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ABSTRACTS AND DISCUSSIONS

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PLENARY SESSIONS

Modification of behavior and disease susceptibility in the offspring of mice pregnant while living in a crowded "mouse city." S. B. FRIEDMAN, L. J. GROTA, and R. ADER, *Univ. of Rochester Sch. of Med., Rochester, N. Y.* (Intr. by R. J. Haggerty.)

An apparatus consisting of multiple interconnecting chambers and tunnels will be illustrated in which mice were allowed to breed until food and space limited the population. When this population level was reached, pregnant females ready to deliver were removed from the apparatus. Control pregnant mice were housed in standard laboratory cages. All offspring were cross-fostered to another set of control mothers and later housed four to six per cage. From 6–8 weeks of age these offspring were subjected to two standard measurements of behavior, emotionality as measured by the open field and exploratory behavior as measured by the cage emergence test. The 27 offspring of mothers living in "mouse city" were significantly more emotional and more prone to emerge from an open cage than the 20 offspring of control mothers. At 13 weeks of age, all offspring were inoculated with 1×10^6 *Plasmodium berghei* organisms to test their susceptibility to a disease pathogen. The offspring derived from mothers pregnant while living in "mouse city" died significantly earlier than the offspring of control mothers. These data suggest that "mouse city" is useful as a model to study the effects of dense and competitive populations upon the behavior and disease susceptibility of offspring born to mothers living in such environments.

Discussion

FREDERIC M. KENNY (*Children's Hosp. of Pittsburgh, Pittsburgh, Penna.*): Was there any difference in the birth weights of the pups in "mouse city"? Did they have mouse intrauterine growth retardation?

DR. FRIEDMAN: No. There was no difference at birth, as far as we could determine. At weaning there was a slight difference, with the "mouse city" offspring being a little less than 1 g heavier.

EDNA H. SOBEL (*Albert Einstein Coll. of Med., Bronx, N. Y.*): I am puzzled about the details of the second set of experiments that you described. You had many pregnant mice in each cage. How did you deal with the pups at the time of birth?

DR. FRIEDMAN: When the animals were estimated to be in their 19th day of pregnancy, they were removed to an individual cage, the same as the "mouse city" pregnant animals. It was only in retrospect that we were sure that we removed them at the right time, and this accounts for not using all of the litters that are born. We do this instead of examining for vaginal plugs to determine time of pregnancy because this may lead to difficulties from the behavioral point of view.

WILLIAM B. WEIL, JR. (*Michigan State Univ., East Lansing, Mich.*): What would have happened if you had cross-fostered your "mouse city" mice on control mothers, and cross-fostered the control mice on "mouse city" mothers and on control mothers, so that your major variable then would have been prenatal experience. Currently you have both postpartum and prepartum problems in your experimental design.

DR. FRIEDMAN: That is right. The difficulty has been to get sufficient pregnancies out of the "mouse city." We are still trying this, and you are correct that a "cross-over" study would be desirable.

DR. SOBEL: Have you considered the possibility that increased adrenocortical function, which Cristian has demonstrated in overcrowded populations, was a factor in the susceptibility of your animals to infection?

DR. FRIEDMAN: We hope to pursue in the very near future two lines as to the possible physiologic mechanisms underlying these differences in susceptibility. One is adrenal function. The other is the role of the spleen, which we have found has a role in the difference in resistance noted in animals housed alone or in groups and infected with *Plasmodium berghei*.

BARBARA M. KORSCH (*Childrens Hosp. of Los Angeles, Los Angeles, Calif.*): Have you excluded the hypertension aspect of the problem, because some years ago Dr. James Henry, on the basis of his "mouse city" experiments, approached me about doing a small study in human infants. We interviewed to try to determine the stresses in the infants' environments, and he measured the blood pressure in these infants.

It was a small series, and because of great technical difficulties we never published it, but there were some indications that under these conditions human infants might also develop systolic hypertension as did those mice.

DR. FRIEDMAN: I have seen that manuscript, which has stimulated us. We are planning to start such a study and have done some of the preliminary work, including the use of the apparatus that you described, that Dr. Henry was kind enough to make for us.

We will be measuring blood pressure in human infants in the

home, with independent measurements of the psychologic and social factors going on within the household. We take no credit for this design. As you say, this is something that you and Dr. Henry previously have done, but we would like, with some modifications, to replicate those findings.

In terms of the animal work, we are currently trying to replicate the blood pressure changes, but are having technical difficulties in the sense of keeping the mouse quiet while we make these blood pressure measurements. I know that it can be done, because I have spent some time in Dr. Henry's laboratory, but eastern mice seem to be a bit more jumpy.

ALLEN S. GOLDMAN (*The Children's Hosp. of Philadelphia, Philadelphia, Penna.*): I wonder whether you noticed any congenital malformations in the offspring, or any abnormality in litter size or fetal weight. When I was studying malformations produced by salicylates in rats, I observed that malformations were produced by very low nonteratogenic doses of salicylate if the mothers were treated with the immobilization stress of being tied down for a number of hours after the injection of salicylates. During the course of these experiments we also used crowding as a stress. I found that malformations could also be obtained with nonteratogenic doses of salicylate when after injection more than nine mothers were crowded in a standard size rat cage. Maternal isolation was even more effective in enhancing this production of malformations by salicylate. A population size of three to seven per cage seemed to be all right but a population size of either above eight, or only one per cage, seemed both to raise susceptibility to malformation by salicylate and to enhance the capacity of salicylate to produce markedly undersized fetuses (Goldman, A. S., *et al.*: J. Pharmacol. Exp. Ther., *142:* 351 (1963)). I wondered whether malformations or fetal growth retardation may occur in the absence of a poison.

DR. FRIEDMAN: No, they were not underweight, and we did not observe any anomalies, but, again, we deliberately tried to keep observations and handling at a minimum to avoid extra stimulation.

You bring up one of the most fascinating aspects of the study, that is, the interaction between physical and psychologic factors. I think that the studies that are going to be most helpful from the clinical point of view are those which are going to take into account physical challenges as well as psychosocial ones.

We are limited, having only one "mouse city." These experiments are long term, and we have four more now building. This should speed our work along considerably.

We have introduced a famine in this city now to determine how the animals as a group adapt to curtailment of half of their food.

S. DOUGLAS FRASIER (*Los Angeles County General Hosp., Los Angeles, Calif.*): Have you looked independently at the effects of crowding and nutrition? You stated that at the end of 24 hr all of the food was gone. Have you conducted experiments where you fed several times during the day, so that there was a surplus of food at the end of 24 hr?

DR. FRIEDMAN: Yes. If one does not pay particular attention to maintaining these animals 7 days a week, you can get results that are hard to explain.

The population stabilizes in this particular apparatus at about 200 animals with about 720 g food. If you provide unlimited food by expanding the hoppers, the population then settles out at a little over 320 animals in this same apparatus. You can limit the population by space if the food is unlimited, or if you limit the food at any level, you can stabilize the population.

These animals stabilize at roughly the same weight as animals that would be raised in traditional cages. It seems that the mice have more wisdom than some of the higher orders, in that they will limit their population, albeit not by a very nice method, so that they can all stay relatively healthy.

Evidence for a sensitive period in human mothers of full term infants. MARSHALL KLAUS, JOHN KENNELL, RICHARD JERAULD, NANCY C. KREGER, WILLIE MCALPINE, and MEREDITH STEFFA. *Case Western Reserve Univ. Sch. of Med., Cleveland, O.*

To determine whether an early critical period exists which affects later behavior, we placed 28 primiparous women in one of two study groups shortly after the delivery of their normal full term infants. Fourteen mothers (routine contact) had the usual physical contact with their infants: a glimpse shortly after birth, brief contact at 3–12 hr, and then 20–30 min every 4 hr for bottle feedings. Fourteen mothers (extended contact) were given their nude babies for 1 hr within the first 3 hr after birth and then had 14 hr of contact in addition to the usual routine during the first 3 days. The mothers' socioeconomic backgrounds, marital state, ages, days hospitalized, attention and time provided by nurses, and infants' weights were similar. Maternal behavior was measured 28–32 days later during (*1*) a standardized interview of the mother, (*2*) an examination of the infant, and (*3*) a time lapse filmed bottle feeding. Extended contact mothers were significantly different from routine contact mothers ($P < 0.002$): more reluctant to leave the infants to the care of anyone else, usually stood and watched during the examination, and showed greater soothing behavior and sensitivity to the babies' cries. In the analysis of behavior using 600 frames from each feeding film, extended contact mothers showed significantly greater ($P < 0.05$) eye to eye contact (8.9 *versus* 2%) and fondling, *e.g.*, stroking and kissing (6.9 *versus* 1.9%). These studies suggest that simple manipulation of care procedures in the early hours following delivery may significantly alter later maternal behavior and are support for a critical period in the human adult.

Discussion

C. HENRY KEMPE (*Univ. of Colorado Med. Ctr., Denver, Colo.*): Does Dr. Klaus think that maybe one of the critical areas is the first 30 min of life? The newborn baby is wide awake at birth, but then tends to go to sleep, say after 30 min, and for the first 2 or 3 hr after that will not be very responsive to eye to eye contact.

DR. KLAUS: We have been especially interested in these first minutes, and, as you have noted, there is beautiful matching. The infant is awake and has an ability to follow objects with his eyes as far as 180°. When we recorded on audiotape mothers' responses during the first hours, they were intensely interested in the infants' eyes and were upset and frustrated whenever the baby was sleepy and the eyelids were closed. Several mothers have told us that they really did not believe the baby was theirs until he looked into their eyes.

We have noted, however, that it may take several minutes for a mother to pull herself together following delivery before being able to take on the baby.

