OBSERVATIONS CONCERNING THE PRODUCTION AND EXCRETION OF CHOLESTEROL IN MAMMALS

III. THE SOURCE OF EXCESS PLASMA CHOLESTEROL AFTER LIGATION OF THE BILE DUCT*

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The rise in plasma cholesterol, occurring after ligation of the common bile duct of most mammals, is recognized. Some observers (1–3) believe this cholesterol to be formed in the liver; others (4) think that the liver may excrete cholesterol formed elsewhere. A study of plasma cholesterol after hepatectomy would decide this question, for a rise similar to that following bile duct ligation should occur if the liver is merely an excretory organ, while no rise could occur if the liver acts primarily as a source of cholesterol. This crucial experiment has not been reported because the survival time after hepatectomy is usually less than the time required for the accumulation of plasma cholesterol in control animals subjected solely to ligation of the bile duct. The liverless dog, for instance, rarely survives more than 24 hours, but 1 or 2 weeks are required (5) for a consistent rise in plasma cholesterol to become evident after duct ligation. Results of work with dogs are therefore inconclusive (6).

We have found (7) that the rat accumulates plasma cholesterol after duct ligation sufficiently rapidly to allow observation of the results of procedures which might prove fatal in other species before a significant change took place. These results indicate that the source of the cholesterol increment in the plasma after bile duct ligation is the liver.

EXPERIMENTAL

Plasma Total Cholesterol after Bile Duct Ligation—The bile ducts of male albino rats were ligated distal to the hepatic branches and above the entrance of the pancreatic duct. Plasma samples at 6, 12, 24, and 48 hours after operation were analyzed by methods previously described (7). The results, presented in Table I, show a 46 per cent increase in 6 hours, almost as great an increase in the next 6 hours, a lesser rate of increase from 12 to 24 hours, and accumulation during the second 24 hour period

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at even a more rapid rate than during the first 24 hours. The rise consisted very largely of free cholesterol.

Castration—The average total cholesterol concentration of plasma, 24 hours after duct ligation in rats castrated 15 days previously, was even higher than that in the control group, possibly because the animals were younger. The data, given in Table II, show the testis to be unnecessary for an increase in plasma cholesterol concentration after duct ligation.

### Table I

<table>
<thead>
<tr>
<th>Duration of ligation</th>
<th>No. of rats</th>
<th>Average weight of rats</th>
<th>Average plasma cholesterol, mg. per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td></td>
<td>gm.</td>
<td>Total</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>294</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>279</td>
<td>86</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>317</td>
<td>111</td>
</tr>
<tr>
<td>24</td>
<td>16</td>
<td>294</td>
<td>124</td>
</tr>
<tr>
<td>48</td>
<td>7</td>
<td>356</td>
<td>275</td>
</tr>
</tbody>
</table>

### Table II

**Effect of Bile Duct Ligation on Plasma Cholesterol after Prior Removal of Various Organs**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of rats</th>
<th>Average weight</th>
<th>Plasma cholesterol, mg. per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td>Before ligation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Castration</td>
<td>9</td>
<td>139</td>
<td>69</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>4</td>
<td>393</td>
<td>65</td>
</tr>
<tr>
<td>Viscerectomy (intestines, spleen, kidneys)</td>
<td>8</td>
<td>389</td>
<td>61</td>
</tr>
<tr>
<td>Ligation of thoracic duct</td>
<td>7</td>
<td>350</td>
<td>60</td>
</tr>
</tbody>
</table>

Adrenalectomy—Four rats were adrenalectomized, and a loose ligature was placed about the bile duct. The ends of the ligature were brought out on opposite sides of the abdomen and secured by clips. The animals were maintained by furnishing 0.85 per cent NaCl as drinking fluid. 4 days after adrenalectomy the opposite ends of the loose ligature were pulled, thus occluding the bile duct without inflicting operational shock on the adrenalectomized rats.

These animals exhibited a rise in plasma cholesterol concentration after duct ligation similar to that observed in control animals, and similarly
confined to free cholesterol. The data in Table II accordingly show the source of the extra cholesterol to be elsewhere.

Viscerectomy—The gastrointestinal tract, spleen, and kidneys were removed from eight rats. The hepatic artery was left as blood supply to the liver. The bile duct was ligated, and the average rise in total cholesterol concentration after 24 hours was found to be only slightly less than that observed in control animals, although the esterified cholesterol rose more than usual. These results (see Table II) indicate that the viscera are not the source of the excess plasma cholesterol.

**TABLE III**

*Effect of Total and Partial Hepatectomy upon Plasma Cholesterol*

<table>
<thead>
<tr>
<th>Hepatectomy with duct ligation</th>
<th>No. of rats</th>
<th>Average weight</th>
<th>Average plasma cholesterol, mg. per 100 cc.</th>
<th>Average liver removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before operation</td>
<td>6 hrs. after operation</td>
<td>24 hrs. after operation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Free</td>
<td>Esterified</td>
</tr>
<tr>
<td>Total ........................</td>
<td>7</td>
<td>317</td>
<td>52</td>
<td>14</td>
</tr>
<tr>
<td>Partial ........................</td>
<td>15</td>
<td>288</td>
<td>61</td>
<td>13</td>
</tr>
<tr>
<td>&quot; with continuous intravenous lecithin injection.*</td>
<td>10</td>
<td>345</td>
<td>51</td>
<td>9</td>
</tr>
</tbody>
</table>

* Lecithin 0.50 per cent, choline 0.25 per cent, inositol 0.13 per cent in water. Four rats received egg lecithin; six received soy bean “lecithin.”

