Pruritus Induced by Crude Bile and Purified Bile Acids

Experimental Production of Pruritus in Human Skin

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Crude bile, when applied to keratin-stripped human skin in a cream vehicle at acid pH, under occlusion, produced noticeable pruritus after a period of 15 to 17 hours. At neutral or alkaline pH, no pruritus resulted. Purified, unconjugated sodium deoxycholate and Chenodeoxycholate in a cream vehicle at acid pH also produced pruritus. Purified cholate sodium and sodium lithocholate, as well as synthetic straight chain anionic and neutral detergents failed to induce pruritus under the same experimental conditions. In complete biliary obstruction, cheno-deoxycholate seems to be mainly responsible for the pruritus.

Although most physicians identify the bile acids as the cause of pruritus in biliary obstruction, evidence for this relationship is indirect and circumstantial and sometimes contradictory. The essential evidence that is lacking has been the failure of workers to induce itching in skin with purified bile acids. The intradermal injection of *impure* bile acids has caused pruritus, but in these cases, substances other than bile acids could have elicited the itching. Efforts to drive purified bile acids into human epidermis and dermis by applying such compounds to skin and by iontophoresis have so far failed to produce pruritus. Herndon, in his review article on this subject, summarizes the situation well in the following statement, "The most serious objection to the claim that bile salts are responsible for pruritus in biliary obstructive states lies in the failure of investigators to reproduce Koch's postulates."

Evidence implicating the bile acids as the cause of pruritus is indirect because from none of the available experimental results can one conclude that the bile acids rather than other constituents in bile are responsible for the pruritus. Thus, the observation that biliary drainage relieves the pruritus merely suggests that a constituent in bile may be inducing the pruritus. Similarly, the relief of pruritus that is obtained when the patient is fed with the anion-exchange resin, cholestyramine resin, which binds bile acid anions, indicates only that an anionic compound in bile is possibly responsible for the pruritus. Contradictory evidence is exemplified by cases in which patients have very high serum bile acid levels but do not experience pruritus. Also, some patients with high serum levels of bile acids and pruritus, may experience relief from pruritus without a concurrent drop in the serum bile acid level. Conversely, the serum bile acid level may return to normal without relief from pruritus.

In an attempt to correlate better a possible causal relationship between bile acids and pruritus, investigators have measured bile acid levels on the skin surface. The correlation was indeed better; for in one such study, patients with hepatobiliary disease accompanied by pruritus were found to have consistently higher skin bile acids than those with such disease but without pruritus. In addition, relief of pruritus by biliary drainage or cho-

Results of applying crude bile in unscented cold cream and cold cream alone at varying pH to keratin-stripped skin. A, Crude bile in unscented cold cream at pH ~ 5; B, Unscented cold cream alone at pH ~ 5; C, Crude bile in unscented cold cream at pH ~ 7; D, Crude bile in unscented cold cream at pH ~ 8.
Table 1.—Results of Application of Crude Human Bile*

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<thead>
<tr>
<th>Agent Applied</th>
<th>Symptoms and Signs</th>
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<tbody>
<tr>
<td></td>
<td>Erythema†</td>
</tr>
<tr>
<td>Crude bile in cream</td>
<td></td>
</tr>
<tr>
<td>pH 5</td>
<td>+++</td>
</tr>
<tr>
<td>pH 6</td>
<td>+</td>
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<td>pH 7</td>
<td>+</td>
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<td>pH 8</td>
<td>+</td>
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<tr>
<td>Cream alone, pH 5</td>
<td>+</td>
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<tr>
<td>Keratin-stripping alone</td>
<td>+</td>
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* Crude human bile in unscented cold cream vehicle, at various pH values, was applied to keratin-stripped human skin. Each observation is the result of duplicate testing in each of five normal subjects and in two patients with obstructive jaundice and pruritus.
† + indicates slight erythema; ++ indicates moderate erythema; +++ indicates intense erythema.
‡ Pruritus developed 17 to 19 hours after application.

