complications encountered include pneumothorax (11 infants), endotracheal tube obstruction (4), hyperventilation (11), infection (3) and abdominal distension (21). Intracranial hemorrhage was present in 24 of 42 (57 %) of autopsies. All autopsies confirmed the diagnosis of HMD. The mean developmental quotient in survivors is 91 at an average age of 2 ½ years. Four have some residual radiographic pulmonary changes. Two lung biopsies demonstrate interstitial fibrosis. Ventilatory assistance was considered to be life-saving in all survivors.

Discussion

R. USHER (Royal Victoria Hospital, Montreal): Dr. MALAN, is there any way by which one can assess your success in reducing mortality from RDS, by knowing the overall mortality rate of the consecutively delivered, live-born, low birth weight populations of maternity hospitals which feed your unit?

A.F. MALAN: Dr. USHER, we are not able to assess the results versus consecutive live births at Vanderbilt Hospital. All I can say is that this population, as I described this morning, of 71 infants, represents the infants who we firmly believed were dying and would have died if we had not intervened. And this represents the, in all, 20 or 25 % group of hyaline membrane babies who would have died otherwise.

IRA H. GESSNER (University of Florida College of Medicine, Gainesville): Are your arterial samples taken from the descending aorta or ascending aorta, i.e. below or above the ductus arteriosus?

A.F. MALAN: Dr. GESSNER, these measurements were made in the descending aorta, and we are well aware of the fact that there may be much higher oxygen values above the ductus.

P.R. SWYER (The Hospital for Sick Children, Toronto): We have the same experience over about 150 cases with comparable survival rates. I think we must now accept that artificial ventilation is a method of treatment which can offer a worthwhile salvage in this condition where facilities are available for satisfactory management of this very complex treatment. I think much remains to be learned about the physiology of artificial ventilation in the newborn and I would like to ask a question pertaining to your statement that negative pressure ventilation is more physiological than positive pressure ventilation. Could you please tell us why this should be so?

A.F. MALAN: Dr. SWYER, we prefer negative pressure ventilation purely on the ground that it seems a more physiological approach. We have not made any measurements of comparative pulmonary function studies or circulatory changes comparing positive and negative pressure ventilation. All the guides we have are some work done in England by Dr. CRAMPTON-SMITH and others on animals showing that with increased positive pressure ventilation thoracic return of blood was diminished, with resultant low cardiac output.

L. STERN (Children's Hospital, Montreal): Dr. MALAN, I have two questions, and I wonder if you would comment on both of them. I would like to reinforce what Dr. SWYER has said, that it seems to be clearly established that in some hands using respirators it's possible to get increased survival with this disease. There now arise two questions out of this. 1. Is survival, in fact, a valid end point of what we are talking about? In other words, is it fair to evaluate a respirator on the basis of the fact as to whether or not the infant survived? Surely his survival in the final analysis depends upon so many other factors in addition; it may be the time of the month that the house staff changes, the kind of nursing and medical care, associated diseases, etc. which are crucial. 2. Are we now also to be content with placing these children in respirators only at the point where we think they will most surely die? This would certainly influence your survival rate. If some method of selection were made whereby one could decide before the child is moribund or has undergone intracranial hemorrhage and repeated apnea, could you not get some better kind of result with this?

A.F. MALAN: Dr. STERN brought up a very important point, in that the result of this sort of therapy is utterly dependent upon an adequate staff being available and how much time and effort the people are prepared to expend. If there is any clear-cut earlier point at which we can intervene with respirators, we either have to wait until all other therapy has failed or go to the other extreme and say that we will ventilate all babies and see what sort of results we get. In these babies we had waited, obviously, until all other therapy had failed. I wish we had had a better assessment earlier; and also to be able to determine earlier which babies may suffer intracranial hemorrhage.

P.A. LIEF (Beth Israel Medical Center, New York): Could you describe the types of respirators used and whether you were using assisted ventilation or controlled ventilation and the degrees of positive and negative pressure used?

A.F. MALAN: The type of respirators used: the negative pressure respirators were Monaghan and Air Shields. The Bird Mark VIII Respirator was used for positive pressure, as well as Circle, which was a bag being inflated by the nurse in time with the negative ventilation. Pressures have been kept less than minus 35 or plus 35 cm of water, and although these machines are available, we are still looking, as I think many others are, for the ideal machine for a newborn infant with such abnormal lungs. In many cases, if we were on hand and knew that the infant was deteriorating rapidly and we had time, the infant was moved to the negative pressure respirator, and with that type of apnea or failure of maintaining adequate oxygen or carbon dioxide levels, the respirator was commenced.

J. DOWNES (Children's Hospital, Philadelphia): My question relates to one that Dr. STERN just posed. If we were to consider earlier mechanical ventilation in RDS, we must also consider the risks of endotracheal intubation. Did you find it necessary in your group to use endotracheal intubation or tracheotomy in these children? Have you successfully altered the pO_2-pCO_2 without the presence of an endotracheal tube?

A.F. MALAN: Many times we were not able to do this. The infants were tubed and moved to the negative pressure respirator. Subsequently the endotracheal tube was removed and adequate ventilation was achieved without an endotracheal tube being present. Infants who survived, as I said, were able to be maintained with negative pressure respiration alone. Infants who died, however, once they stopped responding to negative pressure, it became necessary to intubate them. Otherwise we only ventilated the stomach and not the lungs. To come back to the previous questions, we tried as far as possible to achieve assisted ventilation and not controlled ventilation. Thank you.

28 *The Role of Epinephrine Prophylactic Therapy in Infants of Diabetic Mothers (IDM).* MICHAEL L. MCCANN* and BEVERLY F. LIKLY*, Western Reserve Univ. Sch. of Med. Cleveland, Ohio (introduced by William M.Wallace).

Previous investigators have shown that infants of diabetic mothers (IDM) often develop symptomatic hypoglycemia in the initial hours of life. In addition serum free fatty acid (FFA) levels (CHEN *et al.*) and urinary catecholamines (LIGHT) may be abnormally low. It is hypothesized that these abnormalities are secondary to fetal hyperinsulinism. Since PORTE and WILLIAMS have demonstrated that exogenous epinephrine in adult man suppresses pancreatic insulin out-

put, a trial of prophylactic epinephrine therapy in 14 IDM was initiated. Clinical and biochemical findings were compared to untreated IDM's, in 4 infants who served as their own controls, and in 10 infants who were treated with glucagon. Clinically, infants who were treated with long-acting epinephrine (Sus-phrine, Brewer, 0.01 ml/kg q 6 h) showed improved neurological (Moro, toe grasp, response to pinprick) and respiratory findings (retractions less, resp. rate slower and more regular). Serum insulin (radioimmunoassay method, PEARSON) fell and blood glucose and FFA rose significantly. Epinephrine, in contrast to glucagon, also prevented rebound hypoglycemia (50–85 mg/100 ml after epinephrine vs. 10–25 mg/100 ml after glucagon). Because of the correlation between the clinical changes and the correction of the biochemical abnormalities, it is hypothesized that epinephrine administration immediately at birth and at regular intervals thereafter may be specific prophylactic therapy in IDM resulting in lessened morbidity and possibly lessened mortality.

Discussion

N.M. NELSON (Children's Hospital, Boston): Are you recommending epinephrine therapy as a prophylaxis for symptomatic hypoglycemia or for the respiratory distress syndrome?

M.L. MCCANN: There is an additional slide which summarizes our experience with several treatment regimes and which may answer Dr. NELSON's question. Sixteen untreated infants of diabetic mothers during the first hours of life developed hypoglycemia to mean blood glucose concentrations less than 25 mg per 100 ml associated with a rise in the mean respiratory rate to above 75 per minute. Rapid I.V. glucose injections in 9 infants resulted in 'rebound' hypoglycemia to the same low level of blood glucose two hours later with no effect on the respiratory rates which remained elevated. A large dose of glucagon, 300 μg/kg, given immediately at birth to an additional 8 infants did not prevent either hypoglycemia or tachypnea although both occurred at a later time, that is, at 3 to 6 hours of age compared to 1 to 3 hours of age in the untreated infants. Dr. LIKLY has also administered glucagon at a later age with similar transient and unpredictable effects on blood glucose associated with rebound hypoglycemia. This is probably because glucagon, like glucose, stimulates insulin release. By contrast 5 of the 7 infants who were treated with a combination of short and long acting epinephrine immediately at birth remained normoglycemic and tachypnea did not develop. These infants, who had mean respiratory rates less than 60 per minute associated with blood glucose concentrations above 50 mg per 100 ml, were similar to normal infants. Two other treated infants who did become hypoglycemic either because of insufficient epinephrine or because of a need for additional glucose therapy, also developed tachypnea. Although these numbers of infants are too few to validate a statistical comparison between groups, there is a strong suggestion that if hypoglycemia can be prevented so may tachypnea and at least part of the respiratory distress syndrome. I wish to emphasize that there appear to be two different respiratory problems in these infants. One appears to be related to the metabolic abnormalities and can be prevented. The other is a separate problem related to prematurity and the development of the so-called 'hyaline membrane' respiratory syndrome. Since infants of diabetic mothers are often born prematurely, the two clinical disorders, which may have similar manifestations, are confused. Epinephrine, by suppressing hyperinsulinism, preventing hypoglycemia and elevating free fatty acids, prevents the metabolic component of 'respiratory distress', possibly through central mechanisms. On the other hand, there is little evidence that epinephrine directly improves the peripheral type of respiratory distress which presumably develops as a result of primary lung pathology. It is important to distinguish between these two types of respiratory distress which may occur separately or in combination. The former type can be prevented, but the effects of epinephrine on the latter are unknown.