Ligation of Thoracic Duct—Seven rats were subjected to both bile and thoracic duct ligation, the latter duct being ligated just caudal to the diaphragm. The data in Table II again show the plasma cholesterol rise to be substantially unaffected.

Complete Hepatectomy—Seven rats were subjected to constriction of both the inferior vena cava and portal veins by encircling these vessels with polyethylene tubing of 0.045 inch internal diameter. Within 21 days sufficient collateral circulation developed to allow a complete hepatectomy in one stage. The animals were given subcutaneous glucose and were in good condition for 6 hours, becoming moribund shortly thereafter. The hepatectomized rats (see Table III) showed no significant increase in plasma cholesterol concentration within 6 hours. Their packed cell volume was 46 per cent before operation and 41 per cent afterward.

Partial Hepatectomy—Only the right liver lobe was allowed to remain in situ, and the bile duct draining it was ligated. Such animals maintained good condition, though supplied only with water ad libitum. Table III
PLASMA CHOLESTEROL

shows that free cholesterol rose significantly in 24 hours, while the esterified cholesterol declined. This result indicates that no net rise in total plasma cholesterol concentration took place. The packed cell volume declined from 47 per cent before the operation to 31 per cent afterward.

Partial Hepatectomy and Lecithin Infusion—In order to test to some extent the possibility that the liver might produce not cholesterol itself, but substances which, accumulating in plasma, cause a secondary rise in cholesterol (8, 9), a continuous intravenous infusion of a solution containing 0.5 per cent lecithin, 0.25 per cent choline, and 0.125 per cent inositol was given to rats previously subjected to subtotal hepatectomy. The infusion was given at the rate of 0.5 cc. per hour over 24 hours. Table III shows that the infusion did not increase the cholesterol concentration significantly above the increase in non-infused, partially hepatectomized rats. Packed cell volumes were not obtained on these animals. Duct ligation was performed as before.

Partial Hepatectomy and Bile Infusion—The possibility that the liver normally excretes into the bile substances which, accumulating in plasma after duct ligation, might lead to a secondary increase in cholesterol, was investigated in the following manner. Four pairs of rats were selected. One of each pair was partially hepatectomized, and the bile duct was ligated; polyethylene tubing was introduced into the common bile duct of the other rat. The distal end of the catheter was inserted into the right external iliac vein of the first rat of each pair. The animals were restrained in Bollman (10) cages. In this way unchanged bile from a normal donor was allowed to enter the circulation of a partially hepatectomized recipient, in which the bile duct was ligated, over a period of 24 hours. Previous work (11) has shown that 10 to 15 cc. of bile are excreted through a fistula per rat per day. No experiment was considered valid unless the plasma of the donor rat was not jaundiced after 24 hours, and the bile duct was undilated at autopsy.

The average total cholesterol content of the recipient rats in the four satisfactory experiments was 44 mg. per 100 cc. This value shows no significant increment over those reported in Table III for uninfused, partially hepatectomized, ligated rats, notwithstanding the fact that the infused recipients received an average of 1.8 mg. of cholesterol in the donor bile (11).

DISCUSSION

Our failure to prevent the rise of cholesterol in the plasma of rats subjected to bile duct ligation by (a) prior castration, (b) adrenalectomy, (c) evisceration, and (d) ligation of the thoracic duct indicates that no abdominal organ, with the exception of the liver, is responsible for the rise in plasma cholesterol after ligation of the common bile duct. On the
other hand, the failure of the hepatectomized and partially hepatectomized rat with duct ligation to exhibit a rise in plasma cholesterol indicates that the liver is responsible for this rise. The inability of intravenous infusions of lecithin, inositol, choline, or unchanged bile to change significantly the cholesterol content of plasma of partially hepatectomized rats with bile duct ligation suggests that the failure of cholesterol to rise after hepatectomy is not due to the absence of these (hypothesized) cholesterol-mobilizing substances normally available in the liver. The conclusion, therefore, seems probable that the liver itself directly produces or discharges into the blood stream the excess cholesterol found in the plasma of rats after ligation of the bile duct.

This ability of the liver to produce the excess cholesterol found in plasma after duct ligation does not mean that the liver is necessarily concerned with the formation of the cholesterol found in the plasma of normal, intact rats. In other words, it is quite possible that ligation of the bile duct itself ushers in a train of abnormal mechanisms, one of which may be the production and discharge by the liver of an excess amount of cholesterol.

**SUMMARY**

After ligation of the common bile duct, rats exhibited a rapid and marked rise in the cholesterol content of their plasma. This increase consisted largely of free cholesterol. The rise could not be prevented by prior castration, adrenalectomy, viscerectomy, or ligation of the thoracic duct. Total hepatectomy and partial hepatectomy prevented this rise. Intravenous infusion of choline, inositol, lecithin, or unchanged bile was unable to produce excess cholesterol in the partially hepatectomized animal. It is concluded that the liver itself discharges into the blood stream the excess cholesterol occurring in plasma after bile duct ligation.

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**BIBLIOGRAPHY**

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