Table 2.—Results of Application of Purified Sodium Salts of Free Bile Acids*

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<tr>
<th>Agent Applied</th>
<th>Symptoms and Signs</th>
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<tbody>
<tr>
<td></td>
<td>Erythema†</td>
</tr>
<tr>
<td>Cholic acid, pH 6</td>
<td>+</td>
</tr>
<tr>
<td>Chenodeoxycholic acid, pH 6</td>
<td>++</td>
</tr>
<tr>
<td>Deoxycholic acid, pH 6</td>
<td>+++</td>
</tr>
<tr>
<td>Lithocholic acid, pH 6</td>
<td>+</td>
</tr>
<tr>
<td>Sodium lauryl sulfate, pH 5</td>
<td>+++</td>
</tr>
<tr>
<td>Brij 35, pH 6</td>
<td>++</td>
</tr>
<tr>
<td>Cream vehicle alone, pH 6</td>
<td>+</td>
</tr>
</tbody>
</table>

* Purified sodium salts of free bile acids in unscented cold cream were applied to keratin-stripped human skin.
† + indicates slight erythema; ++ indicates moderate erythema; +++ indicates intense erythema.
‡ Pruritus developed 17 to 19 hours after application.
§ Molar/size is 414.6.
∥ Molecular size is 288.4

lestyramine administration was correlated much more closely with normal skin levels than with normal serum levels of bile acids.7 In spite of these excellent studies, however, one is not closer to establishing that bile acids are the cause of pruritus. For, these results only indicate that skin bile levels, using bile acids as a marker for quantitation, are much more closely correlated to pruritus than are the serum bile levels.

This study presents, for the first time (to our knowledge), direct evidence that bile acids are capable of producing pruritus in human skin under given experimental conditions. In addition, certain bile acids, but not others, appear to induce the itching.

Materials and Methods

All bile acids (sodium salts) were highly purified products. The purity of the compounds used was high, as determined by optical rotations and melting points that correlated closely with standard values in the Merck Index. The neutral detergent, Brij 35, and the anionic detergent, sodium lauryl sulfate ("specially pure") were used.

Human Experiments.—Intracutaneous Injection of Purified Bile Acids.—For these studies 200 μg of the bile acid were dissolved or suspended (if not soluble) in 0.1 ml of physiologic saline solution. The solution or suspension was injected intracutaneously in 0.1-ml aliquots into the flexor surface of the forearm. The above quantity of bile acid was chosen because 200 μg/ml serum was the maximum value of total bile acid measured in the serum of patients with obstructive jaundice.

Bile Acid and Detergent Preparations in Cold Cream Applied to Keratin-Striped Skin.—Crude human bile obtained from normal gallbladders at laparotomy was incorporated into unscented cold cream in a concentration of 10 gm of lyophilized bile to 100 gm of cream. The pH of the bile-cream preparations was adjusted down with either glacial acetic acid or hydrochloric acid, and it was adjusted up with sodium hydroxide. Unscened cold cream has a pH ~ 7. The bile-cream preparations were applied to areas on the flexor surface of the forearm, which had been dermornified by repeated stripping with cellulose tape until "glistening" was visible. Plastic film wrap was used to occlude these bile-cream preparations which were re-applied after 12 hours. The preparations were left on the skin for a total of 24 hours.

Highly purified, unconjugated sodium salts of cholic, deoxycholic, chenodeoxycholic, and lithocholic acids were incorporated into unscented cold cream in a concentration of 25 millimoles (~ 10 gm) per 100 gm of cream. Each preparation was adjusted to a pH ~ 6. The concentration of bile acid used in the cream is based on the highest value of total bile acid that was found in the serum of patients with obstructive jaundice, ie, 200 μg/ml.

The long chain, anionic, synthetic detergent—sodium lauryl sulfate, was also mixed with cold cream in a concentration of 25 millimoles per 100 gm of cream. The pH was left at 5. The neutral detergent, Brij 35, a semisolid, was mixed with cold cream in a ratio of 1:1, by weight. Both the purified bile acid-cream and detergent-cream preparations were applied to keratin-stripped skin in the same way as the bile cream preparations.

The subjects in all these experiments were five normal individuals (four men and one woman, 19 to 35 years old) and two patients (both women, aged 32 and 40), who had obstructive jaundice and pruritus. The study was "double blinded" in that neither investigator nor subject knew which was a control and which was a test application.