D. O'BRIEN (University of Colorado Medical Center, Denver): I would be very interested to know whether you have tried giving mannoheptulose to any of these babies.

M.L. MCCANN: In answer to Dr. O'BRIEN, no, we have not studied mannoheptulose.

M. CORNBLATH (University of Illinois College of Medicine, Chicago): Do you have any evidence that you can measure meaningful immunoreactive insulin levels in the plasma of infants of insulin treated mothers who have insulin antibodies? There is good evidence that these insulin antibodies cross the placenta and have a variable effect on the insulin assay from moment to moment because of variations in binding capacity. It might be that hypertonic glucose or epinephrine also influence this binding and that the insulin changes reported are artifactual.

M.L. MCCANN: Dr. CORNBLATH raises a very important problem, namely the interference in the immunoassay for insulin of maternal antibody to insulin. This results in an artificially high *baseline* for immunoreactive insulin in the baby who acquires maternal antibody transplacentally and which antibody interferes with the added exogenous antibody in the first step of the double antibody system. We were well aware of this problem which is the reason that infants born to non-insulin dependent women (or gestational diabetics), who have no antibody to insulin, were selected as controls. In these latter infants the baseline insulin levels were lower, but the mean increment rises in response to glucose were almost exactly the same as those in infants born to insulin dependent mothers. The data were expressed therefore as increment changes from baseline rather than as absolute concentrations. A comparison of baseline insulin levels between two infants is not valid, but a comparison of a change from baseline (increment) in the same infant who serves as his own control is valid. In addition, the increments in immunoreactive insulin correlated quite well with those increments determined by an entirely separate method which measures C^{14} O_2 and C^{14} glycogen uptake by the rat epididymal fat pad. In this latter method, maternal antibody produces an opposite artifact, namely a lower than actual measurement of insulin-like activity. In this study when maternal antibody in the infant's serum was separated from insulin by the use of an acid alcohol extract, the insulin increment was greater. It is therefore important to either separate the infant's insulin from the maternal antibody or to use appropriate controls, namely infants of gestational diabetics. We did both. Furthermore, the suppression of insulin by epinephrine was greatest in infants of gestational diabetics which suggests that not only is the immunoreactive method measuring a true suppression of insulin in both groups, but that the interference of maternal antibody obscures an even greater suppression than was reported. There is theoretically

an insignificant change in the concentration of maternal antibody from hour to hour since its half life is measured in weeks rather than in hours. I am unaware of data which shows that exogenous epinephrine interferes *in vitro* or *in vivo* with the immunoassay by altering antigen antibody binding. Therefore, all factors considered, in the light of current knowledge the change in serum insulin as a result of epinephrine therapy cannot be considered artifactual, but respresents a real suppression.

JOAN E. HODGMAN (University of Southern California, Los Angeles): I wanted to ask Dr. MCCANN what criteria were used for diagnosis of gestational diabetes, and also whether he could differentiate in any way besides insulin studies between the infants who did not have elevated levels and the babies who did.

M.L. MCCANN: Dr. HODGMAN, the criteria for defining gestational diabetes in the mothers of these particular infants were different from those previously used which were on the basis of intravenous glucose tolerance tests. The mothers in this study had glycosuria, elevated fasting of 2-hour post-prandial blood sugar concentrations and were candidates for insulin therapy. They were definite diabetics. In regard to the infants, there was a good correlation between the rate of glucose disappearance following I.V. glucose and the degree of hyperinsulinism. Ninety minutes after glucose, 1 gm/kg, infants who had hyperinsulinism also had blood glucose concentrations less than 40 mg % associated with fast rates ($\overline{m}K_t = 3.6$ %/min) whereas those infants without a rise in insulin had 90 minute blood glucose concentrations above 40 mg % associated with slow rates ($\overline{m}K_t = 1.3$ %/min) similar to normalinfants.

W.E. HATHAWAY (University of Colorado Medical Center, Denver): I would like to ask a question about a possible complication of the use of epinephrine in these infants. It has been shown that infants of diabetic mothers have an increased incidence of renal vein thromboses. This is a rare complication, but it does occur. It is also known that epinephrine is one mediator of hypercoagulability; both plasma factors (antihemophilic factor VIII) and platelet adhesiveness are increased by epinephrine. I assume that you did not see thrombotic phenomena in these infants, but would you comment on this theoretic complication?

M.L. MCCANN: Dr. HATHAWAY, we have not studied coagulation defects, and I think this is an important problem. We did not find thromboses at postmortem examination in the two infants who expired. I should point out that our perinatal mortality rate ranges, on the average, around 17 % per year, and was 31 % last year in infants who were not treated with epinephrine. Of the epinephrine treated infants, only 2 of 25 expired, and both were not treated prophylactically from birth, but several hours later when they had already developed severe hypoglycemia, hypoxemia and acidosis. This suggests that even a few hours of these severe biochemical abnormalities may produce permanent or irreversible effects. Although the figures are still small, the mortality rate compares very favorably with other good mortality figures and suggests that further trial is indicated in these infants. Experience with the less mature infants, of which we treated only 5 who were less than 36 weeks' gestation, is limited. In these infants other adjunctive forms of therapy may be more important, but replacement epinephrine therapy in a smaller dose appears to be beneficial. In summary, I am suggesting that in these infants there is a highly variable primary and specific dual hormonal abnormality, namely insulin excess combined with epinephrine deficiency (the latter either relative or absolute) both of which can be specifically corrected by epinephrine therapy. Secondarily, the associated biochemical abnormalities (fat and glycogen excess, low blood glucose and free fatty acids) are corrected which results in clinical improvement. Although with this dosage of epinephrine we have observed no side effects other than transient ecchymoses at the injection site, additional careful observations including effects on coagulation changes and acid base balance are necessary before its use can be recommended as a routine prophylactic procedure.

29 *Sodium Transport Inhibition by the Saliva of Patients with Cystic Fibrosis.* JOHN A. MANGOS* and NONA R. MCSHERRY*, Univ. of Wisc. Sch. of Med. Madison, Wisc.
(introduced by Charles C. Lobeck).

Previous studies from our laboratory have shown that rat parotid saliva is formed by the secretion of a 'primary fluid' with plasma-like osmolarity and sodium and potassium concentrations in the acini-intercalated ducts of the gland. Salivary hypotonicity is produced in the striated ducts by reabsorption of sodium at the rate of 6.1 μEq/min/g of wet gland tissue (WGT). Retrograde perfusion of the duct system of the gland either with isotonic saline or with a 4:1 mixture of saline and mixed mouth saliva from 6 healthy children had no effect on the rate of sodium reabsorption. Retrograde perfusion of the duct system with a 4:1 mixture of saline and mixed mouth saliva from 6 patients with cystic fibrosis caused a significant decrease of the rate of sodium reabsorption to 1.8 μEq/min/g WGT ($p < 0.001$). Following retrograde perfusion of the duct system with saline containing ouabain (10^{-3} M) the rate of sodium reabsorption decreased to 2.2 μEq/min/g WGT. These results demonstrate that mixed mouth saliva from patients with cystic fibrosis has a sodium transport inhibitory effect on the rat parotid gland. The magnitude of this inhibition is comparable to the inhibition induced by ouabain, the known inhibitor of sodium transport. This effect may be responsible for the increased sodium concentrations in the saliva of patients with cystic fibrosis.

Discussion

A. ROBINSON (University of Colorado Medical Center, Denver): Have you tried using the saliva of the heteroxygous carrier of cystic fibrosis?

J.A. MANGOS: Yes, we have tried saliva from only three parents, and we could not demonstrate transport inhibition.

E. ROSSI (University of Berne, Berne): Have you studied the concentration of calcium in the saliva, and if yes, did you find any connection between the turbidity and the transport inhibition?

J.A. MANGOS: Thank you, Professor ROSSI. Yes, we have. We took saliva from normal individuals and added calcium from 2 up to 12 mEq/l, in addition to whatever calcium existed in the saliva. We could not demonstrate a transport inhibitory effect following the addition of calcium.

