FAT METABOLISM IN THE DOG FOLLOWING LIVER INJURY PRODUCED BY CARBON TETRACHLORIDE

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Van Dolah and Crandall (1) observed that in the fasting Eck fistula dog the total plasma fatty acids and cholesterol are reduced. The aim of the investigation reported in this paper was to repeat that observation if possible, with another and more gradual type of liver damage. Attention was to be paid to the development of the disturbance in fat metabolism. In addition, a further insight into the rôle of the liver in fat metabolism was sought by the use of physiologic means of altering the blood fat level, and by comparison of the response of normal dogs with that of dogs with liver damage. With this end in view, a study was carried out of the amount and composition (as demonstrated by iodine number determinations) of blood fatty acids following the feeding of linseed oil and of glucose. Normal dogs and dogs treated with carbon tetrachloride were used. The drug used has been reported by Gardner et al. (2) to cause fatty infiltration and central necrosis of the liver when given orally to dogs. Other lesions produced by the oral administration of this substance were inconsequential. Bollman and Mann (3) have shown that long continued administration to dogs leads to cirrhosis of the liver.

Rony and Ching (4) have shown that glucose feeding prevents the alimentary lipemia following a fat meal, acting, they suggest, by increasing the uptake of fatty acids by the tissues. Lichtman (5) reports that glucose causes a fall in the blood fat of normal dogs. Since the liver is actively concerned with the rapid uptake of fatty acids from the blood (Artom (6), Artom and Peretti (7), Aylward, Channon, and Wilkinson (8)), it was hoped that there would be some significant change from the normal in this reaction following liver damage.
Fat Metabolism after Liver Injury

Procedure

Nine dogs were used. These animals had been kept on a basal diet \(^1\) for a period of at least 4 weeks before the start of the experiment. They had consistently shown an alimentary lipemia in several determinations under the conditions given below. These dogs were divided into three groups of three each, and after a preliminary observation of the plasma fatty acid response to linseed oil feeding, two of each group were given carbon tetrachloride daily. The remaining animal was kept as a normal control. The procedure followed at the start of the experimental period was to give each of the test animals 2 cc. of carbon tetrachloride per day in capsules. After 2 weeks trial this amount was increased or decreased so that all treated dogs eventually showed a uniform reaction, as judged by their loss of weight, appetite, yellow tint of the plasma, and general appearance. The final amounts given ranged

\[^1\] Austin Dog Bread, Austin Dog Bread and Animal Food Company, Chelsea, Massachusetts.

\[
\begin{array}{lcccc|ccc|ccc}
\text{Dog No.} & \text{Weight} & \text{Hrs. after fat feeding} & \text{Total plasma fatty acids} & \text{Total plasma cholesterol (fasting)} \\
 & \text{kg.} & \text{mg. per cent} & \text{mg. per cent} & \text{mg. per cent} & \text{mg. per cent} & \text{mg. per cent} & \text{mg. per cent} \\
0 & 1.5 & 3 & 5 & 0 & 1.5 & 3 & 5 & \\
20 & 13.2 & 342 & 107 & 415 & 121 & 490 & 117 & 358 & 119 & 202 \\
21 & 10.8 & 376 & 103 & 414 & 121 & 474 & 123 & 520 & 120 & 232 \\
16 & 13.2 & 465 & 107 & 492 & 105 & 522 & 102 & 402 & 102 & 221 \\
5 & 13.2 & 447 & 103 & 495 & 111 & 644 & 119 & 614 & 116 & 148 \\
9 & 12.3 & 410 & 95 & 483 & 106 & 519 & 114 & 534 & 111 & 136 \\
14 & 10.9 & 362 & 96 & \dagger & 451 & 110 & 411 & 112 & 176 \\
13 & 10.9 & 428 & 90 & 475 & 102 & 541 & 115 & 560 & 113 & 186 \\
\end{array}
\]

* Not included in the averages given in Table II, normal, 0.
† Spilled.
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from 5 cc. per day (Dog 21, 16.8 kilos) to 1 cc. per day (Dog 20, 11.4 kilos). The average daily dose was 1 cc. per 5 kilos of body weight. The plasma fatty acid response to linseed oil feeding was determined on all dogs at 2, 4, 11, and 13 weeks, and to glucose feeding at 12 weeks following the beginning of the carbon tetra-

**Table II**

*Average Values for Normal Dogs and Dogs Treated with Carbon Tetrachloride*

The animals were fed 5 cc. of linseed oil (iodine number, 150) per kilo by stomach tube after a 3 day fast.