The other part of the question is: what is the proper term for this period? It is tempting to consider that this is a form of imprinting or critical period for an adult human. However, this process does not fit the precise definition. Imprinting has only been defined as occurring in an infant animal. This does appear

to be a special attachment period for the mother. We are going to have to find another term for it.

FERNANDO J. DECASTRO (*Cardinal Glennon Memorial Hosp., St. Louis Univ., St. Louis, Mo.*): Could you tell whether these mothers were aware that they were being filmed?

DR. KLAUS: All mothers that we study know that they are being filmed. We have no secrets.

DR. PRINZNETTER (*Chicago, Ill.*): Have you had any experience with mothers who belong to the La Leche group, who are conditioned prepartum to the type of thing that you are talking about? Some of these mothers insist upon nursing their baby in the delivery room.

DR. KLAUS: In this study we controlled the admissions so that the mothers, to the best of our knowledge, came from the same population. While designing this study, however, we did have an opportunity to observe other individual mothers.

If a mother had previously lost a baby, and she had no living children, we have noted in a small sample that she is much more active in the first minutes with her infant, and extremely excited. The mothers that you talk about, who are awake during the delivery, participate in the delivery, and nurse immediately after, are similar to the mothers who have lost a previous infant.

DR. MCFARLANE (*Boston, Mass.*): Did you note the drugs or medications given to the mothers during the labor?

DR. KLAUS: Both groups of mothers had the same amount of premedication.

DR. COCHRAN (*Boston, Mass.*): I hope that your study will change the pattern of how obstetricians tell pediatricians they must conduct newborn services.

What was the reaction in those mothers whose husbands participated or wondered what was going on? Did you have any husband interaction?

DR. KLAUS: First, we agree that other populations should be studied.

We have had difficulty with fathers, because in any study it is obviously much simpler to have two people to study—the mother and infant. The fathers understood that this was a period of time when the mother would be with the baby. The study of fathers poses a number of research problems in that there are three people, and it is another order of complexity.

JULIUS B. RICHMOND (*Judge Baker Guidance Ctr., Boston, Mass.*): I would like to congratulate Dr. Klaus and his group for the persistence with which they pursued this problem of a mother's tie to her child. They have given us only a small indication of the technical difficulties in studying such problems.

How are you doing concerning the study of these mothers and babies following the 1-month period? The reason that I raise this question is that in an organism with the long period of dependency between baby and mother that we see in man the concept of critical period may not be a very good one. We need to be cautious about the significance of this very early brief period, and the early differences in terms of the long range implications for the infant.

On a humane basis, and on the basis of the data presented thus far, I support this kind of program, but I think that it is important that we remain aware of the fact that these differences may wash out.

DR. KLAUS: Your point is well taken. My wife is always telling me that she wants to know what the babies are like at the end of a year, and so, to satisfy all of the investigators and my wife, this summer we are planning to restudy all of the mothers and infants at 1 year of age. It is very important to know what happens to these mothers. Will the mothers continue to care for their infants differently, and what will the infants be like?

HERBERT C. MILLER (*Univ. of Kansas Med. Ctr., Kansas City, Kans.*): There was a study done in Buffalo by a group of psychologists who showed that premature babies in the period immediately after birth gained weight more rapidly if they were handled physically, when compared with a control group that had less handling.

Our psychologists have repeated this study in the past year and their preliminary studies confirm those reported by the Buffalo group.

Did you notice any differences in gaining of weight in the two groups?

DR. KLAUS: We examined the infants at 1 month of age. Surprisingly, the control group gained slightly more weight, but, statistically, it was just significant. If you observe the feedings, there may be a possible explanation. In the midst of a feeding the mothers who have had early and extended contact will break the feeding and often start shaking, kissing, and hugging the baby, as you observed on the film. With a few, it is hard to see how the baby was able to feed.

ROBERT E. GREENBERG (*Chas. A. Drew Postgraduate Med. Sch., Los Angeles, Calif.*): I am also troubled by the implication of biologic sensitivity period.

DR. KLAUS: We agree with your caution; however, the mothers in the control group had their babies at home 24 hr a day from the 4th day on, and yet they were different at 1 month from the mothers given only 16 extra hr of contact in the first 3 days of life. Again I would like to say that "critical period" may not be the proper word for this time period; however, it does appear to be special.

Urea production rate in the mammalian fetus and its metabolic implications. EDWIN L. GRESHAM, ELIZABETH J. JAMES, JOHN R. RAYE, EDGAR L. MAKOWSKI, GIACOMO MESCHIA, and FREDERICK C. BATTAGLIA. *Univ. of Colo. Med. Ctr., Denver, Colo.*

It has been assumed that the fetus, like the newborn, is in a state of positive nitrogen balance and, therefore, produces a relatively small amount of urea. To test this hypothesis, pregnant ewes were prepared with indwelling fetal and maternal catheters. The urea concentration difference between fetal and maternal arteries was consistently positive. Antipyrine and ^{14}C-urea were infused into the fetus approximately 1 week postsurgery, and the placental clearance (PC_u) was found to be 19.6 ± 1 ml/min·kg fetal weight (mean ± SEM) in seven animals. The PC_u was also calculated from the total accumulation of infused ^{14}C-urea in the fetus and liquors. The two methods agreed within ±5%. Since the primary route of fetal urea excretion is via the placenta, in the steady state the transplacental excretion rate approximates the rate of fetal production and is about 1 g/24 hr·kg, a value in considerable excess of an adult animal's renal excretion of 0.2 g/24 hr·kg. The ability of the fetus to synthesize urea was confirmed by a marked rise in the urea production following an infusion of NH_4 lactate into the fetus. A large urea production rate in humans was suggested by the urea concentration difference between fetus and mother of 2.5 ± 0.2 g/100 ml (mean ± SEM) in 14 patients studied. If most of the urea produced is derived from amino acids and/or protein, it would indicate that those compounds serve as a major metabolic fuel for the fetus and would support previous evidence that glucose utilization accounts for only 50% of the O_2 consumed by the fetal sheep.

Discussion

DR. ABEL (*Minn.*): Your opening statement referred to an RQ of 1 for the fetus. Can you reconcile that with your present data in terms of growth, since starch leads to RQ's of 1.3 as it is being converted to fat.

DR. GRESHAM: A more precise answer to your question will be covered in subspecialty section "Child Development II" by Dr. Elizabeth James. She is presenting the data on our study of the respiratory quotient (RQ) and glucose consumption by the sheep fetus. We give these figures on RQ for comparison with the findings of earlier investigators and not necessarily as an indicator of the metabolic fuels being utilized. As you imply, the interpretation of RQ is much more complex in a growing organism where a part of the carbon skeleton, of glucose, for example, may be incorporated in various synthetic pathways and the oxygen freed for other purposes.

CHARLES R. SCRIVER (*Montreal Children's Hosp., Montreal, Quebec, Canada*): I would like to ask you whether you plan to do any studies on the alanine cycle. Cahill and others described the alanine cycle as a major source of carbon chain flux from peripheral to splanchnic tissues, for purposes of fuel metabolism and also for delivery of ammonia. Perhaps this would be of importance to your observations.

DR. GRESHAM: We are extremely interested in the relative importance of individual amino acids in fetal nutrition and are preparing to investigate specific amino acid concentration differences across the umbilical circulation. Hopefully, in the future, we will be able to define more precisely which amino acids are more important to the developing fetus. Unfortunately, we have no information available at this time.

RICHARD E. BEHRMAN (*Univ. of Illinois Coll. of Med., Chicago, Ill.*): I really think that someone should congratulate the group. This is a major contribution, a very elegant study whose results are consistent with what one might expect. We know that the fetus does so well in manufacturing protein there is really no reason not to anticipate that the fetus would catabolize protein as well.

The mitigating effect of addiction to heroin on neonatal jaundice.

MICHAEL I. COHEN, GERALD NATHENSON, HELEN MCNAMARA, and IRIS F. LITT. *Albert Einstein Coll. of Med., Montefiore Hosp. and Med. Ctr., New York, N. Y.* (Intr. by Laurence Finberg.)

Based on a retrospective clinical appraisal, minimal to absent jaundice among 50 infants born to heroin-addicted mothers was noted. The possible role of a narcotic modifying the normal metabolism of bilirubin in the neonate was therefore considered. In a prospective study, 18 infants were evaluated over a 96-hr period by serial determinations of serum bilirubin. Fourteen of these neonates manifested moderate to severe symptoms of the abstinence syndrome while four were mildly affected. Forty-nine bilirubin determinations were carried out and the mean bilirubin levels achieved were as follows: 24 hr, 3.7; 48 hr, 5.4; 72 hr, 5.4; 96 hr, 6.7. These infants were free of any disease except for symptoms related to maternal heroin addiction. The normal excretory pathway for morphine is hepatic conjugation. Chronic narcotic use may stimulate the development of endoplasmic reticulum, enhance glucuronide conjugation, and so lower bilirubin values. To explore this possibility 75 adult male Swiss-Webster mice were addicted over 12 weeks, utilizing daily intraperitoneal injections of morphine sulfate. Saline injections were administered to a control group. Animals from treated and control groups were sequentially killed and bilirubin glucuronyl transferase activity was determined in liver homogenates. A statistically significant increase in transferase activity was noted in the addicted mice. These data suggest the possibility that low levels of bilirubin in neonates with the abstinence syndrome result from the enhancement of glucuronyl transferase activity through prolonged maternal heroin use.

