BILE SALT METABOLISM.

I. CONTROL DIETS, METHODS, AND FASTING OUTPUT.

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(Received for publication, October 1, 1928.)

These experiments are a logical continuation of the research programs of Foster and Hooper (2), Wisner (5), Smyth (5), and Whipple,—a comprehensive study of the physiology of bile salts as they are formed within the body, secreted, and reabsorbed. Since much of the older literature was discussed in these papers as well as in a general review (6) of this subject this need not detain us at this time.

These experiments also give needed information about the open bile fistula as compared with a certain type of closed bile fistula. The experiments noted above reported by Foster, Hooper, Wisner, Smyth, and Whipple were all done with the familiar open bile fistula. Such dogs have an obstructed common bile duct and an open fistula from the gallbladder through the right rectus and by proper technique bile can be quantitatively collected. On suitable diets such dogs may remain in normal health and activity for months or even years. Although the fistula is infected it does not cause impairment of health as long as the drainage is continuous and the diet carefully regulated. The presence of infection may cause interference with certain studies of bile pigments and is always an unpleasant factor, to be viewed with suspicion.

We decided therefore to use the closed sterile fistula method of Rous and McMaster (4) whereby the bile is kept sterile and collected continuously over 8, 12, or 24 hour periods. This method like all others in this difficult field is far from perfect and we note at times uncontrollable fluctuations which in many instances are to be explained by kinks where the cannula is tied into the bile duct, or by bits of mucus or precipitate forming a plug which may tempo-
rarily slow or obstruct the flow through the tubing. Frequent bile cultures were taken during all experiments and the tables show whether the bile was sterile or infected.¹

Infection with common air-borne spore bearers (for example *Bacillus subtilis*) does not cause any clinical disturbance and our experiments show that the bile salt metabolism is unchanged by the presence of these non-pathogenic bacteria in the bile passages, even with the closed fistula.

The experiments tabulated below indicate clearly that there are endogenous and exogenous factors concerned in bile salt metabolism. The level of bile salt excretion is less in sugar feeding days than in simple fasting (2), indicating an important relationship between the body protein breakdown and the bile salt output. From every point of view the bile salts are evidently related to protein breakdown—endogenous in these fasting experiments and exogenous or related to food protein digestion, as noted in the meat products feeding experiments tabulated in Paper II.

This paper includes data (Table 1) to show the bile salt and bile volume output per 8 or 12 hour periods. These experiments show similar figures to those noted in the paper of Whipple and Wisner (8). The output figures during daytime, evening, or night are almost identical when one averages the individual fluctuations of dogs on special or mixed diets. That the bile salt output is so little influenced by the time of food intake is somewhat surprising. It becomes obvious from a study of many tables given in Paper II that there is a distinct lag in output when a dog is fed a diet most favorable for bile salt production. This lag may amount to 24 to 48 hours in the curve of production before the maximum bile salt output is recorded.

Furthermore, after this favorable diet period there may be a "carry over" into a sugar feeding or unfavorable diet period so that the low sugar level may not be attained within 3 or 4 days (Table 23, Paper II). This indicates that this bile salt metabolism must be somewhat complex—not a simple protein splitting but a regrouping of amino acids with synthesis. All this takes time and may explain at least in part the fact that the hour of feeding does not modify the output of bile salt in the subsequent 8 hour

¹ We are indebted to Dr. J. A. Kennedy of the Department of Bacteriology for these cultures.
Smith, Groth, and Whipple

collection period. The question of storage of bile salt factors during favorable diet periods and subsequent depletion of such stores when need arises is discussed in Paper II and probably explains a part of the reaction.

Methods.