30 *Metabolic Studies in Hypophosphatasia and Response to High Phosphate Intake.* ALFRED M. BONGIOVANNI, MANUEL M. ALBUM*, ALLEN W. ROOT* and JOHN W. HOPE*, Children's Hospital Philadelphia, Pa.

A $2^{8}/_{12}$-year-old girl was studied because of loss of bone structure around the teeth. She had rickets and a low serum alkaline phosphatase (0–0.7 BU). The parents and two siblings were normal although the mother's phosphatase was only 0.6 BU. Calcium dynamics were studied by infusion of stable strontium. The exchangeable pool and bone deposition were lower than normal for age, 18.2 and 6.73 liters respectively (normal: 25.7 and 9.6). Phosphoethanolamine was present in the urine. Daily urine phosphate (P) and pyrophosphate (PP) were measured in 25 relatives through 3 generations by ion exchange chromatography. The propositus excreted 20 mM P and 170 μM PP per m^2 daily. Twenty members excreted an average of 15 mM P and 42 μM PP. Five members had elevated PP, above 120 μM. No clear hereditary pattern was established. The patient was treated with 13 g $NaHPO_4$/NaH_2PO_4 daily. The urinary PP rose to 320 μM. Within 6 months there was healing of the rickets which has continued for 18 months. Excess PP may inhibit normal uptake of Ca and P by hydroxyapatite crystals and the administration of excess P leads to removal of PP since both share a common renal transport mechanism.

Discussion

C.R. SCRIVER (Montreal Children's Hospital, Montreal): This is an opportunity to mention the issue now being raised almost yearly at these meetings, namely the problem of genetic heterogeneity in the apparently clear-cut clinical disease. We have had the opportunity to study a child who, from a clinical point of view, would be considered to have classical hypophosphatasia; all the findings described by Dr. BONGIOVANNI in his patient, were mimicked exactly by our patient, with but one exception: our child had normal total plasma alkaline phosphatase activity! I do not know what you would call this disease, but I suppose for lack of better terminology, you could call it 'pseudo-hypophosphatasia', and then run for cover. The important thing about this new disease, in terms of the present discussion, Dr. BONGIOVANNI, is that the patient healed her osseous lesions spontaneously; the only treatment extended to our patient was a craniotomy and measures to offset hypercalcemia. The healing occurred in three or four months without any phosphorus therapy. Perhaps Dr. BONGIOVANNI would like to expand a little bit and say whether he thinks the phosphorus therapy for his patient did really help the progress of the bone lesions. I would perhaps be more optimistic that you did something for the dental lesions.

A.M. BONGIOVANNI: I am not absolutely sure that it did. In our experience with a small group a couple of whom have shown spontaneous healing—and as you and I discussed the other day, there are other things that can produce healing, presumably, like cortisone. This one healed more rapidly, and the dental findings have struck us more than anything else. In addition, Dr. TRYGSTAD in this audience has seen healing on low doses of phosphate and recurrence of rickets in the same subject after withdrawal. He too noted dental improvement.

D. O'BRIEN (University of Colorado Medical Center, Denver): The finding of the increased urinary pyrophosphate in this case seems to have some analogies with what Dr. SOLOMONS has noted, and I wondered if you would consider giving this child magnesium in large doses to stabilize pyrophosphates.

A.M. BONGIOVANNI: We considered it, but we did not try it.

31 *Cysteine/Cystine Transport and Ratios in Leukocytes From Children with Cystinosis.* JERRY A. SCHNEIDER* and J. EDWIN SEEGMILLER*, NIH, Bethesda, Md. (introduced by R. Rodney Howell).

The uptake of cystine-^{35}S by both normal and cystinosis WBC's was very small, with a distribution ratio (cpm per μl intracellular/cpm per μl extracellular) less than 2. The uptake of cysteine-^{35}S, however, was substantially greater, with the mean distribution ratio WBC's of 8 patients with cystinosis double that of 7 control subjects (31.6 ± 7.7 vs. 14.0 ± 2.2, $p < 0.01$). Extracellular cysteine-^{35}S was kept reduced with thiolated Sephadex. The percent of intracellular ^{35}S present as cysteine-^{35}S was measured by lysing the cells in N-ethyl maleimide (NEM) which binds cysteine and prevents its oxidation to cystine. After cystine and cysteine-NEM were separated by high voltage electrophoresis, the percent radioactivity in the latter was much lower in WBC's from cystinosis patients than from control subjects (59.2 ± 4.7 % vs. 94.0 ± 1.4 %, $p < 0.01$). This difference was not influenced by incubation time, cystine content or the differential count of the WBC preparations. When the distribution ratios were corrected to include only cpm from *cysteine-^{35}S*, the ratio in cystinosis WBC's was reduced almost to the level in control WBC's (18.8 ± 5.2 vs. 13.1 ± 2.1, $p < 0.05$). This small difference in distribution ratio may have been caused by a difference observed in the differential counts of the WBC preparations from these 2 groups. The uptake of increased amounts of cysteine-^{35}S, almost half of which is present intracellularly as cystine-^{35}S, represented accumulation of cystine-^{35}S by WBC's from patients with cystinosis.

Discussion

C.R. SCRIVER (Montreal Children's Hospital, Montreal): Did you study cellular efflux of L-cysteine-^{35}S, particularly in the presence of dithiothreitol?

J.A. SCHNEIDER: In our system, dithiothreitol (Cleland's Reagent) caused the leukocytes to clump, and that is why we used another method to keep extracellular cysteine reduced. We have done efflux studies with leukocytes which were pre-incubated in cysteine-^{35}S. These studies have been done in the absence of any reducing agent, and preliminary results indicate no significant difference in the rate of ^{35}S efflux between normal and cystinotic leukocytes.

32 *Dawson's Inclusion Body Encephalitis: Electron Microscopic Study and Treatment with BUDR.* JOHN M. FREEMAN*, GUY M. MCKHANN and ROBERT HERNDON*, Stanford Univ. Sch. of Med., Palo Alto, Cal.

Dawson's encephalitis (subacute sclerosing leukoencephalopathy) has been classed as a degenerative disease of the central nervous system. Despite the presence of type A inclusion bodies and recent electron microscopic evidence of virus-like particles within neurones. No viral etiology has been documented.

Two patients with Dawson's encephalitis, one early and one late in the clinical course, have been studied. Electron microscopy of material obtained at brain biopsy revealed 170–200 A° tubular structures within the inclusions. These tubules are similar in size and morphology to the nucleo-capsid of the type II myxoviruses such as measles and distemper. Elevated measles antibody titers of 1:64 and 1:256 were found in the two patient six years after documented rubeola. These titers were stable over a six week period. Immuno-

fluorescent staining of neurones and glial cells in this biopsy material with fluorescene-tagged measles antibody confirmed the presence of measles antigen in both patients. This disease would thus appear to be a chronic viral encephalitis due to the measles-distemper group of viruses.

Treatment with 5-bromo-2-deoxyuracil (100 mg/kg/day for 5 days) intravenously produced arrest of the progressive course in both patients and documented clinical improvement in one. The mechanism of action of this thymidine analogue in an RNA viral infection is unknown.

Discussion

M.N. GRIFFITH (Massachusetts General Hospital, Boston): Last year we had occasion to treat a patient with a similar disease with labeled IUDR (5-iodo 2′ deoxyuridine) which in structure and mode of action is very similar to BUDR. We gave the drug intravenously and assayed the levels of unaltered drug in the cerebrospinal fluid and found that barely detectable levels of unaltered drug were recoverable from the cerebrospinal fluid following this route of administration. We then gave the drug directly into the lateral ventricle through an Ommaya catheter. This permitted bi-daily injections of $IUDR^{125}$ over a prolonged period and resulted in relatively high levels of the drug in the cerebrospinal fluid. The patient tolerated this procedure well and for the past sixteen months since therapy, his neurological status has remained stable. I would like to ask Dr. FREEMAN whether he has assayed the levels of BUDR in the cerebrospinal fluid or brain and whether he might comment further on this point.

J.M. FREEMAN: We did not do any studies of the level of BUDR within brain or within spinal fluid. BUDR has been used as an adjunct to cancer radiotherapy. It does get into rapidly growing tumors in the central nervous system, and perhaps it gets into areas of inflammation. We have no data on this point.

H.L. HODES (Mount Sinai Medical School, New York): I was very pleased to hear Dr. FREEMAN's paper, which I think is extremely interesting. I should like to mention Dr. JOHN ADAMS' work, reported in the Journal of American Medical Association last year, which suggests that measles virus may stay in the central nervous system for a long time after the initial infection. I am not sure whether Dr. FREEMAN implied that the children he described had measles in the past. I take it that he believes the illness he described is due to something other than measles. Would he explain his ideas on this point? I should like to point out that there is an abstract in the American Pediatric Society notes for this year which describes the case of a child who got encephalitis after vaccination for measles. The statement is made in the abstract that measles virus was isolated from the spinal fluid. I think this is a paper by FORMAN and CHERRY. I wondered whether Dr. FREEMAN would like to comment on the relationship of this finding to his work.