Discussion

ANDREW SASS-KORTSAK (*Hosp. for Sick Children, Toronto, Ontario, Canada*): This is an interesting and important study. I want to ask a question which relates to the mechanism of jaundice in your newborns. There are probably five distinct steps in the handling of bilirubin by the hepatocyte: transfer through the cell membrane, intracellular transport to the site of glucuronidation, coupling with glucuronic acid, transport of bilirubin glucuronide to the site of excretion, and excretion from the cell through the cell membrane. With regard to this it would be important to know whether the blood levels of direct reacting bilirubin were elevated in your newborns. In this respect, using reliable methods, one would consider direct reacting bilirubin levels over 1.0 mg/100 ml abnormal, and levels over 2.0 mg/100 ml as very definitely abnormal.

DR. NATHENSON: No, we did not have any babies in the group that we prospectively studied with significant amounts of direct reacting bilirubin. However, the total amounts of bilirubin were also rather low. Most of the values of direct reacting bilirubin were less than 2 mg/100 ml.

RUTH C. HARRIS (*New York, N. Y.*): Is there a deleterious effect of the morphine on the animal livers? Was there any evidence of morphologic disturbance other than the reticulum changes in relation to damage to the liver?

DR. NATHENSON: No. We did not observe this on electron microscopy.

DR. BARAKIS (*New York, N. Y.*): Did the control take the same medication, and did you check whether the mothers were addicted to other medication?

DR. NATHENSON: Yes, the control infants were not on medication. To the best of our knowledge the mothers of the addicted infants were all heroin users, and we had no information that any of them had also used barbiturates.

DR. EATON (*Montreal, Canada*): I was a little startled to learn of treating withdrawal symptoms in an infant from opiates with camphorated tincture of opium or paragoric. One would think that one would treat it directly with morphine intramuscularly or with methadone given by mouth.

That is not the point that I want to make, though.

The point that I am interested in bringing across is whether this would be a real situation in the infant, because the dosages that you were using are great dosages, 250 mg morphine sulfate/kg in your mice, whereas the standard therapeutic dose of morphine in an adult of 70 kg would be around 10–15 mg, for example.

In an addict it probably goes up higher, and what I was curious about was whether you had attempted to measure the actual morphine blood levels of the infant.

DR. NATHENSON: No, we did not attempt to measure morphine blood levels of the infants. I would point out, however, that in the mice studies, although the levels were quite appreciable, we began to see significant differences in the spread of glucuronyl

transferase activity at levels that were somewhat lower. The ingestion of as much as 600 mg morphine/24 hr in adult addiction has been described. It is almost impossible to establish the quantity of narcotic ingested by heroin users because of the wide variability of active material in the packets consumed.

AARON R. RAUSEN (*Mount Sinai Sch. of Med., New York, N. Y.*): Have you had the opportunity, either in the mouse or in man, to measure the residual bilirubin-binding capacity of the plasma?

DR. NATHENSON: Yes. In one instance, residual bilirubin-binding capacity was found to fall within normal limits when measured by the 2-hydroxybenzeneazobenzoic acid method.

DAVID L. INGRAM (*Yale Univ. Sch. of Med., New Haven, Conn.*): Why did you compare adult mice with infants of mothers who had taken heroin, rather than injecting your morphine into pregnant mice and comparing the liver function in the fetal mice?

DR. NATHENSON: These were still young mice whose base line transferase activity continued to increase during the period of study, but, true, were considered adults nonetheless. We have considered addicting pregnant mice, but have not yet attempted this.

DR. COCHRAN (*Boston, Mass.*): One thing that interested us in our collaborative study was that the dysmature infant had a lower bilirubin level than comparable infants of equal gestation.

Did you take any other infants as controls who you thought were dysmature, but who had not had any morphine addiction in their mothers, and see what kind of levels they got?

DR. NATHENSON: No, we did not. We just analyzed term infants.

The "bronze baby," a complication of phototherapy. ARTHUR E. KOPELMAN, RALPH S. BROWN, and GERARD B. ODELL. *Johns Hopkins Univ., Baltimore, Md.*

A 1470-g Caucasian infant was treated on days 4–6 of life by phototherapy for neonatal jaundice (bilirubin 8. D/21.1 mg/100 ml). She developed unusual complications: dark gray-brown discoloration of her skin, serum, and urine, and acute hemolysis. The bilirubin saturation index of her serum albumin on admission at day 7 was abnormally high and the spectral absorption curves of her serum and urine showed an increased absorbance from 380 to 480 nm associated with the unusual pigment(s). The serum transaminases were elevated and the stools became acholic. Medium-chain triglyceride feedings were necessary for proper weight gain. The serum bilirubin remained elevated for 3 weeks with 30% direct reacting. Complete hematologic studies were within normal limits. Erythrocytes from the patient and control subjects were suspended *in vitro* in 15.0 mg/100 ml solutions of bilirubin containing sufficient albumin to produce 0.5–2.0 M ratios of B/A and irradiated for 0.5–7 hr with blue lights. All cells exhibited classical photosensitized light and after-light osmotic hemolysis. Before hemolysis developed a cation leak of potassium occurred which was demonstrable within 30 min of blue-light irradiation. The membrane injury was dependent upon the presence of oxygen, light, and bilirubin. The rate of cation leak was dependent on the duration of light and the free bilirubin concentration. These studies demonstrate that bilirubin itself is a photosensitizing agent and has photodynamic action. The results suggest that enough light may be transmitted through the skin of premature infants to oxidize circulating bilirubin and also induce photosensitized hemolysis by the bilirubin in erythrocyte membranes. In addition, if the photooxidation products of bilirubin are retained they may be injurious.

Discussion

ROBERT J. MCKAY (*Univ. of Vermont Coll. of Med., Burlington, Vt.*): Have you observed other infants with this syndrome?

DR. KOPELMAN: Yes, we have. We have had two infants with this syndrome transferred to us in the past 1.5 years, the one presented and another, and we have had the opportunity to see three similar infants in Baltimore hospitals during the past year.

We also have heard of a number of similar instances in other teaching institutions, all occurring following phototherapy.

DR. MCKAY: Did they all have evidence of obstructive hepatic disease?

DR. KOPELMAN: All had elevations of the direct reacting bilirubin before being placed under the light, except for one very small premature infant who was transferreed to us who had an intestinal obstruction secondary to meconium ileus.

DR. MCKAY: This paper is of obvious interest, and has widespread implications. Dr. Kopelman has emphasized that caution should be exerted in applying light, particularly to babies with obstructive liver disease in the newborn period.

I have personally seen two such infants, both with obstructive liver disease, long before the days of phototherapy, back in the early 1950's, so that this is not something which has been observed only in conjunction with phototherapy.

Second, I would emphasize that the applicability of these studies to the phototherapy of indirectly hyperbilirubinemic infants is limited, and that to date the experience has been that thousands of such infants have received phototherapy without apparent ill effect.

DR. KOPELMAN: You mention that infants with brown discoloration have been observed before the use of phototherapy. I think that we have seen several infants in which the association between the use of light and the brown discoloration appears to be well documented. I am not certain that the infants seen before light had the same sort of problem that the infants that we are seeing now following phototherapy have. In terms of the applicability to infants treated with phototherapy, I think that it is a big step going from the laboratory bench to the *in vivo* situation, but I have pointed out some reasons why we think newborns might be unusually susceptible to photosensitized oxidations. Of particular importance is our observation and the observation reported by Blum that this type of reaction does not have any threshold in terms of the amount of light needed to cause the reaction. Cells that have a sensitizing agent in the membrane will be damaged by any amount of light. I think that the dangers may be subtle, or that they may be delayed, but I think that they need to be looked for.

JEROLD F. LUCEY (*Univ. of Vermont Coll. of Med., Burlington, Vt.*): It should be recognized and emphasized that this infant had liver disease with an elevated direct reacting bilirubin. When infants with liver disease are exposed to light they do develop a greenish, muddy brown color. This has long been recognized by clinicians to occur in patients with biliary atresia, hepatitis, and biliary obstruction occurring in erythroblastosis fetalis. This color change has not been observed by us in nearly 400 premature infants treated with phototherapy since 1966. I do not believe this child's "anemia" to be unusual or caused by the light as you suggest. The fall in hematocrit on the 1st day of life from 52 to 42 is within the normal range. The late "anemia" observed was probably due to the sepsis, liver disease, or to the bloodletting (24 studies and about 30 ml withdrawn) which you subjected

him to, coupled with the lack of iron therapy in the face of a very good weight gain.

You have not presented any acceptable evidence that the decreased binding capacity observed in this infant was due to phototherapy. There are other causes of a decreased binding capacity such as free fatty acids, bile salts, ampicillin, and perhaps even the Portogen formula.

I would also like to point out that the infant is normal on follow-up.

The amount of light used in your *in vitro* studies is from 600 to 20 times the amount that one would expect to achieve in the tissues of a human infant. The conditions are certainly not comparable.

DR. KOPELMAN: Dr. Lucey said that he has observed this color change due to retention of some colored pigments in infants with liver disease or biliary atresia who were treated with light. These are the colors that you get, but I am not sure that it is just an interesting color change, and there may be some consequences to consider.

These are degradation products of bilirubin. Recently, Ostrow, in studies in the Gunn rat, has shown that the photooxidation products of bilirubin obtained during *in vivo* light treatment are different pigments from those obtained during the *in vitro* photooxidation of bilirubin in the test tube. Studies done by several people to date, which would suggest that photooxidation products are nontoxic, were all done with the *in vitro* preparations of photooxidation products of bilirubin; therefore, they may not give us any reassurance that if these pigments are retained, especially in high concentrations, they may not be dangerous.

The bilirubin saturation of the infant's albumin was very high. In this case the infant had a total bilirubin of 10 and saturation index by the salicylate displacement method of 6.3. During the 1st week at Hopkins the saturation index remained between 6 and 7, even as the bilirubin dropped.