The bile fistula operations were performed after the method of Rous and McMaster (4). The gallbladder is removed and a glass cannula is tied into the common bile duct. From it the bile is led through about 20 cm. of rubber tubing of 3 mm. inside diameter and 1.5 mm. wall, and is brought to the outside through a stab wound in the right flank. The tube is joined to one arm of a 3-way glass connection which connects to a rubber balloon of 350 cc. capacity and by the other arm to an outlet tube which terminates in a glass tip and can be flamed before the bile is removed from the bag. Between collections this tip is kept covered with a rubber cap made by tying a bit of glass rod into one end of a short piece of fine rubber tubing. A number of these rubber caps are made up in advance and are autoclaved separately in Petri dishes. After the cap is placed over the glass tip a part of the cap and the exposed portion of the glass tip are wrapped with iodoform gauze so that at the time of the next collection the cap may be withdrawn without danger of organisms being introduced into the lumen of the glass tip. Several sterile sponges are fastened about the rubber tubing where it emerges from the stab wound. A little vaseline applied to the lower of these helps keep the sponge in close apposition to the wound and helps to prevent access of organisms along the course of the tubing. The rubber bag and sponges are covered over and protected by a light oval helmet-like copper basin thickly padded about the edges to prevent irritation and to give sufficient depth to prevent any possibility that the rubber bag underneath might be compressed and the free flow of bile impeded. This copper basin is about 20 by 10 cm. in diameter and when the edges are built up with padding is about 8 cm. deep in the middle. We found this arrangement superior to the grass baskets used by Rous and McMaster for, in our experience, the baskets are easily destroyed and now and then one of the stems wears loose and punctures the rubber bag, causing contamination of the contents, and, secondarily, generalized infection of the bile passages in the liver.
The copper basin is held in place by a canvas sling, the two terminal tails of which pass through small metal rings soldered to the top of the basin. A canvas jacket is then fitted to the dog. The jacket is tied up the back and is held from slipping by being provided with armholes for the front legs. The jacket is made to bulge outward over the metal basin to fit very snugly about the edges of the latter. If the jacket is properly made it holds the basin securely in place and allows one to dispense with the objectionable sheet of adhesive tape passed over the back of the dog by Rous and McMaster to anchor the basket in place.

Female dogs were used in all experiments so as to minimize the soiling of the jacket by urine. The bile is collected once a day, or oftener if desired, and with proper precautions sterile bile can be obtained from the dog several months before accumulations of bile salts, cells, or mucus stop up the cannula in the common bile duct or before accidental contamination allows access of organisms to the bile passages.

The need of careful and constant supervision of these bile fistula dogs cannot be too much emphasized. This requires almost the entire time of a skilled technician who weighs and observes the clinical condition of the dog. He takes the greatest care with the sterile bile collections which must be done with painstaking aseptic technique. Administration of any materials by stomach tube calls for skill and attention to subsequent feeding to minimize the tendency to vomiting. The whole day’s existence of the dog must be under his eye and the slightest departure from normal is at once brought to the attention of one of the writers.

The standard bread is crumbled into equal weights of water after which the canned salmon is well mixed with it into a sort of mash. This prevents the dog from picking out salmon meat alone. The figures in any table refer to the amounts of bread and salmon actually eaten. In all instances the mixtures contain 10 gm. of whole standard bread for each gm. of canned salmon.

The method of Foster and Hooper (1) for the analysis of bile salt in bile has been modified in several respects, though the general principles involved are the same. Larger quantities of material have been used, thus increasing greatly the precision. The modified technique is given in detail below. The bile salt is estimated from its content of amino nitrogen as determined by the method
of Van Slyke. As a preliminary step the taurine of bile salt must be freed from the cholic acid by hydrolysis, otherwise its nitrogen is not set free in the reaction chamber of the apparatus. Since the bile contains small amounts of certain substances having free amino groups it is necessary to determine these by a preliminary analysis carried out on unhydrolyzed bile. This value is deducted from the amount obtained after hydrolysis, the difference being the nitrogen derived from the taurine of the bile salt. The purpose of the preliminary treatment with hot alcohol is to remove the mucins and any other protein substances which may be present in the untreated bile.

As a preliminary step the bile is centrifuged to free it of any gross particles it may contain. 10 cc. of the bile are precipitated with 80 cc. of 95 per cent alcohol, heated on a steam bath to the boiling point, cooled, and made up to 100 cc. with alcohol. Filter. Two specimens of 40 cc. each are evaporated to dryness on the steam bath. One is washed out quantitatively with distilled water and is made up to 10 cc. with the latter. 3 cc. samples of this are used to determine the amount of amino nitrogen present before hydrolysis. The other specimen is washed out quantitatively with 6 cc. of 8 per cent sodium hydroxide into a 10 cc. volumetric flask. The flask is stoppered with a large piece of rubber tubing partially closed at the top with a tightly fitting piece of glass tubing drawn out to a tip so that evaporation will be minimized during the 6 hour period that the specimen is being hydrolyzed. The actual hydrolysis is carried out by allowing the bulb of the flask to hang down into the steam bath for this period. After the hydrolysis the contents of the flask are approximately neutralized by the addition of 0.5 cc. of 50 per cent acetic acid. The flask is then filled up to the 10 cc. mark with water. 3 cc. samples of this are used for the determination of the amino nitrogen in the Van Slyke micro apparatus. To obtain the mg. of bile acid nitrogen per cc. of bile the number of mg. of amino nitrogen in the 3 cc. sample of the hydrolyzed specimen minus the amount found to be present in the 3 cc. sample of the unhydrolyzed specimen is divided by the factor 1.20. To obtain total taurocholic acid the output of amino nitrogen is multiplied by the factor 36.7.

