Radiologic studies were done by Dr. DAVID PRICE, of our Nuclear Medicine Division, who has had experience with the use of technetium-99m in more than 800 cases, of both adults and children subjected to this liver and spleen scanning method.

The first slide shows the technetium-99m sulfur colloid scintiphotos in this girl. In the upper field on the left are anterior and posterior views of the liver, but there is no splenic uptake visible. In the triangle of three scintiphotos to the right is the uptake in the bone marrow, which shows the unusual finding of active marrow tissue going all the way down into the ankles. The calvarium also clearly shows hypertrophied marrow spaces. The lower group of four shows the scintiphotos after injection of technetium-99m pertechnetate. This is a soluble marker which is quickly demonstrated in the heart and in the abdominal aorta. At succeeding 4-sec intervals, it appears in the kidneys but no spleen is outlined. Finally, at the end of a minute, there is the beginning of spleen visualization in the posterior view, showing a labeled plasma pool within the spleen although perfusion of it was slow and poor.

In the second slide, the older boy was injected with autologous heat-treated red cells labeled with chromium. The liver uptake of this longer-lasting tag at 24 h is strikingly heavy but no splenic uptake is seen even though the spleen was quite large at this time.

The final slide shows the results of studies before and after transfusion. In September, after technetium-99m sulfur injection, there was no splenic uptake of the colloid, although with a greater intensity of the image, there is a faint splenic trace. He came in at this time for treatment of a hemolytic crisis—was transfused with 2 units of packed red cells, giving him over 60% adult hemoglobin. Within 10 days after transfusion and re-injection of technetium-99m, the spleen became visible, particularly in the posterior view where it was easier to visualize because it is a posterior organ. Some 2 weeks later it was still visible; almost 1.5 months later, at which time his adult hemoglobin was still around 30%, it was faintly yet definitely visible.

These studies corroborate the findings reported here by Dr. PEARSON and his associates. They are to be congratulated on adding to our knowledge not only of sickle cell disease but of some of the reasons for frequent infections in these patients and how to combat them.

ROLAND B. SCOTT (Howard University College of Medicine, Washington, D.C.): My colleagues and I have been evaluating a group of older children with homozygous sickle cell anemia who exhibit persistent splenomegaly. The children ranged in age from 8 to 15 years. Isotopic scanning was employed as part of our studies. We also observed that the spleens in these children visualized poorly or not at all.

In regard to your designation of this finding as 'functional asplenia', I would like to point out that our patients with splenomegaly so far have not exhibited greater susceptibility to intercurrent infection particularly of pneumococcal origin than other sicklers without splenomegaly.

It is of particular interest to me that your patients with homozygous sickle cell anemia showed functional asplenia but the variant forms (Hgb AS, SC, S-thalassemia) exhibited normal splenic function by isotopic scanning technique. If the most likely mechanism is a mechanical one, I would appreciate further comment about this differential observation since stasis and vaso-occlusion appear to be a fairly common finding in the SS, SC, and S-thalassemia types.

Dr. SCHWARTZ: I believe that the phenomenon of functional asplenia is related to the degree of *in vivo* sickling, not simply the presence of sickle hemoglobin. The individual with SC disease or sickle-thalassemia is usually not as severely affected clinically as the homozygous sickler. Of interest is the fact that some patients with sickle-thalassemia have 80–90% hemoglobin S, yet still have splenic function. It is known that these people have less *in vivo* sickling than the homozygous hemoglobin S patient, although the reason for this is not clear. In fact, the splenic scan may be more accurate in identifying the sickle-thalassemia patient than is the hemoglobin electrophoresis. There is no increased incidence of pneumococcal sepsis in these people.

I would like to comment on your observation of the lack of infection in your older patients. The young, splenectomized child is the patient most susceptible to overwhelming infection. Your patients are comparable to older splenectomized children. If an asplenic individual is exposed to an intravenous particulate antigen, he will not form antibodies against it. If he is exposed to the same antigen by the intramuscular, subcutaneous, or intraperitoneal route he can produce antibodies. The older patient probably has had immunologic experience with organisms introduced by routes other than the intravenous one. He, therefore, has antibodies to these organisms and is not usually susceptible to overwhelming infection.