In the other infant transferred to us (with intestinal obstruction), the infant had a total bilirubin concentration of 9 and a saturation index of 9. An exchange transfusion was performed at that time because of the elevated saturation index. I agree there are other possible explanations, but I think that it is quite suspicious that if these pigments are retained they may interfere with the binding of bilirubin to albumin.

The anemia, the actual drop in hematocrit from 52 to 42%, occurred within less than 24 hr, and the only blood drawing before that time was microsamples for bilirubin determinations. A large number of hematologic studies were done following the drop in hematocrit.

I would say that, certainly, acute hemolysis has not been a striking finding in infants under the bililamps and, actually, having studied this phenomenon *in vitro,* we are not surprised by this. To get acute hemolysis in erythrocytes you either need a very large amount of absorbed bilirubin in the membranes or you need a very large dose of light. What we would anticipate might occur *in vivo* is that smaller doses of light would damage the erythrocyte membranes, and there might be a delayed or after light hemolysis occurring at a slow rate.

What happens when erythrocytes are treated this way is that there is some shortening of the erythrocyte life span, and we would propose that the place to look for this would be in increased severity of the so-called physiologic anemia at 2 months or so of age, rather than acute hemolysis.

DR. LUBIN (*Philadelphia, Penna.*): I would like to suggest that lipid peroxidation may be an etiologic factor in both the skin discoloration (ceroid pigment) and the hemolytic anemia. In a child with biliary atresia, vitamin E levels are quite low. Phototherapy in the presence of a catalyst like billirubin may result in the release of free radicals and eventual lipid peroxidation.

The kinetics of potassium leak and membrane ATPase activity are identical with the kinetics of vitamin E-deficient cells incubated with a suitable oxidizing agent. Similar skin changes have also been noted in vitamin E-deficient animals exposed to oxidants.

Have you tested the effects of an antioxidant, such as vitamin E, on your *in vitro* system?

DR. KOPELMAN: The only experiment performed was when we completely eliminated oxygen by passing carbon monoxide through the system, and completely inhibited both the potassium leak and hemolysis.

M. M. ORZALESI (*Yale Univ. Sch. of Med., New Haven, Conn.*): In 2.5 years of phototherapy in our institution we have only seen one such baby, and, indeed, it was a baby with a hepatoblastoma, severe hyperhemolysis, and a high direct reacting bilirubin. Therefore, I think that this tends to support the hypothesis that liver damage is a prerequisite for this type of complication.

The second comment concerns the erythrocytes. As you know, we have been performing experiments similar to yours, and we also have found that intense light will produce potassium leak, swelling of erythrocytes, and hemolysis.

We also found that under sufficient light there was a drop in GSH in the erythrocytes, which we have attributed to direct oxidation.

I want to point out, however, that all of these changes, in our hands, although significant, were minimal, and obtained under very extreme circumstances.

I would also like to point out some differences in our experimental setting. We avoided any manipulation of the erythrocytes. We carefully checked the pH and temperature, and we also used erythrocytes suspended in their own plasma, which, of course is a much more physiologic milieu than the one used in your dilution technique. I wonder how these factors may explain the difference in our findings.

Do you think that plasma may exert a protective effect on the photooxidation?

The third point is that when we looked at the changes *in vivo,* in a controlled study of premature infants under prophylactic phototherapy for 6 days, measuring the levels of hematocrit, hemoglobin, and GSH, we could find no difference whatever between control and patient values.

DR. KOPELMAN: Dr. Orzalesi, I think that plasma does have some inhibiting effect on this action. I mentioned that carotenes are naturally occurring quenchers of light, and some amino acids may also serve as quenchers, and might possibly even be photooxidized themselves.

We have done these experiments in human plasma to which bilirubin was added, and have obtained results similar to those I showed previously.

You mentioned that when you exposed cells to bilirubin and light you found only minimal changes. You will recall in the first slide that we also showed only minimal hemolysis after several hours of light exposure.

In this experimental situation there is a large delayed hemolysis which is easily documented 24 hr or so after the light, and I believe that you did not look for the delayed effect in your experiments.

Also, I mentioned that if there was a delayed or after light hemolysis we would suspect that the time to look for that is 2 months or so later, and not during the light treatment.

Hepatic thermography in children. DONALD J. BOON, JOANN D. HABERMAN, and J. RAINER POLEY. *Children's Mem. Hosp., Univ. of Oklahoma Med. Ctr., Oklahoma City, Okla.* (Intr. by H. D. Riley, Jr.)

Thermography is a relatively new technique with which infrared emissions from the body, or parts thereof, are photographed directly. Hepatic thermography appears to be a simple and rapid method to monitor the liver in various disease states and should assist the physician in following the course of hepatic involvement. In fasting healthy children, the heat pattern was equal in both upper abdominal quadrants. However, following the ingestion of a simple breakfast, there was a definite increase in heat over the right upper quadrant which was observed between 30 and 120 min after food ingestion. A heat pattern compatible to that observed postprandially in healthy children was seen in several children with various types of hepatic disease when single thermographs were taken at least 2 hr postprandially. There was increased right upper quadrant heat emission in children with acute leukemia in exacerbation (but not in new remission) and in a child with chronic granulomatous disease of the liver. In fasted patients with acute viral hepatitis temperature changes were not as obvious. Thermal patterns different from those seen in the fasted normal child were detected in patients with chronic active liver disease, but the heat emission did not correlate well with the liver area (collateral circulation?). It was of great interest that a definite right upper quadrant heat emission was detected in patients with the idiopathic nephrotic syndrome (lipoid nephrosis), a disease in which the kidneys are thought to be affected primarily; identical heat patterns were observed whether or not these patients received adrenal steroids. Results of these initial observations have to be substantiated by further studies.

Discussion

FREDERIC M. KENNY (*Children's Hosp. of Pittsburgh, Pittsburgh, Penna.*): Have you had the opportunity to study patients with altered cellular oxidation, such as hyper- and hypothyroidism?

DR. BOON: No, I have not. In relation to that question I should mention that the last patient shown, who had neuroblastoma, had only a diffuse pattern initially and did not then show mottling. She was the only patient over 9 months of age who did not initially show mottling. It was only after the use of the various drugs for cancer chemotherapy, however, that the mottling became apparent.

HARVEY L. SHARP (*Univ. of Minnesota Sch. of Med., Minneapolis, Minn.*): We have very poor tests for evaluating liver function. What clinical applications do you think this test will ultimately provide us?

DR. BOON: Any answer, of course, would be speculation. Since I do not know what the normal distribution of mottling is, for instance, I do not know whether this would be useful in mass screening of school children for chronic diseases. In relation to diagnostic aid to the physician, I can again point out that all our infants with neonatal hepatitis had a diffuse pattern, except the one patient with cytomegalovirus infection, while all patients with total biliary obstruction had only a vascular pattern. Because of the small number of patients in the biliary obstruction group, however, I do not know whether this relation will hold up.

JEROME LIEBMAN (*Cleveland Heights, O.*): As a cardiologist, and clearly knowing nothing about thermography, I am really delighted to note from your pictures that all of your patients have warm hearts.

MONTGOMERY C. HART (*Children's Mercy Hosp., Kansas City, Mo.*): About 1.5 years ago we had a brief, exciting opportunity to use the same equipment that you have used. We were fascinated with the way out possibility that perhaps we could look at pulmonary blood flow changes by using this technique, and we tried this, with notable failure.

But we did, incidentally, have one interesting patient, an infant with a coarctation and with what the neurologist clinically thought was a stroke. With this instrument we were able to show that one side of the skull exhibited decreased radiation, indicating that was the side of the vascular insufficiency.

My question is: have you used thermography in more dynamic blood flow study situations than the ones that you have presented today? If not, what would your thoughts be about the feasibility of such studies?

DR. BOON: I have not done any studies, and if we have any cardiologists interested in doing them, they are welcome to try. I have no recommendation on this.

WILLIAM J. OLIVER (*Univ. of Michigan Med. Ctr., Ann Arbor, Mich.*): Have you had the opportunity to follow any patients with organ transplants? The technique would seem to lend itself quite well to following kidney transplants, for example, in children.

DR. BOON: No.

Renal transplantation in children. RICHARD N. FINE, BARBARA M. KORSCH, ELLIN LIEBERMAN, and CARL M. GRUSHKIN. *Childrens' Hosp. of Los Angeles, Univ. of Southern California Sch. of Med., Los Angeles, Calif.*

Since 1967, 50 kidney transplants have been performed in 44 children aged 1.5–20 years. Chronic glomerular disease (26), including hypocomplementenic glomerulonephritis (GN) and rapidly progressive GN, and obstructive uropathy with chronic pyelonephritis (10) were the major causes of renal failure. Thirty-six allografts were from cadaver donors (CD), 13 of which were from patients <5 years of age and 14 were from live-related (LR) donors. Thirty-seven of the 44 patients and 32 of the 50 allografts are surviving 1 month–4 years posttransplantation. Seven of the CD allografts were second transplants of which five are functioning. Of 28 allografts at risk for >1 year, 22 (79%) functioned for at least 1 year. Ureteral and/or bladder leaks occurred in 11 patients, four of whom had congenitally abnormal bladders. Healing occurred in 10 patients when the prednisone dosage was lowered. The frequency of urinary leaks has markedly diminished since the routine usage of ureteral splints was discontinued. Medical complications included hepatic dysfunction (5) presumably related to azathioprine toxicity in four recipients; diabetes mellitus (2); prolonged aseptic febrile episodes (6) associated with cytomegalovirus infection (CMV) in four recipients; steroid cataracts (6); hypertension (8); stress fractures (6) requiring pinning in one patient; noncompliance (5) leading to allograft loss in two recipients; herpes zoster (3); and CMV infection (10). Growth was observed in seven of 10 children whose bone age was <13 years at the time of transplantation and who survived >1 year with good allograft function. These results justify further

investigation of this procedure for the treatment of end stage renal disease in children.