J.M. FREEMAN: As you have indicated, Dr. JOHN ADAMS has reported the presence of both intranuclear and intracytoplasmic inclusions in patients with measles encephalitis (J. amer. med. Ass. *195:* 150 [1966]). I also understand that he has found these inclusions in children who have died many years after measles encephalitis. Immunofluorescent staining of these inclusions has not yet been reported; nor has electron microscopy. Both of our patients had had rubeola more than six years prior to the onset of their present illness. Neither had an encephalitic course at that time. The particles demonstrated within the inclusion appear similar to the nucleocapsid of the measles virus, and since there was no history of a recent exposure to measles or of anything to suggest reinfection such as a rising antibody titer, it is therefore tempting to assume that the particles seen are the residual of the previous measles infection or represent a reactivation of virus which had been latent for six years. At present there is no data on this point. With respect to a possible infection from the measles vaccine, neither child had received the vaccine.

H.L. HODES: I wanted to make one more remark, if I might. Some years ago Dr. JOHN ADAMS showed a cross-antibody reaction between measles and distemper. My recollection is that he suggested at that time that there might be a causal relation in some of the cases of encephalitis to this particular virus.

J.M. FREEMAN: As you indicate, there is marked cross immunity between measles and distemper viruses, and rinderpest virus as well. This is why we were very excited about this child's exposure to the two dogs with distemper. However, there is no evidence that distemper causes disease in humans. In addition, immunofluorescent studies using the distemper reference immune serum from the NIH, which does not cross-react with measles virus in tissue culture, gives no staining of the inclusions in our patients. This, I think, is good evidence the viral particles are not distemper virus. Ferrets inoculated with brain material from these patients also have remained healthy.

H.W. BAIRD (Philadelphia): Do you have the impression that more children with encephalitis are coming to the attention of your clinic? In Philadelphia it would appear that there are more children with chronic convulsive disorders who have had encephalitic-like illnesses. Is this because of location, or do you think this is possibly a problem of recognition?

J.M. FREEMAN: Well, I think we see at any center where there are pediatric neurologists increasing numbers of children with seizures and what appears to be a degenerative course. Whether this truly represents a chronic encephalitis is a different matter. I think there is no evidence in these children of an encephalitic picture and nothing on pathologic examination to suggest a viral disease.

S.L. KATZ (Children's Hospital, Boston): These results, which you have presented considered in conjunction with those of other workers whom you have cited, are extremely stimulating in opening another avenue of possible etiologic significance in the ill-understood neurologic degenerative disorders. A single complement-fixation antibody titer against measles virus is rarely valuable in dating a measles infection, because there is such great variation in the detectable titers possessed by individuals at any given time after the onset of infection. There are two other types of antibody determination which might possibly assist in your attempt to date the antigenic stimulus; these are virus-neutralization and hemagglutination-inhibition. Additionally, it may be helpful to determine whether the antibody is IGG or contains some IGM. Dr. LENNETTE's laboratory might be willing to help you further in this regard. Your apparent failure to isolate the measles virus should not eliminate it from further consideration. Your electron-photomicrographs showed nucleocapsids interpreted as closely resembling those of measles. However, these may be virons which structurally and antigenically possess the characteristics of measles, but are incomplete and non-infectious. The immuno-

fluorescence reaction strongly supports the presence within the affected cells of measles-specific antigen, but the paradox of a favorable response to the halogenated pyrimidine implies some effect on a DNA-dependent process. This may suggest that measles virus might be present as an epiphenomenon (due to an aberrant host response) or as part of a dual etiology. Finally, in considering possible cross-reactivity, a third agent, related to canine distemper and measles viruses, is rinderpest (cattle plague) virus which has not been identified in continental USA in recent years. A paper by P.D. DeLay *et al.* (Amer. J. Vet. Res. *26:* 1359 [1965]) elucidates some of the relationships among the three viruses. Rinderpest may be studied only at the US Department of Agriculture's Animal Disease Laboratory at Plum Island, Long Island, N.Y., but I am certain Dr. DeLay and his associates would be willing to assist you in excluding (or including) rinderpest as the agent.

33 *Maternal-Fetal Distribution of Radioactive Cortisol and its Correlation with Teratogenic Effect.* Allwyn Levine*, Sumner J. Yaffe and Nathan Back*, State Univ. of New York at Buffalo, N.Y.

The genetic variation in susceptibility to the teratogenic action of corticosteroids in mice remains unexplained. AJAX strain is susceptible while CBA quite resistant to the induction of cleft palate. Primiparous females of each strain received a subcutaneous injection on day 11 of pregnancy of 2.5 mg of hydrocortisone sodium succinate together with a tracer amount (4.9–7.3 mcg) of ring A labeled C^{14}-cortisol. At intervals of 30, 60, 120 and 240 minutes after injection, the mothers were sacrificed, the fetuses dissected out, macerated and aliquots counted in a liquid scintillator. No significant differences between strains in the amount of radioactivity in the fetus was found at 30 min, but the differences at all other time intervals were significant at the 0.001 level. Fetuses of cleft palate susceptible strain retained far greater quantities of the C^{14} label than did those of the resistant strain. The differences noted in fetal steroid concentration suggest that the mechanism responsible for the variation in teratogenicity may involve greater receptor affinity or binding in the fetus of the susceptible strain and is not due to differences in placental permeability (Supported in part by NIH grants HD-01219 and FR-05400.)

Discussion

A. Sass-Kortsak (Hospital for Sick Children, Toronto): Have you measured the disappearance rate in the mother?

A. Levine: No, this was not done.

A.M. Bongiovanni (Children's Hospital, Philadelphia): I wondered if you had given any thought to man in your reflections based on these studies, because, as you know, the incidence of cleft palate in man is very, very low after steroids. Some years ago Dr. Frasier and I combed the world literature, and reached the conclusion that it certainly can produce it in man, but that it is unusual and that man is not highly susceptible.

A. Levine: This has been my experience in perusing the literature as well, that the incidence of steroid-induced cleft palate in man is practically nonexistent.

A.M. Bongiovanni: I was trying to recall Dr. Migeon's data in man. As I remember, the transport is very poor from mother to infant, but I do not think he had information on half life in the fetus.

A.E. Lorincz (University of Florida, Gainesville): Do you happen to have any data regarding localization of cortisone in the fetus, such as relative concentration in the head and neck compared to that in the rest of the body? Such information might be helpful in correlating steroid hormone effect upon acid mucopolysaccharide content of the secondary palate, which has been so elegantly studied by K.S. Larsson in mouse and rat embryos and shown to be related to cleft palate deformity.

A. Levine: These fetuses were dissected at 11 days, and anatomic localization of the drug was not attempted. We just measured the quantitative distribution of the drug, and in effect determined the amount of C^{14} that was present in the fetus. We do intend to repeat these experiments using autoradiographic techniques in an attempt to anatomically localize the distribution of the drug.

A.M. Rudolph (San Francisco Medical Center, San Francisco): You did not mention whether the fetal weights were similar.

A. Levine: The fetuses were not weighed.

A.M. Rudolph: Since you are measuring concentration of steroid in fetal tissue, would not weight be important in determining the total amount of steroid passing into the fetus?

A. Levine: There is an inverse relationship between the fetal weight and the induction of cleft palate. However, with the use of 2.5 mg of steroid, regardless of fetal weight, the incidence of cleft palate formation will be 100 %, in the sensitive strain. In view of the pronounced differences in the slopes of the curves measuring disappearance rates in the two strains, the small quantitative variation in fetal weight probably plays little or no part in the pathogenesis of the malformation.

R.L. Brent (Jefferson Medical College, Philadelphia): Dr. Rudolph's question pertaining to fetal weight in the two experimental groups is important, but although the authors did not weigh the fetuses, a difference in weight would not explain these findings. The most important aspect of many teratological experiments is the understanding of the mechanism of teratogenesis. You and your co-workers are well aware of all of the factors that determine where and how much radioactive cortisone will localize in various sites. The difficulty is in equating localization to etiology, since in a sense we have jumped several steps to assume that cortisone acts directly on palatal tissue. There is no question that this is a logical jump but, nevertheless, it is an assumption that somehow has to be proved.

34 *Graft-Versus-Host Reaction (GVH) Following Intrauterine Transfusion for Erythroblastosis Fetalis.* J. Lawrence Naiman*, Hope H. Punnett*, Marie L. Destiné*, Harold W. Lischner* and James B. Arey, St. Christopher's Hosp. for Children and Dept. of Ped., Temple Univ. Sch. of Med., Philadelphia, Pa.