Interesting papers by Jenke, Enderlen, and Thannhauser (3) have just appeared. We have had no opportunity to use their
TABLE 1.

Salmon-Bread Diet, 8 Hour Collections.

Dog 26-97.

<table>
<thead>
<tr>
<th></th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-21</td>
<td>Salmon-bread 440</td>
<td>8.35 a.m.</td>
<td>24</td>
<td>63.0</td>
<td>0.51</td>
<td>32</td>
</tr>
<tr>
<td>22</td>
<td>Refused food.</td>
<td>8.05 &quot;</td>
<td>5.5</td>
<td>19.0</td>
<td>0.51</td>
<td>9.7</td>
</tr>
<tr>
<td>23</td>
<td>Salmon-bread 220</td>
<td>12.10 a.m.</td>
<td>8</td>
<td>9.0</td>
<td>0.69</td>
<td>6.2</td>
</tr>
<tr>
<td>24</td>
<td>Salmon-bread 352</td>
<td>12.05 a.m.</td>
<td>8</td>
<td>14.5</td>
<td>0.54</td>
<td>7.8</td>
</tr>
<tr>
<td>25</td>
<td>Salmon-bread 176</td>
<td>8.20 a.m.</td>
<td>16</td>
<td>33.0</td>
<td>0.71</td>
<td>24</td>
</tr>
<tr>
<td>26</td>
<td>Salmon-bread 440</td>
<td>8.20 a.m.</td>
<td>24</td>
<td>37.0</td>
<td>0.28</td>
<td>10.4</td>
</tr>
<tr>
<td>27</td>
<td>Salmon-bread 440</td>
<td>8.00 &quot;</td>
<td>8</td>
<td>21.0</td>
<td>0.49</td>
<td>10.3</td>
</tr>
<tr>
<td>28</td>
<td>Refused food.</td>
<td>8.03 a.m.</td>
<td>8</td>
<td>20.0</td>
<td>0.68</td>
<td>13.6</td>
</tr>
<tr>
<td>29</td>
<td>Salmon-bread 303</td>
<td>8.04 &quot;</td>
<td>24</td>
<td>25.0</td>
<td>0.56</td>
<td>14.0</td>
</tr>
<tr>
<td>30</td>
<td>Salmon-bread 440</td>
<td>8.04 a.m.</td>
<td>8</td>
<td>33.0</td>
<td>0.42</td>
<td>13.9</td>
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<tr>
<td>31</td>
<td>Salmon-bread 83</td>
<td>8.05 a.m.</td>
<td>8</td>
<td>31.0</td>
<td>0.35</td>
<td>10.8</td>
</tr>
<tr>
<td>2-1</td>
<td>Salmon-bread 193</td>
<td>8.04 &quot;</td>
<td>8</td>
<td>8.0</td>
<td>0.39</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.59 &quot;</td>
<td>8</td>
<td>13.5</td>
<td>0.45</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Average values of 8 hr. periods.

<table>
<thead>
<tr>
<th>Day</th>
<th>Evening</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>23</td>
<td>18</td>
</tr>
</tbody>
</table>

method but hope to report further on this point. Their tables do not include food intake, so that it is impossible to compare their figures with ours as the importance of food protein is not considered. Whenever given, the dog weight figures indicate a rapid loss of weight—for example, a loss from 20 to 14 kilos in 2 months. This means usually serious bile fistula infection or unsuitable food intake and introduces a very serious complication.

**Experimental Observations.**

The bread used in these experiments (Table 1) is prepared in this laboratory for use with anemic dogs as described elsewhere (7). It consists of wheat flour, starch, bran, sugar, canned salmon, cod liver oil, canned tomatoes, yeast, and a salt mixture. It contains 1.16 gm. of nitrogen per 100 gm. This bread is mixed and baked by one of the technicians. It supplies a complete diet capable of maintaining an adult dog in health for long periods if not indefinitely. The bread is palatable and eaten readily but small amounts (10 per cent by weight) of canned salmon are added to the bread as an appetizer. The figures given in Table 1 show the amount of bread and salmon which was eaten daily.