Discussion

KENTON R. HOLDEN (*NIH, Bethesda, Md.*): You presented ranges for patient and transplant survival, but do you have the mean patient and transplant survival data available? Was there a higher incidence of chronic rejection and nephrotic syndrome in the patients who had had glomerulonephritis as their original disease and/or was there a difference in the pathologic studies of those transplanted kidneys *versus* transplanted kidney failures from patients who had anatomic variants which caused their original disease?

DR. FINE: The incidence of chronic rejection was no different, and the incidence of the clinical nephrotic syndrome did not differ in the two groups.

In addition, the pathologic studies of the rejected kidneys did not show any evidence of transmission of the primary disease in those patients whose original disease was glomerular in origin.

If you plot an actuarial table, our 1-year survival data for live-related donors would be 84%; for cadavers, 75%; and 2-year data, about 68% for both.

WALTER HEYMANN (*Cleveland, O.*): Were they all posterior subcapsular cataracts?

DR. FINE: Not being an ophthalmologist, I think that they were all posterior subcapsular cataracts. We have not had specific ophthalmologic consultation on all of our patients as yet.

RICHARD HONG (*Univ. of Wisconsin Med. Ctr., Madison, Wisc.*): I wondered whether you had encountered any malignancies in the series.

DR. FINE: No.

CLARK D. WEST (*Children's Hosp. Research Found. Cincinnati, O.*): We have performed 24 transplants in Cincinnati and in general I can say that the experience has been a happy one. Our overall survival for both kidneys and recipients is 83%. The first patient in the series was transplanted 6 years ago and is still doing well. Our series differs from that of Dr. Fine in that all but two of the patients have had living donors.

A large number of our patients have been able to be maintained on a single dose of prednisone every other day rather than daily prednisone. This regimen has been profitable in that the patients have grown, probably better than they would have grown on daily prednisone. Further, the progress of cataracts which have developed on daily prednisone has been slowed by changing the patient to an every other day regimen and surgery on the cataracts has not been necessary. With several patients it has not been possible to convert to this regimen and possibly it would be more difficult in patients receiving cadaver kidneys. When it has been possible, however, it has been to the patient's distinct advantage. I wonder whether Dr. Fine has experience with this regimen.

DR. FINE: We have used every other day dosage for only one patient. We were not happy with our results, and, therefore, subsequently have not used this form for our other patients.

I have been somewhat loathe to embark upon every other day therapy, because I have seen patients at 2 and 3 years post-transplantation where lowering the dose of prednisone from 15 mg/24 hr to 12.5 mg/24 hr has precipitated an acute rejection, and, when you reverse the rejection, if you are fortunate enough to catch it early enough, you really never get the same function you had before even though you may get the serum creatinine down to 1.3 or 1.5. It is not down to 0.9 or 0.8, as it was before the rejection episode.

ROBERT SCHULK (*Rochester*): You mentioned your patients' behavioral difficulties, and I wondered whether you had ever selected patients for transplant or dialysis in accordance with these factors. Have you ever turned down a patient for these reasons?

DR. FINE: When we embarked upon our program, and I asked Dr. Korsch to work with us, one of the prerequisites was that she would in no way have anything to do with deciding who was going to live and who was going to die.

DONALD E. POTTER (*Univ. of California Med. Sch., San Francisco, Calif.*): Your results are certainly commendable. Like Dr. West, I too am concerned about the growth of these children. Your data showed that six of 11 children showed some or normal growth. On a previous slide, however, you also showed that there were six children who had poor function, and I assume that these children were on high doses of prednisone, and were not growing very well.

We have been using every other day steroid therapy since the comments from Dr. West's group last year at this meeting. I think that we have transferred about a dozen children to every other day prednisone, and have seen normal growth in at least half of those children.

DR. FINE: I feel that every other day therapy may have some validity. I think the problem is: what is normal growth? I think this is a difficult question to answer when you have a child who may be significantly retarded in growth at the time that you transplant him.

The syndrome of trisomy for the short arm of chromosome no. 9, confirmed by fluorescence microscopy.

PARK S. GERALD and WLADIMIR WERTELECKI. *Children's Hosp. Med. Ctr., Boston, Mass., and Med. Univ. of South Carolina, Charleston, S. C.*

The recent introduction of fluorescent staining of chromosomes by Caspersson makes possible the identification of each member of the human complement. Translocations involving chromosomes of the C group, which heretofore could not be resolved in detail, can now be specifically analyzed. Two unrelated families ascertained through propositi with similar congenital malformations have been found to carry a morphologically identical translocation involving a C and a D chromosome [t(Cp−; Dp+)]. Fluorescence microscopy in one family identifies chromosome no. 9 as the translocated C chromosome. These propositi are thus trisomic for the short arm of chromosome no. 9. Both propositi exhibit severe mental retardation, oculomotor abnormalities, cup-shaped external ears, markedly hypoplastic phalanges of digits II and V, markedly hypoplastic finger- and toenails, and various bony abnormalities. Our experience indicates that trisomy (or monosomy) for portions of specific C group chromosomes will result in syndromes as definable as have been associated with aberrations of B, E, and G group chromosomes.

Discussion

ANDREW LORINCZ (*Birmingham, Ala.*): We can now modify the microscope to function as a microspectrofluorophotometer, and we can apply this instrumentation to this kind of study.

What is the chemical interaction of chromosomal material that permits this differential fluorescence? The fluorescence that we can see visibly can be resolved by physical, chemical, optical techniques.

DR. GERALD: Caspersson, who is an expert in microspectrophotometry, is busily engaged in exactly this practice.

R. B. YOUNG (*Med. Coll. of Virginia, Richmond, Va.*): I would like to support your comments regarding the vulnerability of the short arm of the no. 9 chromosome. In 1962 Drs. Edwards, Fraccaro, Davies, and I reported in the Annals of Human Genetics, *26:* 163, a family in whom the father had a balanced reciprocal translocation with attachment of the short arm of the no. 9 chromosome to the long arm of a B chromosome. Karyotypes on his only two children showed the 9/B translocation chromosome. They both showed severe mental retardation and clinical findings which were very similar to the cases that you have just presented. We assumed from the chromosomal architecture that the translocation involved the short arm of the no. 9 chromosome and the long arm of a group B chromosome. It would have been very helpful to have the fluorescent staining technique available at that time to assist in the identification of these chromosomes.

DR. GERALD: I might add that, if the original chromosome preparation were stained with Giemsa, it could be destained and now examined with fluorescence.

DR. SUH (*Chicago, Ill.*): Did you look for the absence of the patella in such a patient?

DR. GERALD: These children can have small patellas, according to Lejeune, so there is that connection. They do not have any of the other manifestations of nail-patellar syndrome, such as radiosynostosis, so I would think it unlikely there is any real connection although one would be intrigued to search further for an association.

NICHOLAS M. NELSON (*The Milton S. Hershey Med. Ctr., Hershey, Penna.*): It seems in this field, more than in others, it is as the Germans say, "Die Methode ist alles."

FREDERIC M. KENNY (*Children's Hosp. of Pittsburgh, Pittsburgh, Penna.*): The facial features, the epicanthine folds, and the cardiac lesion are all reminiscent of idiopathic hypercalcemia. Did you measure calcium or, alternatively, have you applied fluorescent staining to the patients with idiopathic hypercalcemia?

DR. GERALD: I may have confused you. There was no cardiac lesion present in these children, so that would differentiate it in my mind. We have not done either calcium balance studies or calcium determinations in our patients, nor have we looked at patients with idiopathic hypercalcemia.

KENNETH M. HOFFMAN (*Univ. of Maryland, Baltimore, Md.*): Relative to your data that this was prevalent in certain racial backgrounds, I would be interested in the statistics to back this up.

DR. GERALD: Both Dr. Walzer and Dr. Lubs have made a similar observation. Dr. Walzer has now looked at 7000 newborns, which include roughly 25% blacks, and in this total population he has found eight individuals who have this pericentric inversion. Seven of the eight are black.

I have not done a chi-square test on this, but I have taken support from Dr. Lubs, who has found a similar excess in black populations of this inversion.

DR. HOFFMAN: I would think that consanguinity would play a part here. Has this also been evaluated in the other reports?

DR. GERALD: You mean admixture of black genes in not obviously black individuals.

The whole problem of the definition of the race of an individual is extremely difficult. We used the degree of skin pigmentation to estimate race of origin. We have seen, now, one individual in our entire population who is white who happens to have the inversion. We have not been able to trace this family to see whether there is an admixture of blacks in them or not.

KENNETH W. DUMARS (*Univ. of California, Irvine, Calif.*): Could we see again the slide of the fluorescein karyotype completed upon the patient that you presented? I am curious. Where is the third short arm of the no. 9 chromosome in the karyotype? As you presented the slide, I saw no evidence of the partial trisomy.

DR. GERALD: I did not show you the karyotype of the child. What I showed was that translocation carrier father. The child has a normal karyotype except that the chromosome no. 18 has abnormally long upper arms.