Intrauterine transfusion has become an accepted measure in the salvage of the fetus affected with severe Rh hemolytic disease. Because of the increasingly young gestational age at which such transfusions are being performed, concern has arisen that the immune mechanisms of the fetus may not be sufficiently mature to prevent a GVH reaction by donor lymphocytes. We recently observed a possible example of this. A white male infant was born at 33 weeks' gestation after receiving 3 intrauterine transfusions between 27 and 31 weeks' gestation. He received several exchange transfusions and appeared to have recovered but at 8 weeks

of age developed diarrhea, jaundice and hepatomegaly. Peripheral blood revealed pancytopenia with absent reticulocytes and granulocytes. Bone marrow revealed no granulocyte precursors; instead there were immature monocytes and many histiocytes which had phagocytosed intact lymphocytes. The infant failed to gain weight and died of pneumonia at 12 weeks of age. Culture of peripheral blood lymphocytes during life revealed two cell populations distinguished by variations in length of the Y chromosome, so-called 'Yy' chimerism. The Y chromosomes of the father (and infant) were long, whereas those of the donor of the first intrauterine transfusion were short. Failure to reject the engrafted lymphocytes could not be ascribed to an underlying immunological deficiency syndrome, as evidenced by a rise in serum IgA and IgM, *in vitro* lymphocyte response to phytohemagglutinin, and immunofluorescent demonstration of IgG, IgA and IgM synthesis. Calcified bodies suggesting Hassall's corpuscles were seen in the thymus.

Discussion

FLOSSIE COHEN (Child Research Center of Michigan, Detroit): Two years ago we had an opportunity of reporting a similar type of case to this Society; a baby who received four intrauterine transfusions, starting at 22 weeks' gestation, who happened, fortunately, to be a female and who had received blood from male donors. We were able to show cells with XY sex chromosomal complement up to a ratio of about 8 % in this infant and were able to follow this child to about 11 months of age when the cells were gradually rejected. She was perfectly well until 11 months of age, with no difficulty at all, and as far as I know she is perfectly well up to the present time. At that time we were prompted to make calculations with Dr. BILLINGHAM regarding dosage of cells necessary for a 'graft versus host reaction'. Compared to animal experimentation the dosage of cells given was too small to possibly produce such a phenomenon. As it happened, in our case, being in the earlier days of intrauterine transfusions, the child had received whole blood with its total complement of white cells. In your case I would like to know what percentage of cells were donor cells. It seems to me that the data you have presented have all the earmarks of severe infection and/or hepatitis which caused the demise of this infant. The elevated immunoglobulins and lack of evidence for an immunological deficiency syndrome would seem to be the most important arguments against a 'graft versus host reaction'. However we define 'graft versus host reaction' in animals, all evidence at the present points to its not existing in the absence of infection, and the transference of this disease to humans has not yet been satisfactorily documented. Even in the well-authenticated case reported from our laboratories (KADOWAKI *et al.*, XX/XY lymphoid chimerism in congenital immunological deficiency syndrome with thymic alymphoplasia. Lancet, Dec. 4, pp. 1152–1156 [1965]), of a male infant showing persistence of maternal leucocytes verified by the presence of approximately 40 % of XX sex chromosome cells in his blood, it was not possible to say whether the runt disease like the clinical picture was the result of the thymic alymphoplasia itself with tolerance for the maternal leukocytes as an incidental finding or whether these leukocytes were in any way contributing to a 'graft versus host reaction'. It seems to me that of the two possibilities, 'graft versus host reaction' and infection, the latter has all to favor it as a sequelae of intrauterine transfusions. Embryological tissue forming an excellent nidus for the growth of viruses etc. would thereby make hepatitis and other infections a real threat. Why did your case require 6 exchange transfusions after birth?

J.L. NAIMAN: I am aware of your demonstration of persisting donor cells in infants following intrauterine transfusion, a finding that has been confirmed in Pittsburgh and other centers. Whether such lymphocytes maintain a peaceful coexistence or whether they react in an aggressive manner against the host lymphocytes is another matter, however. The factor determining which of these courses is followed can be found in the original prerequisites for the GVH reaction. As you recall, in order for graft lymphocytes to react against host lymphocytes, they must lack a transplantation antigen present in the host. I would suggest that such was not the case in your infant and therefore no illness resulted. Regarding the percentage of donor cells in our patient, this was impossible to determine because of the overlap in Y chromosome size from donor to infant. I would guess that there was a minority of donor cells. As far as hepatitis causing the hematologic reaction in our infant, we certainly considered this. As you know, hepatitis has been associated with aplastic anemia in a number of reports. Upon reviewing these, I was unable to find any mention of the striking histiocytic and phagocytic reaction that was seen in our patient. Furthermore the serum glutamic oxaloacetic transaminase activity in our patient was normal and there was no evidence of hepatitis at autopsy. Regarding the dosage of lymphocytes in donor blood and its potential for inciting a GVH reaction, I think it is difficult to compare mice and men. Dr. Billingham has seen our data and has since recommended the removal of lymphocytes from blood intended for fetal transfusion.

J.H. GITHENS (University of Colorado Medical Center, Denver): The authors have carried out a very careful study of this patient and have recognized a significant complication of intrauterine transfusion. I would like to speak in support of their diagnosis. This patient showed hematological and pathological findings that were very similar to those that we have observed in children with thymic alymphoplasia or thymic dysplasia that had graft-versus-host reactions following transfusion of blood containing viable leukocytes. The finding of numerous histiocytes in the marrow is very characteristic of the graft-versus-host reaction, as is the infiltration of the thymus and other organs such as the spleen with histiocytes. I would like to ask whether this baby may have had some form of thymic dysplasia, either congenital or acquired, and, therefore, was more susceptible to the development of the graft.

Dr. FUDENBERG has reported one child with a variant of thymic alymphoplasia who had absence of delayed hypersensitivity and did show Hassall's corpuscles in the thymus. I would like to ask one further question: How do you separate the small lymphocytes from the red cells for transfusion in utero or into children with thymic alymphoplasia?

J.L. NAIMAN: I wish I knew. This is an easy thing to recommend to others, as long as you do not have to do it yourself. There are various techniques that are recommended. One in the American Association of Blood Banks Technical Manual recommends a low speed spin for about 15 minutes with the blood bag upside down and harvesting the bottom 80 % of cells. In the few bloods we have tried this way there was no great reduction in the number of white cells and lymphocytes. Washed cells have been recommended, but

experience with this at the Philadelphia General Hospital has led to disastrous results, in that several of the fetuses have been born a few weeks later with severe hemorrhagic syndromes. The recent enthusiasm for frozen blood may bear fruit in this situation, in that it's quite likely that white cells are destroyed in such blood, although this is something that has to be tested.

J.M. BOWMAN (Winnipeg, Canada): I am just going to comment. We now have 26 survivors following intraperitoneal transfusion; 11 were initially transfused before 26 weeks' gestation. On two occasions we felt on clinical grounds that we might have some graft-versus-host reaction initially. These were babies with severe pulmonary infections. Both recovered, and we were unable to prove that we had any chimerism at all. We have tried to remove leukocytes by various types of centrifugation and red cell packing to the limit, squeezing off all the plasma, buffy coat and top layer of red cells, but we always wind up with about 3000 lymphocytes per mm^2 left in our blood. Because of our failure to find any evidence of chimerism, and because of the risk of infection, we have not washed our red cells at all.

J.L. NAIMAN: I think perhaps one of the reasons why we may not be able to demonstrate chimerism is that, as in our child, this may be a self-limited reaction with varying degrees of severity. If the child had recovered from the reaction it would not be possible to demonstrate chimerism. We have evidence to suggest this from our patient who did improve hematologically in association with rejection of the donor lymphocytes. Why he died, I am not certain. I am sure there were many other disturbances that followed upon the initial immunologic problem and that this predisposed him to the severe Pseudomonas infection terminally. The case that I mentioned from Italy, although it is not fully documented in the letter that we have, did experience a severe Pseudomonas infection at 3 months of age, from which he recovered.

35 *A Model for the Investigation of Host Resistance to Virus Infection.* LOWELL A. GLASGOW and STANFORD B. FRIEDMAN*, Univ. of Rochester Sch. of Med., Rochester, N.Y.

