

8786 P

Effect of Varying pH on Toxic Effect of Bile Salts on the Normal Gall Bladder.*

HANS G. ARONSOHN AND EDMUND ANDREWS.

From the Department of Surgery, The University of Chicago.

On injection of bile salts into the dog's gall bladder we obtained, as reported in previous papers,^{1, 2} marked changes of the gall bladder wall, which on gross and microscopic examination, closely resemble those found in human cholecystitis. In the following study, these findings are subjected to thorough criticism because of their importance in explaining the origin of human cholecystitis.

First, a sufficient number of experiments, more than 70, have been performed to allow us to draw conclusions. Secondly, the technique used (retrograde catheter-cannula) has been described in a previous paper³ and an ample series of controls has been run. Furthermore, the unimportant rôle played by bacterial infection is seen from previous work, as injection of overwhelming numbers of bacteria into the dog's gall-bladder causes little or no reaction on the gall-bladder wall.⁴

A possible objection against our contention that the cholecystitis obtained had been caused by the toxicity of the bile salts, is that these bile salts might bring about their effect by changing the pH of the gall bladder bile, thus converting the bile itself to a toxic substance. There are two ways of controlling this. One would be to change experimentally the pH of the gall bladder bile by adding acids or alkali and to observe the effect this change might have in the gall bladder wall. The other consists of injecting bile salt solution after adjusting its pH to that of gall bladder bile. Both methods have been applied. Large amounts of strongly acid or alkaline solutions are necessary to overcome the buffering action of bile. Death of the animal and total gangrene of the gall bladder follows the injection of 5 cc. of glacial acetic acid into the dog's gall bladder. The pH after adding this acid to the gall bladder bile and thoroughly

* Work done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Aronsohn, H. G., and Andrews, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 87.

² Andrews, E., and Aronsohn, H. G., *loc. cit.*

³ Aronsohn, H. G., and Andrews, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1629.

⁴ Aronsohn, Hans G., *Am. J. Surg.*, 1936, New Series **32**, 18.

mixing was about 2. Similarly, total gangrene of the gall bladder wall was obtained by adding 10 cc. of Na_2CO_3 20% to the gall bladder bile with a final pH of 10. However, here death of the animal did not occur, and the effects were wholly local. The following detailed study shows the extent of the buffering potentiality of bile.

On aspirating the total gall bladder bile, washing the gall bladder with a citric phosphate buffer solution of pH 4 and leaving the gall bladder filled with this solution, in 2 experiments practically no change at all occurs in the gall bladder wall. In 2 other experiments, again the total gall bladder bile was aspirated and a citric phosphate solution of pH 3 left in the gall bladder. In one of these, the gall bladder, containing a colorless thin fluid, showed a moderate degree of acute cholecystitis. The other, however, was refilled with dark bile and revealed a normal gall bladder wall. It is quite obvious that the bile which had reentered the gall bladder through the hepatic duct by its buffering action had kept the acid mixture from developing an irritating action on the wall of the gall bladder.

The second series of experiments consists of the introduction of bile salts previously adjusted to the pH of the gall bladder bile into the dog's gall bladder and reveals similar results.

Various difficulties were encountered in performing these experiments. The first and chief difficulty was that adding of acid to a bile salt solution very easily causes precipitation of the bile acids. Special precautions are necessary to prevent this precipitation and keep the bile salts in solution. Furthermore, we have to consider that many acids, especially inorganic ones, if brought in contact with the gall bladder wall, themselves set up a reaction. We could overcome these difficulties by using for the adjustment a weak citric acid solution, which, as seen from controls, would not cause any change on the gall bladder wall even in higher concentration than that used. As an example the adjusting procedure for sodium desoxycholic is given as follows: 12 gm. sodium desoxycholic are dissolved in 100 cc. aq. dest. This solution which has a pH of 8.4, is heated to body temperature and 0.1 M citric acid added one drop at a time. The precipitate which occurs is dissolved by shaking. After the desired pH is reached (determined first roughly by a set of indicators) exact pH determination is done with Hastings colorimeter.

Table I shows the experiments with adjusted bile salts and the controls with citric acid.

Summary. 1. Strong acids or alkali of pH lower than 2 or higher

TABLE I.

Injected Solution	pH	Result
1. Sod. desoxychol. 10, 8%	pH after adjust. with 0.1 mol. citr. acid:	7,3 severe cholecystitis
2. " " 9%		7,2 " "
3. Cholic acid 12, 6%		7,8 medium "
4. " " " 5%		7,4 " "
5. Purif. B. S. 15%	pH without adjust. •	5,7 moderate "
6. " " 15%		5,7 " "
7. Citric acid-NaOH	1 M. citric acid adjust. with NaOH 10%	7 normal gallbladder
8. " " "		7,4 " "

than 10, cause a gangrene of the gall bladder wall. 2. When an acid solution at pH 3 is injected into the empty gall bladder and bile through patent ducts is allowed to reenter the gall bladder, the buffering action of this bile is sufficient to convert the acid bile mixture into a harmless solution. 3. The toxicity of bile salts on the gall bladder wall is not changed by adjustment to the pH of gall bladder bile previous to injection. 4. Citric acid solution, in concentration even higher than that used for the adjustment, does not affect the gall bladder wall.

Conclusion. The toxic action of the bile salts on the normal gall bladder is not affected by alteration of the pH unless it exceeds the normal pH range of gall bladder bile.

8787 P

Relative Toxicity of Different Bile Salts on the Normal Gall Bladder.*

EDMUND ANDREWS AND HANS G. ARONSOHN.

From the Department of Surgery, The University of Chicago.

In a previous publication¹ we have shown that an experimental increase of the bile-salt concentration in the bile produces changes in the gall bladder wall similar to "human cholecystitis" Sixteen experiments had been performed, using "commercial bile-salts" (Armour and Company), and so-called "purified bile-salts", *i. e.*, the commercial variety freed from non-alcohol soluble impurities by alcohol precipitation. The latter proved the more effective prepara-

* Work done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Andrews, E., and Aronsohn, H. G., *loc. cit.*