Electron microscopic demonstration of colloidal gold uptake into abnormal lysosomes of cultured fibroblasts from patients with Hurler's syndrome and with type II glycogenosis. GEORGE HUG, WILLIAM K. SCHUBERT, and SHIRLEY SOUKUP. *Children's Hosp. Research Found., Cincinnati, O.*

"Abnormal lysosomes" visible by electron microscopy (EM) in liver biopsy specimens contained heteromorphous material in six patients with Hurler's syndrome, and glycogen particles in eight patients with type II glycogenosis. EM of primary and secondary skin fibroblast cultures of these patients revealed morphologically similar abnormal lysosomes in the cytoplasm of the fibroblasts. The vacuoles could also be identified by light microscopy in 1 μ thick sections of liver or of fibroblast cultures. When colloidal gold (100 μg/ml) was added to the culture media of six control fibroblast cultures, EM showed that the electron-dense gold particles entered the cells by means of pinocytotic vesicles and then became segregated in lysosomes. In fibroblast cultures from the patients with Hurler's syndrome or with type II glycogenosis the gold particles accumulated in the respective abnormal lysosomes. The results suggest that membranes surrounding abnormal lysosomes can fuse with membranes surrounding pinocytotic vesicles. Thus, contents of pinocytotic vesicles and of abnormal lysosomes may become admixed. This mechanism might explain the normalization of hepatic ultrastructure observed in a patient with type II glycogenosis after he had received exogenous glycogen-degrading enzymes.

Plasma estradiol in prepubertal children, pubertal females, and in precocious puberty, premature thelarche, and in feminizing ovarian tumor. M. R. JENNER, R. P. KELCH, S. L. KAPLAN, and M. M. GRUMBACH. *Univ. of California, San Francisco, San Francisco, Calif.*

The concentration of plasma estradiol (E_2) was determined, utilizing a radioimmunoassay, in 11 normal prepubertal children, 31 pubertal females, and five patients with precocious puberty, three with premature thelarche, two with hypogonadism, and one with feminizing ovarian tumor. The sensitivity of the assay was 15 pg. Pooled male plasma values ($n = 17$, $\bar{x} = 17.6 \pm 0.8$ SEM pg/ml) by this method agree with previous reports. Using 2.0 ml plasma, 11 prepubertal children (2 months–9 years) had values of <10 pg/ml. A gradual rise in plasma E_2 was observed from *stages II* through *IV* (Tanner): *stage II* $\bar{x} = 14.5 \pm 3.4$, *stage III* $\bar{x} = 20.2 \pm 4.4$, *stage IV* $\bar{x} = 54.6 \pm 17.4$ pg/ml. Mean plasma E_2 in eight menstruating females (11 7/12 to 13 10/12 years) was 58.5 ± 19.3 pg/ml, not significantly different from *stage IV*. Mean values for *stages II + III versus* IV + V were $\bar{x}$ II-III = 17.6 ± 2.7, $\bar{x}$ IV-V = 56.6 ± 12.6 pg/ml, $P = 0.005$; these values also correlated with the rise in plasma FSH. In contrast, five pa-

tients with idiopathic precocious puberty and three with premature thelarche (3 9/12 to 7 7/12 years) had plasma E_2 values from <10–39 pg/ml. An 8-year-old girl with a granulosa cell tumor had a plasma E_2 of 413 pg/ml, which fell to 12 pg/ml 2 months after operation, with concomitant regression of secondary sexual characteristics. In patients with gonadal dysgenesis and functional anorchia, plasma E_2 was not different from the blank. These results indicate that plasma E_2 levels correlate with serum FSH and sexual development and that the method is useful for quantification of circulating estradiol in children.

Discussion

DR. PAGE (*Portland*): The patient that you said had premature thelarche had some estrogen effect noted on the vaginal smear?

DR. JENNER: Yes, there was moderate estrogen effect.

DR. PAGE: All of us think that we have seen patients who have only breast development. Is it your opinion that what we call premature thelarche simply represents early sexual precocity that arrests, as so many of the more complete sexual precocity patients do, prior to the onset of generalized sexual development?

DR. JENNER: It is interesting in this patient that 1 year following this episode there was hardly a nubbin of breast tissue palpable. We feel in this particular girl, at least, despite the fact that she was 7 years old, there was regression, and that the estrogen stimulation was probably transient.

DONALD B. CHEEK (*Johns Hopkins Hosp., Baltimore, Md.*): You have correlated plasma estrodiol with chronologic age during growth. This is not the best way to look at these data, since estrodiol *per se* may be a powerful predictor of maturation age. Body length is a predictor of maturational age, and you would obtain a better correlation if you plotted your data against length (or height age). Actually, those of us interested in human growth know that certain determinants (be they anthropometric or chemical) predict maturational age. For example, in girls, body potassium (^{40}K) predicts developmental age better than body length (Cheek, Migeon, and Mellits: In: Human Growth, p. 541 (Lea and Febiger, Philadelphia)). Recently we have found that body length, bone age, and creatinine excretion if combined in a *multivariate equation predict maturational age in girls with a* small standard deviation (Dorst, Mellits, and Cheek: Bone age: contribution to the prediction of maturational and biological age. J. Phys. Anthrop. (in press)). It may be that estrodiol would also be a significant component of such an equation. In any event, inspection of estrodiol against the determinants mentioned might prove rewarding. The reason for preoccupation with "maturational age" is that one is in a position to assess "catch-up growth" or growth retardation in terms of time—or the effect of therapeutic treatment on growth retardation. The Tanner method for assessing stages of puberty does not hold up in the USA.

I would like to know also whether your data correlate with body fat. A spurt in the growth of adipose tissue can be defined in the girl at puberty (Mellits and Cheek: Monogr. Soc. Res. Child Develop., *35:* no. 7, 12 (1970)), and a role for estradiol would not be unexpected in that direction.

Last, I would like to know whether you have investigated any girls with obesity. We find (Cheek *et al.:* Pediat. Res., *4:* 268 (1970)) that obese girls have increased lean body mass for body length but some have bone age advanced more than 2 years, excess muscle mass, and increased number of nuclei in their muscle mass, when body length is the base line. Their growth suggests a suppression of estrogens and an excess of androgens in circulation. The situation is in need of study.

DR. JENNER: We have not measured the subcutaneous fat in the study just shown. We also have not looked at girls with obesity with regard to secretion of androgens or estrogens.

FREDERIC M. KENNY (*Children's Hosp. of Pittsburgh, Pittsburgh, Penna.*): Dr. Comacho and his co-workers showed a fall in testosterone during hydroxyprogesterone seen in sexual precocity. I think that it would be interesting to know: did you find a fall in estrogen during that treatment of your girls?

DR. JENNER: We have one patient to report on, and there was a significant fall in the plasma estradiol level.

JOHN F. CRIGLER, JR. (*Children's Hosp., Boston, Mass.*): In the use of tracer of estradiol, have you found any loss on the chromatograph in terms of transformation of that steroid at these very low levels?

DR. JENNER: No. We have had good recoveries, considering just the chromatographic step, so we had no suspicion that there was a breakdown into other steroid products.

MALCOLM M. MARTIN (*Georgetown Univ. Sch. of Med., Washington, D. C.*): Do you have any information on the correlation between your plasma levels of the 17β-estradiol and changes in the vaginal smears? At what level of circulating estrogens would you expect to find such a change?

DR. JENNER: We have not looked at that at the present time. Our suspicion is that there is some continuation of the presence of cornufication in the urocytogram or the vaginal smear, even with low levels of estradiol.

One year's experience with disseminated intravascular coagulation (DIC) in a children's hospital. JUNE M. WHAUN, JOAN URMSON, and FRANK A. OSKI. *Children's Hosp. of Philadelphia, Philadelphia, Penna.* (Intr. by D. Cornfeld.)

Forty-eight patients with DIC were observed. Diagnostic criteria included combinations of the following: thrombocytopenia, burr cells, decreased fibrinogen, factors II, V, VIII, prolonged thrombin time, and the presence of fibrin split products (FSP). Twenty-nine patients (60%) were under 1 month of age. In 32 patients (66%) DIC was associated with sepsis; 23 were neonates. Other causes included the hemolytic-uremic syndrome, cavernous hemangioma, sickle cell anemia, battered child syndrome, and ventricular-jugular shunts. Consistent laboratory findings included anemia (94%), thrombocytopenia (94%), positive FSP (86%), prolonged thrombin time (85%), factor II (85%), burr cells (82%), and low factor V (74%). Overall mortality was 64%. Neonatal mortality was 76%. Mortality in patients treated with heparin was 50%. Because of this high mortality, it would appear that only prompt recognition and treatment of those situations associated with DIC (*e.g.,* birth asphyxia, severe acidosis, obstetric complications, premature rupture of membranes, T-E fistula, and bowel obstruction) will improve survival. These infants should have platelet counts and smears performed on admission. Prompt exchange transfusions with heparinized blood may prove beneficial. DIC is, in most instances, a manifestation of severe infection or profound illness. Prompt treatment of the primary disease will usually determine the patient's outcome.

Discussion

ANDREW SASS-KORTSAK (*Hosp. for Sick Children, Toronto, Ontario, Canada*): Was the diagnosis of sepsis in your material always verified by positive blood cultures? Second, what percentage of your infants with sepsis verified by blood culture developed hemolysis and jaundice?

DR. WHAUN: In our study all newborns with sepsis had diagnosis proved by positive blood culture. These infants were in our neonatal unit which receives many referrals to our surgery department or to our cardiology department. Most of them had anemia (97%) with fragmentation (90%). Although you can see some minor degree of fragmentation of erythrocytes in normal newborns, to some minimal degree, the newborns in this study usually had severe enough fragmentation and erythrocyte hemolysis to require transfusions. At the beginning of the study some of them were merely treated with simple transfusions at the hands of the surgeon. Some of them were jaundiced but I do not have the percentage at hand.

ARTURO ABALLI (*Long Island Jewish Hospital, Jamaica, N. Y.*): This paper confirms the reports that we have been making for a number of years regarding alterations found in sick newborns that bleed.

We have classified these cases as secondary hemorrhagic disease of the newborn, because, although we appreciated the importance of disseminated intravascular coagulation, we feel that there were some atypical factors.