Table 1 shows a continuous observation period of 11 days during which 8 hour collections were the rule. This dog did not eat as regularly as could be desired and there are 2 days on which it refused food. Such fasting days were followed by days of low output of bile and bile salts. However, the dog made up on diet intake on subsequent days and its weight showed only a decrease from 11.5 to 11.2 kilos. There are many days when the bile output is quite regular per 8 hour period and it may be noted that the average values show but little difference between the day, evening, and night collections. The night collections are very slightly below the day and evening periods. It is especially noteworthy that the output in the 8 hours following midnight is practically as great as at other times despite the absence of food and despite the fact that the dogs are at rest during this part of the day. The bile fistula was sterile during this entire period, yet on two occasions we note no collection during an 8 hour period. We must explain this by a transient obstruction due to a kink or to a small plug of mucus or sediment in the bile. Low diet intake is probably in part responsible for the low output of the last 2 days.
There are considerable fluctuations in bile concentration shown by the figures of amino nitrogen in 1 cc. of bile. Some factors which influence bile concentration will be discussed in the other papers of this series but we must admit that in general we cannot explain such variations as they occur from day to day.

Table 2 shows a continuation of observations on Dog 26-97 as in Table 1. The bile cultures remained sterile throughout. We observe the rapid fall in bile secretion and bile salt output when the dog is fasting, yet there is a considerable “carry over” from the bread period into the fasting period so that the fasting level is not reached until the 3rd or 4th day without food. We note a great concentration of bile salts in whole bile during fasting so that the fasting bile contains 3 or 4 times as much bile salt per 1 cc. of bile. It is clear that in this experiment the night output falls below the day output. We may note the following facts as bearing on this observation. There is no change in bile concentration but only in

<table>
<thead>
<tr>
<th>Date</th>
<th>Wt.</th>
<th>Dist.</th>
<th>Hr. of collection</th>
<th>Vol. of bile</th>
<th>Amino nitrogen.</th>
<th>Total taurine acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-2</td>
<td>11.2 Salmon-bread 121</td>
<td>8.00 a.m.</td>
<td>24</td>
<td>44</td>
<td>0.43</td>
<td>19.0</td>
</tr>
<tr>
<td>3</td>
<td>“ 314</td>
<td>8.05 “</td>
<td>24</td>
<td>62</td>
<td>0.38</td>
<td>23.5</td>
</tr>
<tr>
<td>4</td>
<td>“ 8.00 “</td>
<td>24</td>
<td>54</td>
<td>0.50</td>
<td>27.0</td>
<td>995</td>
</tr>
<tr>
<td>5</td>
<td>“ 8.00 “</td>
<td>24</td>
<td>31</td>
<td>0.45</td>
<td>14.0</td>
<td>515</td>
</tr>
<tr>
<td>6</td>
<td>“ 11.57 p.m.</td>
<td>16</td>
<td>13</td>
<td>0.66</td>
<td>8.6</td>
<td>317</td>
</tr>
<tr>
<td>7</td>
<td>“ 11.00 a.m.</td>
<td>12</td>
<td>4.9</td>
<td>0.74</td>
<td>3.6</td>
<td>132</td>
</tr>
<tr>
<td>7</td>
<td>“ 12.02 “</td>
<td>12</td>
<td>6.5</td>
<td>1.32</td>
<td>8.6</td>
<td>317</td>
</tr>
<tr>
<td>7</td>
<td>“ 8.50 “</td>
<td>8</td>
<td>3.0</td>
<td>1.10</td>
<td>3.3</td>
<td>122</td>
</tr>
<tr>
<td>8</td>
<td>“ 11.57 p.m.</td>
<td>15</td>
<td>2.6</td>
<td>1.50</td>
<td>3.9</td>
<td>144</td>
</tr>
<tr>
<td>8</td>
<td>“ 8.05 a.m.</td>
<td>8</td>
<td>1.4</td>
<td>2.10</td>
<td>2.9</td>
<td>107</td>
</tr>
</tbody>
</table>

Average values per hr. during fast.
Day and evening:.............................. 0.40 1.02 0.49 18.2
Night and morning:......................... 0.24 1.10 0.26 9.5

bile salt output. We are dealing with very small volumes of concentrated bile and we may argue that bodily activity may favor the outflow of this syrupy bile sufficiently to explain these differences. We hesitate to believe that the fasting organism produces more bile salt during moderate activity than during rest but prefer to think of activity squeezing out more completely from the biliary tree this thick syrupy concentrated bile.

DISCUSSION.

