We have also been able to confirm—probably the same specimen that you analyzed—the difference in distribution of acid mucopolysaccharides in the Hurler amniotic fluid.

There is only one problem that is still a little bothersome to us, and that is the question of expressing the results in terms of volume, since I am not quite sure whether there might be differences in concentration, water content, of amniotic fluid which might artifactually influence the results.

Dr. MATALON: I have discussed this problem previously with you, Dr. SCHAFER, and I agree with you that one might add more criteria for expressing these data.

I would like to stress again that in the Hurler amniotic fluid the problem is not a problem of concentration only, but it is again a problem of total different distribution. In none of the amniotic fluids did we find any suggestion of this amount of heparitin sulfate; as you will recall, normal amniotic fluids have almost 80% of their polysaccharide as hyaluronic acid.

The second thing you mentioned is that you detected some heparitin sulfate. We did the same thing you mentioned, and found a reactive substance in all the amniotic fluids; however, this substance was not true heparitin sulfate, because it gave reaction in the zero time, which heparitin sulfate does not.

5 *Intravascular Coagulation and Fibrinolysis in Human Renal Disease.* E. RICHARD STIEHM, JON I. SCHEINMAN, CARL W. TRYGSTAD, LINDA S. KUPLIC and DAVID T. UEHLING, Dept. of Ped., Univ. of Wisc. Med. Sch., Madison, Wisc. (introduced by Arthur J. Moss).

JAMES J. CORRIGAN, Jr. (Emory University School of Medicine, Atlanta, Ga.): What happens to the fibrolytic split products in inflammatory disease in general? Can you venture a guess as to what the half-life of the split products might be? And, in studies that we have done and reported by others, we have noted that there is a high titer of a material that is antithrombin in the alpha-2-macroglobulin fraction in addition to the antiplasmin; so that thrombosis in the nephrotic syndrome is probably not simply on the basis of elevated antiplasmins.

Dr. STIEHM: Fibrin split products increase in inflammation and infection. Using a sensitive technique like hemagglutinin inhibition, elevations of fibrin split products are frequently noted; this may be a useful test for sepsis. The half-life of fibrin split products is from 3 to 6 h. Limited studies on antithrombin levels in the nephrotic syndrome have disclosed no abnormalities; I will await your data with interest.

LYMAN A. PAGE (Stanford University School of Medicine, Palo Alto, Calif.): Is defective fibrinolysis seen as nonnephrotic hypoalbuminemia and analbuminemia?

The second question is whether you have tried to correlate the presence of fibrin split products in the urine with the extent of renal failure. It seems to me you are drawing some important inferences from the absence of correlation between urinary and plasma fibrin split products.

Dr. STIEHM: In patients with chronic renal disease with low albumin, there was no defect in fibrinolysis. We have not studied any patients with analbuminemia.

In any type of chronic renal failure urine fibrin split products may be elevated; therefore, when one is using urine fibrin split product levels as an indicator of transplant rejection, one should take into consideration the degree of azotemia. A urine fibrin split product level is significant only if it is markedly above what we would expect from other uremic patients with the same degree of renal failure. Currently we are relating levels of azotemia with urine fibrin split product levels.

HAROLD M. MAURER (Medical College of Virginia, Richmond, Va.): Have you used the ethanol gelation test to detect intravascular coagulation? How sensitive is this test compared with the test for fibrin split products in serum?

Dr. STIEHM: We have had limited experience with this procedure. The ethanol gelation test is positive in marked intravascular coagulation with defibrination. I do not believe, on the basis of limited studies, that it is of value in the study of subtle and localized intravascular coagulation.

GERALD S. GILCHRIST (Children's Hospital of Los Angeles, Los Angeles, Calif.): I noticed that 37% of the patients had fibrin split products in their serum. Larger numbers have it in their urine. Can you explain the large percentage of serum positive patients in the light of the apparent defect in fibrinolysis? Secondly, with regard to the hemolytic-uremic syndrome, I find difficulty in accepting your classification of the syndrome as a manifestation of generalized intravascular coagulation. Most studies would support the concept that this is a localized phenomenon with no consistent evidence of vascular involvement outside of the kidney.

Dr. STIEHM: There is a disagreement whether fibrin split products can occur in the presence of a defective fibrinolytic system. In my experience, I have never seen a fibrinolytic system so defective that fibrin split products are not generated even in the presence of markedly decreased plasminogen levels. Fibrin split products may be falsely negative if insensitive techniques such as agar diffusion are used. Secondly, I regard the hemolytic-uremic syndrome as an illness not limited to the kidney, with central nervous system and peripheral vascular involvement. At autopsy I believe there is evidence for intravascular coagulation.

JACK RESNICK (Naval Hospital, San Diego, Calif.): What type of correlation do you find between the biopsy evidence of fibrin or fibrinogen in the kidney, and the urine and serum fibrin split products?

Dr. STIEHM: We have not as yet had extensive experience correlating urine fibrin split products with biopsy evidence of deposited fibrinogen. We have found poor correlation between serum levels of fibrin split products and immunofluorescent evidence of a fibrinogen.

6 *Functional Asplenia in Sickle Cell Anemia—A Reversible Defect.* ALLEN D. SCHWARTZ, HOWARD A. PEARSON, JOSEPH H. ZELSON, RICHARD P. SPENCER and SORRELL L. WOLFSON. Depts. of Ped. and Nucl. Med., Yale Univ. Sch. of Med., New Haven, Conn.

LOUIS K. DIAMOND (University of California Medical Center, San Francisco): Shortly after hearing of this work of Dr. SCHWARTZ and Dr. PEARSON, we had the opportunity to study two children in one family with severe sickel cell anemia. The younger, a 12-year-old girl, had a spleen that was easily palpable two to three finger breadths below the costal margin. The older boy, 16 years of age, had an even larger spleen at an age when it is usually fibrosed and not palpable. He had had more than 30 admissions in the first 10 years of his life, several of them with pneumonia, one of them with pneumococcal pneumonia and septicemia.

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 reinjection 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 vasoocclusion 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 nonspecific 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