First, even in the very severe cases with all of the typical findings of intravascular coagulation, liver damage was demonstrated on autopsy in a number of these cases. The coagulation findings tend to be atypical in many of these cases.

For instance, fibrinogen is frequently not low. Factor VIII remains high in many patients. Fibrin thrombi are rather rare at autopsy in babies that show extensive hemorrhage.

For that reason, we believe that there are still other factors that contribute, and I feel that there is some reason to retain the term "secondary hemorrhagic disease of the newborn."

I feel, unfortunately, that we do not have any good parameters yet to diagnose the condition early. We have found in our material that most of the typical findings will not develop until the patient's condition has deteriorated rather markedly, and the condition is too far advanced.

DR. WHAUN: If the diagnosis is really so obvious that the nurse on the ward can diagnose it, the child is too far gone even for therapy.

GILBERT W. MELLINS (*Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.*): In order to assess the magnitude of the problem it would be helpful to know what percentage of your fatal cases had morphologic evidence of intravascular coagulation as compared with a group of fatal cases who did not have abnormal tests of hemostasis?

DR. WHAUN: I am afraid that an answer to that question is a little bit hard for me to come by, because I only saw the postmortem findings on the DIC children who died that were in my study. I do not have the ones who did not have DIC.

I apologize for the fact that this is a most inadequate answer, because in many of the cases, even at postmortem, there were very few with microthrombi, and there appeared to be no thrombosis, infarcts, or gangrene in 60% of those autopsied.

WOLF ZUELZER (*Children's Hosp. of Michigan, Detroit, Mich.*): You said that patients die of DIC associated with sepsis, and I would be inclined to turn this around, and say that patients are dying of sepsis associated with DIC. The physiologic significance in terms of the outcome of the disease, it seems to me, is not too well established.

DR. WHAUN: I think that I should underline what you have said about those dying with sepsis and DIC: I do not mean to imply that DIC is a disease. It is associated with an end stage, just before you get to rigor mortis.

The second point is that, in many of our children, whether a patient develops DIC or not depends on three different mechanisms going on in the body at the same time. There is a coagulation system, a fibrinolytic system, and a reticuloendothelial system to clear the body, the blood, either of these activated clotting products or the products of fibrinolytic degradation. What you are going to find at postmortem is going to depend on which of those three systems was active or inactive. I do not know whether autopsy is going to be that helpful, because you are going to find some cases such as I referred to in the earlier part of the presentation. We had eight children, some of whom died, who had *no* evidence of gangrene, thrombosis, petechiae, or bleeding. The only clue to possible DIC was that these children had a low platelet count. We zeroed in on these. As the study progressed, we were consulted at earlier and earlier points in time for that particular patient's disease. Initially, we were called in about 1 hr before the body was taken to the postmortem room, and by the time we came back with the results the child had expired. There were children who were severely septic, or acidotic, and very dehydrated, and they were bleeding from every orifice.

As time went on and the house staff became more experienced and erudite, we were called in earlier and earlier, to the point that now we seem to be doing coagulation studies on all tracheoesophageal fistulae as they are entering the front door of the hospital.

JAMES J. CORRIGAN, JR. (*Univ. of Arizona Coll. of Med., Tucson, Ariz.*): It is very important to realize that elevated levels of factor VIII, factor V, and fibrinogen are a normal event in a patient who has fever or any type of inflammatory disease. For someone to reconstruct this and say that this may be due to a rebound from DIC and therefore go ahead and use heparin on that basis is really standing on very thin ice.

I would also emphasize that the use of heparin is still investigational as you have already well pointed out. The proposed success that you had with heparin in some cases and the failures in others may be telling us that what we are looking at is not DIC, or that we are not using the right criteria for that diagnosis, or that perhaps the type of thrombotic or consumptive process that is occurring is not treatable by heparin, for instance, like a primary vasculitic effect that you propose for the hemolytic-uremic syndrome.

I would just like to underscore that you have to treat the primary initiating event if you ever expect to stop the DIC.

If you take the classical definition of DIC, that is, reduction of factors II, V, and VIII, hypfibrinogenemia, thrombocytopenia, fibrinogen, platelets, with or without fibrin split products, how many of your patients had that?

DR. WHAUN: Most of the ones who were septic had classical DIC, as outlined in the criteria. As I went on to enlarge on my scoring system, if you only allot half-points to the rebound, you still cannot get enough points to make a diagnosis unless you have other things that are strongly supporting the diagnosis of DIC. Just half-points will not get you anywhere. We might have up to about five points, I think, but we will not get a diagnostic score of seven or greater. This is a sort of a tempest in a teapot. The primary difficulty with disseminated intravascular coagulation is: how do we diagnose it, and when do we diagnose it? We are diagnosing it far too late at present.

JOSEPH W. ST. GEME, JR. (*Harbor General Hosp., Torrance, Calif.*): Just one question, about shock, in the pathogenic role of this disease, particularly in the neonate with sepsis.

DR. WHAUN: The role of shock is important in the etiology of the manifestation of disseminated coagulation, and this would be treated under the heading of conservative therapy. The management of the acidosis and dehydration is very important. One should not sit back and wait for the coagulation studies while the child is rapidly deteriorating, with cyanosis and pallor and poor circulation. So one should really be right on top of the situation in the neonatal unit, where you have many sick neonates, to be monitoring each child all of the time. This is where the nursing staff may be able to help you.

Viral hepatitis: recent studies on its natural history and prevention. SAUL KRUGMAN, JOAN P. GILES, and JACK HAMMOND. *New York Univ. Sch. of Med., New York, N. Y.*

In a recent report (J. A. M. A., *212:* 1010 (1970)) we described new clinical, epidemiologic, immunologic, and prophylactic aspects of viral hepatitis. These observations stemmed from studies involving immunodiffusion (ID) and complement fixation (CF) tests for detection of Australia or hepatitis-associated antigen (HAA) and antibody (anti-HAA). More recent studies with radioisotope precipitation (RIP) and hemagglutination (HA) techniques revealed the following findings: (*1*) HAA continued to be associated exclusively with serum hepatitis (SH); it was not associated with infectious hepatitis (IH); (*2*) HAA was usually transient in icteric SH infection; it was commonly persistent in anicteric SH infection; (*3*) anti-HAA was not detected by ID or CF assays following primary SH infection; it was consistently detected by RIP assays approximately 2 weeks–2 months after first detection of HAA, and it persisted for more than 1 year after infection.

Studies to be completed by April 1971 will reveal the effect of passive and active immunization for the prevention of serum hepatitis (MS-2 type). Passive immunization was attempted by inoculation of immune serum globulin containing a very high titer of anti-HAA. Boiled, noninfective, antigenic MS-2 serum was used for active immunization.

Discussion

DAVID H. CARVER (*Johns Hopkins Hosp., Baltimore, Md.*): Dr. Dexter Seto and I have repeatedly demonstrated that sera containing the Australia antigen confers on cells in tissue culture refractoriness to superinfection with a high multiplicity of Newcastle disease virus.

The first slide is a demonstration of control cells infected with Newcastle disease virus. Newcastle disease virus replicated and hemagglutinin developed in the cells. You see erythrocytes hemadsorbed to the surface of the tissue culture cells.

This slide shows sister cells first treated with a prototype serum containing Australia antigen, and then, 10 days later, challenged with Newcastle disease virus. You can see that the tissue culture cells here are generally free of erythrocytes, indicating that the Newcastle disease virus did not replicate in these particular cells and form hemagglutinin.

This effect has been passed. The effect is unaffected by heating either the sera or the passage materials at 60° for 1 hr. The effect is prevented with human antisera to Australia antigen.

Control sera from normal individuals do not produce the effect.

These data will appear in mid-June in Science. We are currently doing further experiments to delineate whatever relations exist between this effect and the possible replication of serum hepatitis virus in tissue culture.

DR. KRUGMAN: These observations by Dr. Carver and Dr. Seto are extremely important and I would like to congratulate them. I neglected to state, because I assumed that you all knew, that as of today, April 29, 1971, there has not been a single confirmed report of the cultivation of either hepatitis A or hepatitis B viruses.

I hope that this work can be confirmed, because the solution of the hepatitis problem will be dependent on the successful cultivation of the hepatitis viruses in tissue culture, such as was done with poliomyelitis, measles, and rubella viruses.

CHARLES A. JANEWAY (*Children's Hosp., Boston, Mass.*): Have you tried to demonstrate that the same phenomenon that you have shown for serum hepatitis, in other words, heating for 1 min at 100°, rendering the material noninfective, but retaining antigenicity, can be repeated for the type A epidemic hepatitis virus?

DR. KRUGMAN: The MS-1 serum which contains hepatitis A virus was inactivated by boiling for 1 min. Unlike hepatitis B virus, however, it was not antigenic.

I believe the difference between the two results may, in part, be a quantitative phenomenon. It has been estimated that infectious serum containing hepatitis-associated antigen may contain 10^{11}–10^{13} viruslike particles. It would be difficult to achieve this quantity by cultivation of poliomyelitis, measles, or rubella viruses. I believe that hepatitis A virus may prove to be similar to these conventional viruses in this respect. Hepatitis B virus is not a conventional virus. This is a phenomenon that is part of serum hepatitis, but in all probability it does not occur with infectious hepatitis.

DALE E. DIETZMAN (*NIH, Bethesda, Md.*): Many patients, as you know, have Australia antigen which is persistent in their serum, and some of these patients have simultaneous antibodies.

Also, Dr. Purcell has reported that following transfusion patients developing Australia antibody were not protected against posttransfusion hepatitis.

Can you comment on the fact that antibody is not always protective against serum hepatitis?