It has been recognized that males manifest a greater susceptibility to a number of infectious diseases. This study was initiated to develop a model in experimental animals which would permit: 1. an investigation of the determinants of this enhanced susceptibility; 2. an attempt to define the role of antibody and interferon in host resistance to virus infection. Male mice were significantly more susceptible to encephalomyocarditis virus (EMC) than matched females (32/45 vs. 16/46). Determination of the pathogenesis of EMC virus in both groups demonstrated that: 1. the course of the viremia was similar, although virus appeared to be cleared slightly earlier in the resistant female; 2. interferon levels in the serum were identical following infection and did not correlate with the clearance of virus; 3. clearance of viremia in both groups was associated with the appearance of neutralizing antibody; 4. virus replication occurred in the CNS at an identical rate; 5. there were higher titers of virus in the hearts of male mice although this was not reflected in the degree of myocarditis in pathology sections. The development of difference in susceptibility between sexes correlated with sexual maturation. Male castrates were as resistant as control females (9/26 vs. 6/26). Castration of female mice did not alter their normal pattern of resistance (8/26). Male or female castrates treated with testosterone both manifested susceptibility of control males (20/26 and 25/31) in comparison with control males and females (19/25 and 8/25). These data strongly suggest a function of testosterone as one determinant of host resistance, and that this variation in host resistance occurs in the absence of detectable differences in the interferon or immune response of the host.

Discussion

H.C. KEMPE (University of Colorado Medical Center, Denver): I am delighted that the EMC virus is coming back. We were assigned this virus 22 years ago, and it was one of the interesting models, because we thought we had found the cause of viral myocarditis, and then it turned out not to be the cause of viral myocarditis in man, but in chimpanzees. My question is: If I remember right our correlate was with central nervous system titers, and the best correlate we found, in terms of final best, related to the amount of virus in the brain, rather than in any other organ. You did show data on heart and pancreas, but not on brain. Would you tell us what that one was?

L.A. GLASGOW: I believe that the second slide illustrated the assay of virus from brain tissue, not pancreas. I am sorry if I did not make that clear in the presentation.

R.H. PARROTT (Children's Hospital, Washington, D.C.): You have me a little worried about next year's cold tablets. They might have estrogen in them. (Laughter) But I do wonder if you were able to study infant and toddler mice. It seems to me as pediatricians and as microbiologists interested in childhood viral illness, we do see a difference in the male and female predilection for croup, pneumonia, and many of the viral infections; and yet I do not think there are massive differences in the testosterone and estrogen levels in infancy and childhood. Would you comment on this?

L.A. GLASGOW: I think I can at least relieve your worry concerning estrogen therapy. In other experiments we have evidence that exogenous estrogen therapy actually enhances susceptibility of both male and female animals. With regard to your question of the susceptibility in suckling animals, we found that as we move back toward birth, the sex difference does tend to disappear; and that both male and female suckling animals manifest an enhanced susceptibility. We felt, therefore, that our initial studies would be more productive in the adult animal, where the greater differences in mortality occurred. We are interested in the problem of the development of resistance with maturity and do plan to investigate this more thoroughly in the future.

H.L. HODES (Mount Sinai Medical School, New York): I was very interested in Dr. GLASGOW's paper. It is beautifully controlled work. I would like to say something about the antibody. I think that even if you showed no quantitative difference, there may very well be qualitative differences. In man, for instance, we have shown in a study of polio antibody by radioimmunodiffusion, that all males have 90 % or less in the IgA globulin fraction. There are a group of females who have as much as two-thirds of their polio antibody as IgA globulin. This group is about 40 % of the adult female population. The remaining females have the same pattern as do males. Girls are similar to the adult women. However, the boy population is not like the adult male population, but is like the girl and adult female population. We believe the child male population is like the females, and that something happens at puberty to change this. This suggests that the production of IgA is controlled by hormonal factors which

modify a gene on the x chromosome. But the point I am making is that IgA antibody might be more effective or less effective in stopping the multiplication of the virus in the myocardium than is IgG antibody. I think that a qualitative analysis of the antibody might add very much interest to your study.

L.A. GLASGOW: That is an excellent suggestion. Thank you.

D.J. LANG (Massachusetts General Hospital, Boston): Frequently we study circulating antibody, although in viral infections, cellular factors may be at least of equal importance. I wondered whether you had an opportunity to study the cellular response to this infection in males and females, either in peripheral blood or perhaps the peritoneal cavity.

L.A. GLASGOW: We do not have, at the present time, any evidence of differences in the cellular response. The response of parenchymal cells to the virus infection, however, may be strikingly different. In the pancreas, for instance, the quantity of virus present in male and female animals, is identical during the fourth and fifth day following infection. The pathology sections of pancreatic tissue from infected females showed a mononuclear, interstitial pancreatitis with edema and minimal evidence of cell necrosis, while in the male there was a striking degree of cell necrosis. There is no difference in the peripheral white count or differential. There has been some evidence that EMC virus does not react with peritoneal macrophages. We have not studied this in detail other than to show that EMC virus is not cleared from the serum as rapidly as viruses such as Newcastle disease virus, which are cleared by the reticuloendothelial system. In addition, we have been unable to obtain evidence that EMC virus induces an interferon response in these cells.

S. BERKOVICH (State University of New York, Brooklyn): Like Doctors GLASGOW and FRIEDMAN, we have been and still are very interested in sex. Our studies have been done with a strain of Coxsackie B1 virus that has proved to be particularly virulent for the adult mouse. Approximately 15 % of our infected 'Swiss albino' animals succumbed. Following gonadectomy, the death rate for both sexes decreased to about one-third. The protective effect of gonadectomy, therefore, was not sex-linked. The administration of testosterone following orchiectomy did not alter the susceptibility of the test, male animals (BERKOVICH and RESSEL: Proc. Soc. exp. Biol., N.Y. *119:* 690 [1965]). The test virus was far more lethal to adult Charles River CD-1 mice. Moreover, twice as many male animals succumbed (65 % as compared to 27 % among infected females). Castration significantly increased the resistance of the male (death rate –32 %). By contrast, the resistance of the female was decreased (death rate –55 %). It is apparent that the virulence of the virus and the effect of gonadectomy on susceptibility were sex-determined characteristics among the CD-1 mice. Treatment of male and female castrates and normal females with testosterone significantly lowered resistance. However, almost all of the estrogen treated animals of both sexes succumbed to infection with the Coxsackie B1 test virus. The response of gonadectomy in the mouse is strain-controlled (CHAI and DICKIE: Biology of the laboratory mouse, p. 397 [McGraw-Hill Book Co. 1966]). It is our belief, therefore, that the variable results we have observed in different strains of castrated mice following Coxsackie virus infection may merely reflect the dissimilar genetic makeup of the animals studied.

J.W.ST. GEME, Jr. (UCLA Medical School, Torrance): Dr. GLASGOW has, with his colleague Dr. FRIEDMAN, presented again such very fascinating and excellent data. This is a complex system. Cognizant of Dr. BERKOVICH's comments and the recent literature of the past five years there has developed considerable confusion regarding the relationship of estrogen and testosterone to protection and enhancement *in vivo*. Evidently the macrophage does not seem to play a very significant role in this system *in vitro*. If one cultivates these cells or tissues, thusly converting your beautiful model *in vivo* to a simple model *in vitro*, and then relates very quantitatively the plaque-forming units of EMC virus to the number of host cells—one might evaluate the concept of receptor density, the rate of virus synthesis and, perhaps, interferon. Can you detect any differences *in vitro*, related to sex or gonadal steroids, which may involve biochemical phenomena such as enhanced protein synthesis, enhanced enzymatic activity, and ultimately increased virus synthesis?

L.A. GLASGOW: We would very much like to be able to develop and carry this model into an *in vitro* situation, where we could examine it in more detail. We have carried out just a few preliminary experiments, primarily consisting of plaquing EMC in the presence of various concentrations of testosterone. This did not seem to have any effect. I think, though, that these were pretty sketchy, preliminary studies, and we plan to go back and look at this in a little more detail.

36 *Prognosis in Patients with Ventricular Septal Defect and Severe Pulmonary Vascular Disease.* PATRICIA M. CLARKSON*, ROBERT L. FRYE*, JAMES W. DUSHANE*, EARL H. WOOD* and WILLIAM H. WEIDMAN, Mayo Clinic and Mayo Foundation, Rochester, Minn.

Patients with ventricular septal defect and severe pulmonary vascular disease are often not benefited by surgical closure of the defect. Little is known of the prognosis of such patients not operated on. Follow-up data were available on 55 patients who underwent cardiac catheterization at the Mayo Clinic before 1962 and had a proved diagnosis of ventricular septal defect and severe pulmonary vascular disease (pulmonary-to-systemic resistance ratio of 0.70 or greater). None had been operated on. Ages at the time of catheterization ranged from 3 to 57 years. None had congestive failure in infancy, all complained of fatigue and dyspnea, but none was severely disabled. All had physical findings of pulmonary hypertension, and 35 were cyanotic at rest. Five years after catheterization, 44 patients were alive; all but 2 of the 11 that died had been over age 20 when catheterized. Survivors were limited in activity, but none was severely disabled and all were in school or gainfully employed. Thirty-eight patients were alive 10 to 16 years after catheterization, and only seven of these were severely disabled. Longevity could not be related to degree of systemic arterial oxygen saturation or severity of calculated pulmonary resistance at the time of catheterization. Hemoptysis was found to be an unfavorable prognostic sign. These patients with severe pulmonary vascular disease lived longer and were more active than comparable patients who had surgical closure of the ventricular septal defect.

