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Discussion

Dr. Robert E. Hermann (Cleveland, Ohio): Dr. Berry, Dr. Jones, Members, and Guests: I enjoyed this interesting paper by Dr. Pitt and his colleagues very much and I appreciate the opportunity to review the manuscript. They have focused in this study on the role of bacteria on the formation of gallstones and bile-duct stones.

I believe most of the evidence to date accumulated in the many institutions that are studying the lithogenicity of bile, in other words, the formation of stones, have shown that there are probably several factors that contribute to the development of stones in the biliary system. Among these are first and foremost, the degree of saturation or supersaturation of cholesterol for primary gallstones, the role of stasis or inadequate emptying for both gallstones and bile-duct stones, and the role of infection, which Dr. Pitt and his colleagues have focused on today.

It is always difficult, it seems to me, to determine whether the infection is primary, an initiating factor, or if it is a secondary factor.

Dr. Pitt, I wonder if you could speak to this a bit further. Do you feel stasis comes first or does the infection comes first? Because in your studies most of the stones, the brown stones or stasis-type stones, in the bile duct are those that are associated with bacteria, how much of a role do you believe stasis plays? Second, once the bile duct has bacteria in it and you correct the obstruction by connecting it to the intestinal tract for better drainage and bile flow, does the bile duct or the bile ever become sterile again? In other words, I am concerned about the role of stasis and whether, once infection is there, can you ever overcome this problem?

Dr. R. Scott Jones (Charlottesville, Virginia): I wanted to ask a question and make a comment.

The first question concerns the bacteria. In the beginning of Dr. Pitt's presentation, he mentioned the Maki hypothesis for the formation of pigment stones and the basis for that is that certain bacteria produce an enzyme, beta-glucuronidase, that hydrolizes conjugated bilirubin to produce the deconjugated form. The deconjugated form is much less soluble in water than is the conjugated form, and my question, therefore, is: In the bacteria that you recovered from the bile of the patients or from the stones, did you test these to determine if they were the species of bacteria that produced beta-glucuronidase, certain strains of E. coli, or did you, in fact, measure for the enzyme in the bacterial isolates?

The comment that I wanted to make was concerning the finding of ileal disease in patients with pigment stones. We usually think that ileal disease participates in causing gallstones by producing abnormal bile

acid losses, decreased bile pool, and decreasing, therefore, the solubility of cholesterol in bile, thinking that that would produce cholesterol stones. But here you have showed us that there was a significantly greater amount of ileal disease in patients with pigment stones. I find that very interesting, and I would like for you to tell us, if you would, how this fits into the pathophysiological thinking, and what is it about ileal disease that leads to the development of pigment stones?

I thought this was a fascinating study, a quantitated study, and a very interesting study that increases our understanding of the pathogenesis of gallstones.

DR. HENRY A. PITT (Closing discussion): With respect to Dr. Hermann's comments, I would agree that multiple factors play a role in the pathogenesis of gallstones. He questioned which comes first, stasis or infection; and he wondered whether you really need both to cause brown-pigment stones. To answer this question, Cetta and his Italian colleagues recently reported in *Hepatology* two patients who had negative cultures at the time of initial common duct exploration. Over the ensuing 10 days, bile cultures became positive before the T-tube were removed. Each of these two patients went on to form brown-pigment primary common-duct stones. These cases suggest that bacterial infection precedes brown-pigment stone formation.

Nevertheless, I believe that both stasis and infection are necessary for brown-pigment stone formation. For example, in patients with biliary-enteric anastomosis the bile is almost always infected. However, without stasis, stones will not form and cholangitis will be a problem.

With respect to Dr. Jones's comments, he is right about glucuronidase. Phospholipases also may be important in breaking down phospholipids into fatty acids, such as palmitate, which may then bind with calcium. Calcium palmitate was found only in the brown, and not in the black, pigment stones.

Both glucuronidases and phospholipases can be found in bacteria as well as in hepatic and gallbladder tissues. A group from Scandinavia has recently reported that various bacteria, including *E. coli,* bacteroides species, and clostridia species, can produce glucuronidases. In our patients with brown pigment stones, we found no anaerobes, but we did isolate *E. coli.* We did not, however, measure biliary enzyme levels in this study.

With respect to the association of ileal disease and pigment, as opposed to cholesterol, gallstones, I was not surprised. Older human studies had suggested that ileal disorders decrease bile salt-pool size and increase cholesterol saturation of bile. Therefore, most people have assumed that patients with ileal disorders are prone to cholesterol gallstones rather than pigment gallstones. However, we and others have performed ileal resection in the prairie dog and have noted that pigment gallstones form. In our studies at 4 weeks after ileal resection, 94% of animals had pigment sludge and 45% had pigment stones in their gallbladders. When we studied biliary lipid metabolism, we found a dramatic decrease in bile-salt pool size. However, the cholesterol saturation index of both hepatic and gallbladder bile was unchanged by ileal resection.

We did find increased levels of bilirubin and calcium in the gallbladder, as well as increased gallbladder fluid absorption in the animals that have had ileum resection. We believe that the increased water absorption may have been a response to conserve fluid in these animals that were experiencing diarrhea after ileal resection. The increased levels of calcium and bilirubin in bile may be the result of altered gallbladder absorption. The next step is precipitation of calcium bilirubinate and subsequent pigment-gallstone formation. Thus we were gratified to see that our clinical data supported our earlier animal studies.