DR. KRUGMAN: At least two immunologically distinct types of hepatitis have been identified. It is possible that additional types will be recognized. Consequently, it is conceivable that these patients could have had antibody to viral hepatitis, type B, which did not protect them when exposed to another type of hepatitis. The recent reports about the existence of various hepatitis B subtypes may have some bearing on this problem.

Moreover, the available evidence indicates that immunity to hepatitis B infection may be partial rather than complete under certain circumstances. This phenomenon may occur when reinfection is caused by multiple transfusions of large quantities of infectious blood. In general, however, in our experience, the presence of antibody before exposure to our MS-2 strain of hepatitis B virus has been indicative of immunity to the disease.

Clinical and immunologic response of infants and children to administration of low temperature-adapted respiratory syncytial virus. HYUN WHA KIM, JULITA O. ARROBIO, CARL D. BRANDT, ROBERT M. CHANOCK, and ROBERT H. PARROTT. *Children's Hosp. of the District of Columbia and George Washington Univ., Washington, D. C.*

The RS virus produces serious respiratory tract disease in

young infants; serum antibody does not protect against such disease. Based on evidence that local respiratory tract immunity plays a major part in resistance, attenuated RS virus strains have been developed for local administration. The first was a live 26° grown RS virus (strain A2). Thirty-nine infants and children, 6 months–13 years of age, received this virus by the oropharyngeal and nasopharyngeal route. The RS virus was recovered from 22 individuals and 17 of these showed a significant rise in CF and/or plaque reduction serum antibody. Twelve of those from whom virus was recovered and an additional three from whom virus was not recovered showed a threefold or greater rise of RS nasal neutralizing activity. Thus 26 of the total showed some evidence of having been infected with the RS strain. The rate of infection was significantly greater in infants and children under 2 years of age than in older children. Infection in individuals older than 8 months of age was asymptomatic. Three infants infected with the vaccine strain had relatively minor respiratory tract illness.

The findings from this study indicate that the low temperature-adapted virus retains a low level of virulence which is expressed only in individuals undergoing primary infection. Nonetheless, these studies have shown that it is possible to induce local and systemic immune responses to RS virus by means other than fully virulent natural infection.

Discussion

JOSEPH W. ST. GEME, JR. (*Harbor General Hosp., Torrance, Calif.*): It sounds as though you are very close, particularly with your concluding comments, to the development of a fully attenuated strain, particularly the TS mutant with the addition of a mutagen. I have several questions about the work that was presented with the present strain and the new.

Concerning particle size, is there some way of getting around residual virulence, the ability to replicate in the lower respiratory tract, by simply modifying, if you will, the biophysics of the application of the near fully attenuated virus? Is there some way of restricting its distribution to the upper respiratory tract, rather than the lower respiratory tract?

With reference to the virus which you just mentioned in closing, did those youngsters develop only nasal antibody in the absence of serum antibody? The suggestion has been that some of the respiratory viruses which have been manipulated in this fashion replicate primarily in the upper respiratory tract and induce only or, rather, selectively nasal antibodies. The virus particles that find their way, either because of their capacity to replicate at the higher temperature of the lower respiratory tract or because of their particle size, will induce, because of their availability to systemic immunopoietic cells, serum antibody, rather than just nasal antibody.

Are you very close to the development of a fully attenuated strain which will induce just local antibody, and consequently protect?

DR. PARROTT: We constantly wonder whether, in fact, we need to have a fully attenuated strain. We think so. We think even a very mild cold in an infant undergoing immunization against bronchiolitis-bronchopneumonia is probably not acceptable. But if a fully attenuated vaccine is not found it might eventually be acceptable that a cold be exchanged for bronchiolitis or bronchopneumonia. Currently, our program goal is a vaccine which produces no illness in the infant.

With respect to the 16 who received the TS mutant, we do not yet have our data on nasal secretion antibody, so I cannot really answer your question. With the cold-adapted RS A2 strain, there was the induction of both serum and nasal antibody. In the older children at least, we have to assume that the virus did not replicate extensively over the respiratory tract; nonetheless, there was serum antibody response. I would predict that there will be serum and nasal secretion antibody response with the TS mutant. The big question with the TS mutant is the same as with RS A2-26°: will there be illness in the infants?

With respect to particle size, I think that it is unlikely that we have modified the viral particle size. If what you are referring to is the medium of administration, then, yes, I think, having found the appropriately attenuated virus, the method of administration may become important. As I understand it from those who are concerned, for example, with respiratory therapy in cystic fibrosis of the pancreas, the size of the medium which delivers whatever you want to deliver, namely, in this instance, the virus, can make a difference in the extent to which it would pass down the respiratory tract.

I would be a little afraid, if you had a virulent virus and all that you did in the process of immunization was limit delivery of virus to the nose, that you would still get into trouble. That is what happens with natural virus. A sneeze puts it in the nose, and then it goes.

Characterization of "natural" infection and vaccine-induced immunity to rubella virus. PEARAY L. OGRA, DONALD KERR GRANT, GABRIEL UMANA, and JUDITH L. DZIERBA. *State Univ. of New York at Buffalo, Buffalo, N. Y.* (Intr. by David T. Karzon.)

Antibody response to rubella virus in serum and nasopharyngeal secretions was studied in children following (*1*) natural infection (*2*) intranasal inoculation with live rubella vaccine (RA-27/3), or (*3*) parenteral administration of live rubella vaccine (HPV-77DK/12). The techniques of hemagglutination inhibition, density gradient centrifugation, and radioimmunodiffusion using ^{32}P-labeled rubella virus as the antigen were employed to determine the antibody activity in γG, γA, and γM immunoglobulins in serum and secretions. Serum antibody responses following natural infection or immunization were approximately similar, and characterized by initial appearance of γM, which was subsequently replaced by γG and to a smaller extent by γA antibody. Secretory antibody response following natural infection was characterized by regular appearance of γA antibody in nasopharynx 5–6 weeks after the onset of illness. No fall-off was observed in antibody levels for as long as 10 months. Intranasal inoculation with RA-27/3 elicited appreciable levels of γA rubella antibody in nasopharynx 4–6 weeks after immunization. However, the titers were two- to threefold lower than those observed after natural infection. No change was observed in antibody levels for 3–4 months after immunization. Significantly, little or no nasopharyngeal antibody response was detectable after parenteral immunization with HPV-77DK/12. A few children manifested transient rubella antibody responses in nasopharynx which disappeared within 1–2 months. These observations may explain the relatively poor nasopharyngeal immunity to reinfection with rubella virus observed in children previously immunized with parenterally administered rubella vaccine.

Discussion

E. RICHARD STIEHM (*UCLA Ctr. for the Health Sciences, Los Angeles, Calif.*): Was it not necessary to use enormous quantities of anti-IgG antiserum to absorb out quantitatively all of the IgG in the secretions? Also, since serum IgA may reflect locally produced IgA, did you correlate serum IgA with secretory IgA with the hope that it may predict secretory IgA antibody level.

DR. OGRA: We tried gradient centrifugation so that we could separate out 19S (γM) and 7S (γG and γA) immunoglobulin fractions in the specimens. The 7S fractions which contained least amounts of γG or γA immunoglobulins were then selectively absorbed with antiserum to γG or γA, respectively. This was done to obtain a predominantly γG- or γA-rich immunoglobulin-containing fraction. Although I must admit that these fractions were not absolutely pure, the amount of other immunoglobulins after such absorption was relatively small. Separation by gradient centrifugation and immune absorption were employed simply as an additional control to evaluate antibody titers obtained by radioimmunodiffusion.

In response to your second question, I might add that one cannot predict secretory γA antibody levels on the basis of serum γA levels. Many children who manifested a secretory γA response failed to elicit any detectable serum γA rubella response. Although secretory γA is generally believed to be produced locally in the mucosal immunocompetant tissues, there is no evidence to indicate that all serum γA is derived from secretory sites.

JOSEPH A. BELLANTI (*Georgetown Univ. Med. Ctr., Washington, D. C.*): The lower levels of γA-associated antibody in the serum following the parenteral use of rubella vaccine, I think, is in keeping with the local origin of γA which is seen following either natural infection or vaccine-induced immunity when antigen is given intranasally.

Did the child whom you studied, who was previously immunized with the live vaccine, who then became reinfected following natural exposure, develop a clinical rash? Was it modified in any way? Was it atypical either in its form or in its distribution?

DR. OGRA: None of the immunized children who acquired natural reinfection developed any clinical disease.

STANLEY A. PLOTKIN (*Univ. of Pennsylvania, The Wistar Institute, Philadelphia, Penna.*): I am not quite ready to conclude that the route of administration is the chief factor in determining whether or not antibody appears in the nasal cavity; so far, the data concerning reinfection or lack of it suggest that parenteral RA-27/3 may have, in fact, the same effect in producing nasal antibody as intranasally administered RA-27/3.

SAMUEL L. KATZ (*Duke Univ. Med., Ctr., Durham, N. C.*): Dr. Ogra, was it possible for you to detect any viremia in those three children whom you studied with evidence of nasopharyngeal reinfection? That feature of the reinfection phenomenon which most concerns us is its extent. Does it represent restricted local replication of virus or is there any evidence of dissemination? In that regard, did you have opportunities to culture blood, urine, feces, or any other secretions or materials which might help to delineate this?

DR. OGRA: Specimens of blood and urine collected 7 days after reinfection failed to yield any infectious virus. Unfortunately, we could not study blood and urine samples for virus isolation immediately after reinfection. It is difficult to say whether they did or did not develop transient viremia initially. In view of appreciable serum antibody levels, however, and the absence of any clinical disease, the possibility of a minor or major viremia seems remote.

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