Animal Experiments.—The common bile ducts in five adult male mice were tied so as to obstruct fully the flow of bile. Five other mice that underwent the same operative procedure but without ligation of the common bile duct served as controls. Following surgery, the mice were observed daily for 14 days for signs of "increased scratching activity." Itching was "quantitated" by the number of times per hour that a mouse scratched himself. To be counted, the duration of a bout of scratching had to be 3 seconds or more. Serum bilirubin determinations were performed two times per week on these animals.

Observations

Table 1 gives the results of the experiment in which crude human bile in a cream vehicle at various pH val-
ues was applied over a keratin-stripped area of skin for a period of 24 hours. From these data, it is apparent that crude bile produced some irritation of skin accompanied by severe pruritus only at acid pH values. Severe pruritus is defined as itching of such intensity that the subject complains spontaneously and bitterly about it; it is generally accompanied by efforts to scratch off the occlusive dressing. This reaction occurred in six out of the seven subjects. The seventh subject admitted that there was itching only on questioning. Moderate pruritus is defined as itching of such intensity that the patient complains only casually about it. This was the reaction of four subjects who mentioned the itching spontaneously, while three subjects complained that the test site itched more than the control site only when they were questioned directly.

The Figure demonstrates a typical result. The circle of irritation (intense erythema, + + +), A, on the right forearm resulted after applying the crude bile in unscented cold cream at pH ~ 5. Area A was also intensely pruritic. Areas B, C, and D all had much milder degrees of irritation (+) than area A and the erythema resulted from applying unscented cold cream alone at pH ~ 5, crude bile in cold cream at pH ~ 7, and crude bile in cold cream at pH ~ 8, respectively. It should be pointed out that the cellulose tape "stripping" procedure alone produced a minimal degree of irritation (+) when covered with plastic film wrap for 24 hours, with no cream applied.

In Table 2, it is clear that only the purified, dihydroxy bile acids—chenodeoxycholate and deoxycholate—produced pruritus accompanied by an increase in irritation greater than that induced by keratin-stripping alone. All seven subjects spontaneously volunteered the information that the test sites itched, while the control sites did not itch. Interestingly, cholate and lithocholate also produced irritation of skin at the concentration used, but failed to elicit any pruritus.

The synthetic detergents, Brij 35 and sodium lauryl sulfate, both induced irritation in the skin equal to that induced by the bile acids, but without accompanying pruritus. Thus the detergent properties of these bile acids and the irritation resulting from their application to the skin are probably not responsible for the pruritus.

Table 3, illustrates that intracutaneous injection of sodium salts of purified bile acids does not produce pruritus. It is interesting that the inflammation produced by lithocholate (a known pyrogen) was unaccompanied by pruritus. The reason for these negative results and, probably, for the failure of the iontophoretic and other techniques, was possibly due to the speedy dilution of the bile acids in tissue fluid after injection and their subsequent rapid removal by the blood. The successful results obtained with the bile acid-cream preparation were probably due to the more prolonged exposure of the bile acids of the C fiber nerve endings at the dermoeipidermal junction. This was presumably accomplished by a continuous percutaneous absorption of the material through skin without barrier layer, thereby building up a sufficiently high bile acid concentration in the skin for a sufficiently long exposure to stimulate pruritus in the nerve endings.

The test mice failed to show an increase in scratching activity, even when the serum direct-reacting bilirubin was very high. Although it is speculative to comment, it seems that the absence of pruritus is due to the nature of the target site, that is, the nerve endings. This speculation is perhaps supported by our observation that the concentrations of histamine that produced pruritus in human skin failed to produce increased scratching activity when injected intracutaneously in the mice.

Comment

The fact that itching in human skin can be elicited by the application of the sodium salts of certain purified bile acids supports the conclusion that the bile acids are responsible for the pruritus in hepatobiliary disease. These data also suggest that free (unconjugated) bile acids are capable of producing pruritus. This observation is consistent with the findings of Schoenfield et al, that free bile acids constitute 85% of the total bile acids.
on the skin surface in hepatobiliary disease with pruritus. Also on normal skin, the same high percentage of bile acids are un conjugated, as opposed to serum and biliary tract bile where bile salts are mainly conjugated; for this reason free bile acids were used in this investigation.