Discussion

A.M. RUDOLPH (University of California, San Francisco): I think this is an extremely valuable and im-

portant study. Many of us have felt for some time that it is undesirable to subject patients with severe pulmonary vascular disease and ventricular septal defects to surgery, and this certainly bears this out. I would like to ask one question: Do you have any information regarding where these people have lived? In other words, was the survival related to any altitude difference?

W.H.WEIDMAN: Of the 58 patients only two lived at an altitude of 5000 feet or above, and these two patients were living at time of follow-up.

J.I.E.HOFFMAN (University of California Medical Center, San Francisco): Could you tell us two things? First, what was the time of death of the 70 % who died after operation; that is, were they immediate operative deaths or were they delayed deaths? Second, do you think this difference in prognosis between operated and non-operated groups would hold if you were to talk about children under three years of age?

W.H.WEIDMAN: In answer to the first question, half of the patients died in the hospitalization period. The other half died during the five years following surgery. The point you bring up is an interesting one. Could one prevent pulmonary vascular disease by very early surgery? The answer is not yet certain. (Slide) In our experience, some patients with mild to moderate pulmonary vascular disease have progression of the disease after closure of the ventricular septal defect. It is difficult to know pre-operatively which patients will improve and which will not. In general I would certainly support the idea that a child with a significant ventricular septal defect should have the defect closed as soon as it can be done safely by the surgeon.

J.L.BRAUDO (Michael Reese Hospital, Chicago): I should like to ask Dr. WEIDMAN: What is the place of anticoagulants in the treatment of these patients who have not hemoptysis and whether it would not be advisable to put the youngsters on anticoagulants over a prolonged period of time.

W.H.WEIDMAN: None of the patients was treated with either heparin or other anticoagulant. I do not know whether such treatment is advisable or not.

J.LYNFIELD (New York University Medical Center, New York): Dr. WEIDMAN, do you have any data about progressive changes in the series of patients that you have studied by serial catheterizations to determine the rate of progression of the pulmonary vascular disease in those individuals who are already known to have it?

W.H.WEIDMAN: Yes, we do, and in some it does not seem to progress. In others it progresses very rapidly changing from a situation which we would consider operable at the age of four months to becoming an inoperable situation at the age of three years. Others seem to progress very slowly.

E.ROSSI (University of Berne, Berne): May I ask how many cases you have had with spontaneous closure of the ventricular septal defect, and what was the highest value of the pulmonary pressure in these cases?

W.H.WEIDMAN: I do not know how many we have had. Many of the spontaneous closures occur with the smaller muscular defects, and we do not study most of these patients, but we have had a number—and I do not know how many—who have closed in the smaller group. We have had at least one patient where the pulmonary artery pressure was 50 % of systemic that closed spontaneously, but I know of none in this group that have.

37 *Circulatory Changes in Newborns with Erythroblastosis Fetalis with and without Hydrops.* RODERIC H. PHIBBS*, PAUL JOHNSON* and WILLIAM H.TOOLEY. Dept. Ped. and Cardiovascular Res. Inst., Univ. Calif., S.F. Med. Ctr., San Francisco, Cal.

In 18 erythroblastotic infants—11 with moderate to severe hydrops (H) and 7 non-hydropic (NH), aortic (PAO) and central venous (PIVC) pressures—arterial pH and blood gas tensions were monitored serially beginning at 7 min (average) of life. Volumes (plasma T-1824; RBC Cr^{51}) were also measured in the first 2 hours of life, and were expressed as ml/kg non-edematous weight. All infants were prematurely delivered by cesarean section or after induced labor. Fetal distress and neonatal asphyxia were common in both groups. There was no significant difference in mean blood volumes between the 2 groups H = 96 ml/kg, S.D. 8.2; NH = 80 ml/kg, S.D. 10.9 (normal 91 ml/kg; S.D. 5.2).

Moderate to severe asphyxia was present in all 11-H and 3-NH infants—mean pH 7.15, PCO_2 66 and PO_2 45 mm Hg breathing 100 % O_2 at mean age 7 min. In this group, when PAO and PIVC were measured before correction of acidemia, both were elevated; when pH was raised intravascular pressures fell strikingly. Pressures became normal in 5 (3 NH, 2 H) and in the others (9 H) fell to subnormal levels which returned to normal only when blood volume was expanded therapeutically. In the 4 NH infants without asphyxia, 3 had normal PAO and PIVC, 1 had low PAO and PIVC which returned to normal with therapeutic blood volume expansion.

We conclude that the presence of H and a high PIVC is not necessarily an indication of heart failure, hypervolemia or need for phlebotomy. In H and NH erythroblastotic infants, elevated intravascular pressures may result from asphyxia and can be misinterpreted as evidence of heart failure unless properly evaluated. (Supported by USPHS Grant HE-06285.)

Discussion

N.M.NELSON (Children's Hospital, Boston): The relatively nonhydropic baby originally had a pO_2 of 99. Then you administered two separate doses of alkali which changed the pH from 7.1 to 7.2, but his pO_2 went down to 49 mm Hg. I wonder if you could interpret that for us, and tell us whether you think this is related to the Bohr shift or increased venoarterial shunting.

R.H.PHIBBS: The blood pressure measurements suggest that there is both pulmonary and systemic vasoconstriction associated with moderate asphyxia in these infants and that the vasoconstriction may be relieved by correction of the acidosis. If the systemic vasoconstriction were relieved more rapidly than the pulmonary, then arterial pressure in the systemic circulation would fall to a normal level sooner than in the pulmonary circulation and transiently 'right to left' shunting of blood through either the foramen ovale or the ductus arteriosus or both would be facilitated by these pressure differences. In the infant illustrated on the first slide who was, incidentally, only moderately asphyxiated but distinctly hydropic this appears to be the explanation for the transient fall in arterial oxygen tension. You will recall that the central venous pressure did not reach a normal level until about 15–20 minutes after the aortic pressure did and it was during that period that the arterial oxygen tension fell. After this period the arterial oxygen tension rapidly rose to a much higher level. I did not put the values for oxygen saturation on the slide; however, the early fall in arterial oxygen tension is due to decreased saturation and not just to the Bohr effect as the pH was raised as you suggested.

J.M. Bowman (Winnipeg, Canada): I would like to ask Dr. Phibbs how many of his 24 fetuses with hydrops fetalis ultimately survived. And I do not believe at any time that he mentioned the hemoglobin levels of his hydropic babies. We have not carried out blood volume studies on the four liveborn hydropic babies, delivered after intrauterine transfusion that we have managed. However, one of them and another infant, prehydropic, with a cord hemoglobin level of 2.8 g %, both with severe respiratory distress, had very marked increases in portal venous pressure. They were both intubated initially and carried on positive pressure artificial respiration, but were extubated promptly. Both of these babies at the end of seven and six exchange transfusions respectively were left with volume deficits of 350 ml, two and a half times the expected blood volume of normal babies at their gestational age (34 weeks). We felt that these infants certainly did have very markedly expanded blood volumes. We removed the relatively large amounts of blood and they survived.

R.H. Phibbs: What we are reporting is our physiological observations on a group of infants who were managed in various different ways immediately after birth. Based on these observations we think we have a better idea of what optimal care should be during this period, but we are not reporting on the success of such a program of management since not all of these infants were handled in the same way. Taking the group as a whole between 1/5 and 1/6 of the hydropic infants survived. To date, however, our experience has been that it is those who have not had large phlebotomies at birth that make up the majority of the survivors. I would hasten to add that just because we have a clearer understanding of the circulatory derangement of these babies it does not follow that we should expect a sudden decrease in the overall mortality rate in hydropic newborns. Obviously this is only one part of the pathophysiology of hydrops and as we deal with the hydropic infant more adequately immediately after birth, I expect more of these infants will live long enough to manifest other complications of the disease which we have only rarely encountered before. Regarding the cord hemoglobins these vary considerably with the lowest ones falling just under 3 g % as in the infant which you just described. There was not time to show a plot of cord hemoglobins vs. blood volume, but there was no statistically significant correlation between the two which was interesting because it was different from what had been reported by others in the past.

Audrey K. Brown (Medical College of Georgia, Augusta): Dr. Phibbs, after you deal with the asphyxia, how do you deal with the anemia? Do you use packed cells or do you subject them to exchange?

R.H. Phibbs: These infants initially had small exchange transfusions with packed cells, keeping the blood volume constant. And I might add, we did not even change the blood volume during the exchange. We had two catheters which were, of course, responsible for the hematocrit.