J. LAWRENCE NAIMAN (St. Christopher's Hospital for Children, Philadelphia, Penn.): Have you tried to open up the spleen pulp with fresh frozen plasma?

PHILIP L. CALCAGNO (Georgetown University Hospital, Washington, D.C.): My question is the same one. Have you tried plasma, saline, or expanders? It would seem to me these might give a good handle to pathogenesis.

Dr. SCHWARTZ: We tried to open up the spleen with fresh plasma in several children, but we could not.

7 *Response to Rubella Vaccine Among Seronegative Children with Congenital Rubella.* ALFRED L. FLORMAN, LOUIS Z. COOPER, PHILIP R. ZIRING and SAUL KRUGMAN. New York Univ. Sch. of Med., New York.

E. RICHARD STIEHM (UCLA Center for the Health Sciences, Los Angeles, Calif.): Many patients with rubella syndrome have a systemic immunologic deficiency. Were your sero-negative rubella patients tested for their response to other antigens to exclude a non-specific diminution of antibody response, rather than specific tolerance to rubella virus?

Dr. FLORMAN: Several of our children by history had had regular measles vaccination. It was possible for us to test their sera and find that they had responded with perfectly fine titers of antibody against rubeola.

JANET HARDY (Johns Hopkins Hospital, Baltimore, Md.): Dr. FLORMAN and his associates have attempted to answer some interesting and important questions. Individual variation in response to immunologic stimuli may perhaps lead to the emergence of a number of different answers. In this regard I would like to comment on a patient followed by Dr. JOHN BORDLEY and me, with the help of Dr. JOHN SEVER. This was a child born early in 1964 whose mother presumably had inapparent rubella approximately one-third the way through her pregnancy. She has a history of exposure. When her child was just over 1 year of age her serum rubella HAI titer was 1:512. At this age the child was found to have

a severe hearing loss (50 dB in the right ear, 80–90 dB in the left ear), small body size, and mild peripheral pulmonic stenosis. His rubella HAI titer was 1:128; further serologic determinations at 30 and 36 months of age showed no detectable HAI antibody in a dilution of 1:4, nor did he have neutralizing antibody at 30 months.

At 5 years of age he was exposed to, and developed, clinical rubella, with lymphadenopathy and rash. Attempted viral isolation 5 days after the appearance of the rash was not successful but some 3–4 weeks later his rubella HAI titer was 1:512. Subsequently, he experienced a further depression of auditory sensitivity and he now has a severe hearing loss in both ears. He is a highly intelligent child—IQ 137 (Leiter nonverbal test) and there is no question about the validity of the test results.

One further comment pertains to our experience in an epidemiologic study carried out in 1967 by Dr. JOHN GRANT, Maryland State Department of Health. This study was a survey of all the children born in 1964 in several areas in Frederick County, Maryland to determine the number of children with congenital rubella. Of some 600 children screened, 8–10 had defects compatible with congenital rubella, but more than 50% had no rubella HAI antibody. This points out the problem of the limited usefulness of serologic study in the retrospective diagnosis of congenital infection in both the clinical and epidemiologic setting.

Dr. FLORMAN: I think you have touched on the really crucial point, and that is: are these children who are seronegative susceptible to reinfection by wild virus? Your case is very pertinent.

JOSEPH W. ST. GEME, Jr. (UCLA Medical School, Harbor General Hospital, Torrance, Calif.): Is it possible that these children have very low levels of neutralizing antibody?

Dr. FLORMAN: We have not yet tested these children who no longer have detectable HI antibody for the presence of neutralizing antibody.

Dr. HARDY: May I comment on that? Because we have, and there is no neutralizing antibody.