The observation that chenodeoxycholate and deoxycholate but not cholate produced pruritus correlates with the results of other investigators. Thus, feeding pure cholic acid to individuals produced a high serum cholic acid level, without pruritus. In addition, the intravenous injection of radioactively labelled un conjugated cholic acid in patients with Laënnec cirrhosis did not elicit pruritus. These studies show no evidence contradictory to the results of this study.

There was a greater amount of total dihydroxy bile acid derivatives (chenodeoxycholate plus deoxycholate) than of trihydroxy derivatives (cholate) in skin surface film in both the normal subjects and in the patient with obstructive jaundice with pruritus (Table 4). This finding suggests that the dihydroxy derivatives may be the more important causative agents of pruritus in obstructive biliary disease.

Another interesting finding shown in Table 4 is that in the patient with severe pruritus and obstructive jaundice from carcinoma of the pancreas, only the chenodeoxycholate level was substantially elevated. This observation, coupled with the data from this study, further suggests the important etiologic role of the dihydroxy acids in producing pruritus; furthermore it indicates that in complete obstructive jaundice, this particular primary bile salt is probably mainly responsible for the pruritus.

In incomplete biliary obstruction, as in the case of sclerosing cholangitis (Table 4), the elevation of both deoxycholate and chenodeoxycholate is a finding which, coupled with present data, indicates that these bile acids are probably the prurigenic agents. The same two compounds, therefore, probably cause the pruritus in most cases of incomplete obstruction, whether it is extrahepatic or intrahepatic, as for example in the cholestasis associated with pregnancy. In addition, the greater affinity of cholestyramine for dihydroxy than for trihydroxy bile acids produces a decreased dihydroxy-trihydroxy bile acid ratio on the skin surface, a ratio that is closely correlated with relief of pruritus. This observation also lends support to the idea that the dihydroxy acids probably produce the pruritus.

The rationale for the prurigenic properties of bile acids varying with pH is possibly related to the pKa of the responsible bile acid. Perhaps the glycine conjugate of chenodeoxycholate or deoxycholate, which predominate in bile, with its pKa ~ 4, is better absorbed at pH ~ 5 than at pH ~ 7 or pH ~ 8 because the larger number of neutral species of molecule present at a lower pH improves nonionic diffusion into skin. Similarly, free (unconjugated) bile acids, with their pKa ~ 6, would be better absorbed at pH ~ 5 or pH ~ 6 than at a higher pH if electrically neutral species of molecule are preferentially absorbed. The rationale for assaying pruritus at varying pH is based on the work of K.N. Jeejeebhoy, MD, (oral communication) in which it was shown that bile irritated the esophagus of rabbits only at acid pH.

The physical and chemical properties of the bile acid detergents differ quite widely from the synthetic detergents. Thus, bile salt solutions fail to show a clear critical micellar concentration, as shown by conductivity studies, as do typical ionic detergents. In addition, the bile salt solutions have a different molecular arrangement of polar and nonpolar regions than the ionic detergents. In view of these differing properties, it is not surprising that the biological characteristics of these agents also differ. Thus, the bile acids stimulate pruritus, while the synthetic detergents do not do so. In addition, the synthetic long-chain anionic detergent used in these experiments sometimes produced superficial necrosis of the skin after several days, whereas the bile acids never produced more than short-lived irritation.

It is quite likely, though not proved in this study, that the dihydroxy bile acids produce pruritus by a relatively specific pathophysiologic mechanism rather than by a nonspecific chemical injury. The main reason for this speculation is that detergent agents, such as lithocholic acid, and synthetic long-chain anionic and neutral detergents, such as sodium lauryl sulfate and Brij 35, respectively, produced inflammation in the skin to the same degree as deoxycholate and chenodeoxycholate but did not elicit pruritus. The specificity of the pruritus is further suggested by recent preliminary work in this laboratory indicating that a lower concentration of deoxycholate than was used in this investigation produced pruritus without inflammation greater than that produced by the cream vehicle alone.

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References

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