Audrey K. Brown: How much did you use?

R.H. Phibbs: Whatever it took. In some cases 75 to 100 cc.

J.F. Lucey (University of Vermont, Burlington): These observations are extremely important. In hope that they will enable us to improve the survival rate in severe hydropic fetalis. The estimated mortality rate in this group of infants treated with a conventional exchange transfusion is about 90 %. You have demonstrated they have a normal blood volume and severe acidosis. Certainly it no longer makes sense to recommend that these infants be phlebotomized. This procedure and the failure to correct the severe acidosis may well have been responsible for the high mortality rate in the past. Several years ago Dr. Edith Potter commented in her book that she thought these infants actually had small blood volumes. We should have paid more attention to this observation.

R.H. Phibbs: That is right. We just recently came across Dr. Potter's statement in her textbook.

G.B. Odell (Johns Hopkins University, Baltimore): I think it is of interest that you may have at the time of birth a small circulating blood volume; but I would hasten to add that as you exchange with donor blood, you suddenly change the baby's circulating plasma proteins from something under 3 g % to something close to 6 g %. The real importance of phlebotomy is that during exchange the baby will start reabsorbing fluid from his extravascular spaces, particularly the pooling sites of ascitic fluid. This will greatly expand blood volume and result in cardiac overload. Phlebotomy has to be done more often in the middle of the exchange than at the beginning of it.

R.H. Phibbs: I think this is a very pertinent point you have raised. The observations we have presented in the first 20 or 30 minutes of life were before there was sufficient correction of plasma proteins to cause a major redistribution of body fluid. The point you raised suggests why it is also valuable to continue this sort of monitoring of intravascular pressures after the initial period of resuscitation. Our own experience has been that as fluid is drawn into the intravascular compartment due to correction of the plasma proteins, the central venous pressure does not rise significantly. Perhaps this is because as the plasma protein concentration is raised and edema fluid mobilized, a brisk diuresis commences.

Audrey K. Brown: Since you are using a small packed red cell exchange, how are you correcting protein in the absence of plasma?

R.H. Phibbs: We used a small exchange with packed cells initially; however, this was followed by larger exchange transfusions with whole blood later on and this then raised the plasma proteins as Dr. Odell pointed out. In some instances in which hypoproteinmia and edema were severe, we also used salt-poor albumin early.

R.C. Neerhout (UCLA, Los Angeles): I was wondering if you can be certain that in these severely ill infants the blood volume measurements are indeed accurate, or whether there could be sequestration or poor mixing. Have you followed any of these after you have corrected their metabolic aberrations and rechecked the blood volume to see if it is still small, or do they show a difference as far as effective circulating blood volume after you have corrected the metabolic abnormalities?

R.H. Phibbs: This is a technical point which is quite important. If an infant has poor peripheral circulation and 'pooling of blood' as you suggested it may take much longer for complete mixing of the red cell and plasma tags and also in such infants there may be an increased rate of loss of the plasma tag from the intravascular compartment. These can produce errors in measuring the blood volume in such infants. The only way to avoid these errors is to take multiple samples after injecting the tags so that one can see when mixing is complete, then get the rate of loss of the plasma tag after complete mixing and extrapolate that to the concentration at time zero. These technical details may

explain the discrepancy between our findings and some of the older reports describing large blood volumes in hydropic infants. In some of the older studies, the plasma tag was injected and a single sample taken rather late. In the severely ill infant mixing of the tags in the circulation was quite slow and the rate of loss of the plasma tag from the circulation was often accelerated. When blood volume measurements were repeated later, the blood volumes were remarkably similar to the early measurement, but the rate of mixing of the tag was much faster. In other words, as you suggested, initially there was pooling as indicated by the slower rate of mixing, but this did not seem to introduce an error in the measurements when appropriate precautions were taken.

38 *Cortisone-Induced Growth Failure in Neonatal Rats.* MYRON WINICK* and ANTHONY COSCIA*, Cornell Univ. Med. Col., New York N.Y. (introduced by W.W. McCrory).

Growth failure in neonatal rats following a single injection of cortisone has been ascribed to thymic destruction. However, specific early degeneration of thymus gland has not been demonstrated. Moreover, the cellular changes in other organs accompanying this growth failure are unknown. Within 24 hours of injection there is marked destruction of thymic and splenic cells as evidenced by a 50-fold reduction in DNA content. Beginning about the 5th day following injection, DNA, RNA and protein content is proportionally reduced in 7 other organs studied. Thus protein/DNA and RNA/DNA ratios remain normal. All organs, therefore, contain fewer cells of normal size. By three weeks of age, thymus and spleen have partially regenerated and show reductions in DNA, RNA and protein in proportion to other organs. In male animals these differences persist to adulthood; in females only to puberty. Thereafter in the female, experimental values for DNA, RNA and protein gradually approach and then finally reach control values. These data demonstrate that the initial event after injection of 1 mg of cortisone in 2-day-old rats is thymic and splenic destruction. This is followed by partial regeneration of these organs. Later cell division is slowed in the other organs, resulting in a stunted animal whose organs contain fewer cells of normal size. This stunting persists in the male and disappears in the female after puberty.

Discussion

F. KENNY (Children's Hospital of Pittsburgh, Pittsburgh): Were the adrenals of the treated animals altered in size or in histological appearance when compared to the normal controls?

M. WINICK: If you are referring to histological changes, we do not know. We have not looked at them histologically. However, in the experiments done by SHAPIRO where the experimental technique was essentially the same as ours, they did note histological changes, i.e. suppression of the adrenals.

L.I. GARDNER (State University of New York, Syracuse): This comment is a corollary on the one just brought up about the adrenals. In mice, voles, golden hamsters and shrews there is a special juxtamedullary adrenal zone which is generally called the X zone. The X zone regresses in male mice before puberty, but in females it progressively enlarges; at the first pregnancy there is rapid regression. In unmated females the X zone gradually regresses (DEANE, H.W., ed: The adrenocortical hormones, Handbuch der experimentellen Pharmakologie, pp. 47–48 and 593–598 [Springer, Berlin 1962]). In cats there are some similar changes between sexes in the adrenal reticularis (Ibid. p. 598). It is possible that the interesting sex differences found by Drs. WINICK and COSCIA in neonatal rats in response to cortisone injection may somehow be connected with the previously reported sex differences in adrenal-cortical histology in small mammals. I was wondering if you had an opportunity to see whether there was any correlation between the previous histological observations relating to known sex differences in the appearence of the adrenal cortex of small mammals and your experimental work?

M. WINICK: No, Sir, we have not looked. I did not know about the difference in appearance, and we will look in the future.

A. DRASH (Children's Hospital of Pittsburgh, Pittsburgh): Cortisone is not a physiologically occurring steroid in the rat. It would appear very important to know whether your studies are simply pharmacologic in nature or whether they may have some physiological application. In order to determine the physiological significance of your observation, it would seem necessary to carry out these studies using the naturally occurring glucocorticoid compounds found in the rat. Have you carried out such studies?

M. WINICK: No, we have not. But we are not saying or even trying to intimate at all that this is physiologic. The dose, 1 mg, in a newborn rat is very, very high. It is a massive dose. So we believe this is one means of pharmacologic injury.

D.B. CHEEK (The Johns Hopkins University, Baltimore): Of particular interest to me is the demonstration that the female rats showed 'catch up' growth. In our studies on human muscle and cell growth we became aware, some years ago, of a distinct sex difference both with respect to the increase of size of muscle cells and cell number increase. And while in the human the emphasis on muscle cell number increase is more remarkable than in the rat, we still find similar sex differences in the growth of muscle tissue in rats. The increase in the number of muscle cells in the female is less remarkable than in the male during growth. This may place the female in a more advantageous position for subsequent 'catch up growth' if an early insult presents. In view of the fact that other steroids when injected into rats early in postnatal life (e.g. androgens into female rats) can alter the subsequent pattern of growth (sex type), I wonder if you would speculate that some alteration in the programming of DNA within the cell has resulted here.

M. WINICK: No, I neither have any ideas, nor would I speculate. But thank you for your comment.

W.B. WEIL, Jr. (University of Florida, Gainesville): Along that line, Dr. WINICK, what about muscle? It is a major constituent of the body in terms of mass.

A. WINICK: Muscle acts essentially the same way as heart—at least in the muscles that we have studied.

J.A. GRUNT (Yale University School of Medicine, New Haven): I wonder if you have had a opportunity to study the length of the animals that you have been looking at, particularly during the period prior to weaning; and then for how long subsequent to weaning have you looked at them this way?

A. WINICK: Again, we did not do this. This has been done, in the experiments of SHAPIRO. The problem was that we did not know we were going to run into the sex difference. Had we known we would have followed the length.