PEARAY L. OGRA (State University of New York, Buffalo, N.Y.): I would like to raise one question in regard to the disappearance of rubella virus antibody in these children. It has been reported in the past that there are children with congenital rubella syndrome who do not have any detectable neutralizing or hemagglutinating antibody in the newborn period. Is it possible that some of these children who had no detectable HI antibody after 6 months—that the pre-existing serum antibody was all transplacentally acquired maternal antibody—failed to have an immunological response to start with? Secondly, did you try by any means to identify immunologically the immunoglobulin class with which this antibody activity was associated? Presence of γ M antibody would suggest active fetal production rather than maternal transport.

Dr. FLORMAN: As you noticed in our charts, we excluded all determinations on patients' sera except those that were obtained after 6 months of age, because we too were concerned that the determinations done earlier might represent transplacentally acquired antibody. Consequently, all these children had responded with detectable levels of antibody earlier in life, even those who by the age of 2–5 years had lost it.

DOUGLAS E. COX (Wayne State University Medical School and Children's Hospital of Michigan, Detroit, Mich.): Is your twin, which was discordant for con-

genital rubella and became seronegative before 5 years of age, dizygous by placentation and membranes or by difference in some genetic marker?

(Subsequent communication with the authors (*viz.*, Dr. LOUIS Z. COOPER) has confirmed clear documentation of dizygosity by both criteria.)

- 8 *Rubella: Reinfection in Vaccines and Natural Immunes Exposed in an Epidemic.* DOROTHY M. HORSTMANN, HARVEY LIEBHABER, DONALD M. ROSENBERG and SCOTT B. HALSTEAD. Yale Univ. Sch. of Med., and Univ. of Hawaii Sch. of Med.

PEARAY L. OGRA (State University of New York, Buffalo, N.Y.): We have some data which are very similar to what Dr. HORSTMANN has presented today. I think that the basis of herd immunity may really lie in the mechanisms of mucosal immunity, rather than in the circulatory antibody. Presently, we are studying comparative antibody responses in serum and secretions following natural or vaccine-induced rubella virus infection in a large group of children. Preliminary data suggest that although the antibody responses in serum are generally similar, following either type of infection, the responses in the secretions are strikingly different between the two groups. Those children who have been immunized with rubella vaccine usually fail to develop secretory antibody in their nasopharyngeal secretions. On the other hand, the natural disease almost invariably results in γ A antibody production in the secretions.

I was wondering if Dr. HORSTMANN had looked at the secretory immune responses among her patients.

Dr. HORSTMANN: We are very much interested in this aspect of the problem, and are currently investigating it, particularly in relation to Dr. STANLEY PLOTKIN's RA 27/3 vaccine. Unlike the HPV 77 derivatives and the Cendehill vaccine, RA 27/3 induces infection and serologic immunity when given intranasally. We do not know whether this vaccine will stimulate a greater secretory antibody response in the nasopharynx than the others, but it seems possible that it might do so. If so, it would have a distinct advantage, for as Dr. OGRA indicated, the superiority of natural over vaccine-induced immunity may be associated with local mucosal antibody and resistance.

HARRY M. MEYER, Jr. (National Institutes of Health, Bethesda, Md.): Four years ago, our reports of rubella virus attenuation to this Society included data indicating that experimental animals immunized with either natural rubella or attenuated virus could undergo modified reinfection after challenge. The following year at these pediatric meetings, we showed that persons with antibodies as a result of earlier natural rubella or vaccination could also experience anamnestic increases in antibody. We noted that these reinfections were subclinical, highly abbreviated from a virologic point of view, and generally occurred in persons with relatively low antibody titers. Since 1967, several groups have confirmed and extended these findings.

This past spring, we had the opportunity to reexamine this matter in considerable detail. Epidemic rubella entered the institution of our earlier studies involving 5 cottages. Each contained susceptible children, vaccinees, and others naturally immune. We examined and collected specimens from each person every day. In brief, 22 of 33 susceptibles were infected; all evidenced signs of rubella. Five of 22 vaccinees and 1 of 66 naturally immune children had subclinical reinfections