We wish to compare these experimental periods (Tables 1 and 2) with those in subsequent papers—for example, Tables 21 and 27 (Paper II). These other diet periods with standard bread and salmon give a remarkably constant total bile salt output—about 100 mg. of bile salt per kilo of body weight per 24 hours. This gives us a fixed base-line and the evidence is strong that the production is pretty uniform throughout the whole day, regardless of the hours of feeding or sleep. If anything is to be said it is to the effect that the early morning hours may show a very small decrease as compared with the early evening bile salt output when digestion is at its height.

These observations are entirely in harmony with those of Wisner and Whipple (8) who made 24 hour collections subdivided into 6 hour intervals. We may compare our experiments on a diet of standard bread plus salmon with their experiments in which a diet of potato, rice, and milk was used. The output per kilo per 24 hours is very close to 100 mg. Wisner and Whipple (8) concluded that there was little if any difference between the various 6 hour periods and if anything could be said it was to the effect that the night bile output might be slightly less than the normal day output. This shows absolute parallelism between the closed sterile fistula and the open infected fistula in this type of experiment. In comparing the fasting and sugar feeding experiments in this and subsequent papers we may refer to the older experiments of Foster, Hooper, and Whipple (2). During fasting or sugar feeding (Table 2, and Tables 23 (Paper II) and 32 (Paper III)) we note very low values for bile salts—about 300 to 400 mg. of bile salt per 24 hours or 30 to 40 mg. per kilo of body weight per 24 hours. Foster, Hooper, and Whipple (2) record higher values or about 100 mg. of bile salt per kilo of body weight per 24 hours, for fasting dogs and
about 80 mg. of bile salt per kilo of body weight per 24 hours for sugar-fed dogs.

In correlation of these results we may submit the following argument. The older experiments of Foster, Hooper, and Whipple (2) were done with an open fistula which we believe introduces no change in bile salt production, but their experiments dealt with 6 hour collections and the calculation for 24 hours is given on this basis. We know that the fasting bile is concentrated and syrupy and some bile could be licked from the open fistula during night periods of non-collection. Also these open fistula dogs were set up each morning to drain for 30 minutes to permit any excess of accumulated night bile to escape before the 6 hour collection was begun. It is possible, if not probable, in view of the data here given (Table 2) that the syrupy night bile did not completely drain during this 30 minute preliminary drainage and therefore increased the 6 hour output.

It is obvious that fasting will reduce the output of bile and bile salt to low levels. The output of bile salt in our experiments cannot be reduced below 30 to 40 mg. of bile salt per kilo of body weight per 24 hours. This we may term the base-line of endogenous bile salt production and as it continues over long periods of time we must assume that this bile salt must come from body tissue breakdown. The fact observed by Foster, Hooper, and Whipple, that the output of bile salt decreases with sugar feeding as compared with fasting periods, is further evidence that body protein breakdown is the source of some fraction which is built into the finished bile salt within the liver.

We note also in sugar feeding (Table 23, Paper II) or fasting that the bile salt output falls slowly over a period of 2 to 3 days when the sugar diet or fasting begins. Again when the dog is given a favorable diet following a period of sugar feeding or fasting there is a slow rise during 2 to 3 days to the expected favorable diet level. This may indicate a certain storage of materials suitable for bile salt production to be gradually depleted as fasting continues. When a favorable diet follows fasting we may postulate a storage of a part of this material which will not appear as bile salt until subsequent emergency needs arise.
SUMMARY.

Bile salt secretion in dogs is remarkably uniform during the 24 hours in spite of a single daily feeding.

Daytime (8.00 a.m. to 4.00 p.m.) or evening (4.00 p.m. to 12.00 m.) or night-time (12.00 m. to 8.00 a.m.) all show practically equivalent bile salt output. There may be a very slight difference — lower at night-time during the period of bodily quiet, when compared with the evening collection which includes maximal digestive activity.

The base-line output of bile salt on a diet of standard salmon bread is very close to 100 mg. per kilo of body weight per 24 hours. Individual dogs may show values as low as 80 or as high as 130 mg., but this level is fairly constant for any given dog.

Fasting reduces the bile salt output to very low levels. Sugar feeding likewise shows very low levels, even lower than fasting.

Bile salt output continues even during long fasting periods. This fasting base-line is about 30 to 40 mg. of bile salt per kilo of body weight per 24 hours. This represents the endogenous portion of the bile salt formation. There is good reason to believe that the breakdown of body protein contributes an essential component for such bile acid synthesis.

BIBLIOGRAPHY.

BILE SALT METABOLISM: I.
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