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>Group</th>
<th>No. of dogs</th>
<th>No. of dogs</th>
<th>No. of dogs</th>
<th>No. of dogs</th>
<th>Total cholesterol</th>
<th>Rise in fat</th>
<th>Change in I No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>wks.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preliminary</td>
<td>N</td>
<td>8</td>
<td>399</td>
<td>99</td>
<td>450</td>
<td>109</td>
<td>502</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>5*</td>
<td>284</td>
<td>78</td>
<td>336</td>
<td>91</td>
<td>319</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>3</td>
<td>428</td>
<td>91</td>
<td>508</td>
<td>102</td>
<td>568</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>5*</td>
<td>284</td>
<td>78</td>
<td>336</td>
<td>91</td>
<td>319</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>N</td>
<td>3</td>
<td>434</td>
<td>100</td>
<td>459</td>
<td>102</td>
<td>478</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>4*</td>
<td>250</td>
<td>82</td>
<td>270</td>
<td>84</td>
<td>305</td>
<td>98</td>
</tr>
<tr>
<td>11</td>
<td>N</td>
<td>3</td>
<td>325</td>
<td>106</td>
<td>396</td>
<td>122</td>
<td>439</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>6</td>
<td>235</td>
<td>100</td>
<td>251</td>
<td>103</td>
<td>290</td>
<td>103</td>
</tr>
<tr>
<td>13</td>
<td>N</td>
<td>2†</td>
<td>353</td>
<td>106</td>
<td>373</td>
<td>108</td>
<td>401</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>5</td>
<td>259</td>
<td>99</td>
<td>297</td>
<td>98</td>
<td>337</td>
<td>101</td>
</tr>
</tbody>
</table>

N represents normal; T, treated.

* Dog 21 which gave no clinical signs of liver damage at a level of 2 and 3 cc. of CCl₄ per day was excluded from the average. The fasting values for total fatty acids were 318 mg. per cent, iodine number 92, for the 2nd week; 375 mg. per cent, iodine number 105, for the 4th.

† The determination for Dog 16 was lost.

chloride administration. All animals were kept on the basal diet throughout the experimental period.

It was found, in accordance with the work of Rony and Ching (4), that without a fasting period of some days the lipemia following a fat meal was inconstant in its appearance. Consequently the following procedure, with which a lipemia was invariably secured, was employed. The dogs to be used were fasted for 3 days.
Fat Metabolism after Liver Injury

After a fasting blood sample was taken, 5 cc. of linseed oil (iodine number, 150) per kilo of body weight were given by stomach tube, and subsequent blood samples were drawn at 1.5, 3, and 5 hours after the fat meal. These samples were oxalated, centrifuged, and the plasma analyzed for total fatty acids (Bloor (9)), iodine number of the total fatty acids (Yasuda (10)), and total cholesterol.

**TABLE III**

<table>
<thead>
<tr>
<th>Lipid Fractions in Plasma of Dogs Fasted 3 Days; Given Carbon Tetrachloride Treatment for 10 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog No.</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>Treated</td>
</tr>
<tr>
<td>29</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>26</td>
</tr>
</tbody>
</table>

* In the light of recently completed determinations in this laboratory, these values would appear to be low. However, since the analyses were made on both normal and treated dogs at the same time, with identical techniques and solutions, it is believed justifiable to include them for comparative purposes.

† Values obtained by subtracting phospholipid and cholesterol ester fatty acids from total fatty acids. Cholesterol ester acids were calculated by assuming 50 per cent of the normal total cholesterol to be in the ester form; the decrease from 165 mg. per cent of total cholesterol in the treated dogs is assumed to be entirely in the ester form.

(Bloor et al. (11)). As examples of the rise in plasma fatty acids obtained by this method, in Table I are given the results of the preliminary determinations mentioned above. Since in over 100 determinations on normal and treated dogs no consistent change in the total cholesterol was found, only the fasting values are listed.

Dog 14 was killed accidentally the 3rd week after the carbon tetrachloride treatment was begun, and Dog 5 died from the ef-
fects of the drug in the 8th week. Dogs 24 and 26 were substituted for these animals, but experiments were not performed on these substitutes before the 11th week of treatment. Dog 20 died in the 12th week, and was not replaced.

The data obtained from the feeding of the linseed oil to normal and treated dogs are given in Table II. Since the response observed was quite uniform, and to save space, only the averages of the individual values for each time period are given.

### Table IV

**Effect of Glucose Feeding on Total Plasma Fatty Acids**

3 gm. of glucose per kilo of body weight were given after a 3 day fast to normal and carbon tetrachloride-treated dogs.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Hrs. after glucose feeding</th>
<th>Change in fatty acids</th>
<th>Change in % T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>113</td>
<td>421</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>107</td>
<td>364</td>
</tr>
<tr>
<td>Average</td>
<td>416</td>
<td>107</td>
<td>364</td>
</tr>
<tr>
<td>Treated</td>
<td>2</td>
<td>106</td>
<td>268</td>
</tr>
<tr>
<td>12 wks.</td>
<td>26</td>
<td>102</td>
<td>324</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>108</td>
<td>343</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>97</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>101</td>
<td>296</td>
</tr>
<tr>
<td>Average</td>
<td>326</td>
<td>103</td>
<td>300</td>
</tr>
</tbody>
</table>

To study further the effect of liver damage on fat metabolism, the phospholipid fatty acids in the plasma of five normal and five treated dogs (10th week of treatment) were determined by the method of Bloor (12). An attempt was made to fractionate the solid and liquid fatty acids of the plasma phospholipids, with the microtechnique of Sinclair (13), but the results were too variable to be reported at the present time. The total phospholipid fatty acid values obtained are given in Table III.

It is known that the blood cholesterol decrease due to liver dam-
age is at the expense of the ester fraction (Trumper and Cantarow (14)). Assuming that 50 per cent of the cholesterol in normal dog plasma is in the ester form, it is possible to calculate the “neutral fat” in the plasma. This value represents the difference between the total fatty acids found and the sum of the phospholipid fatty acids and the (calculated) fatty acids bound to cholesterol. The fractionation of the various lipid constituents of the plasma of the normal and treated dogs, according to this scheme, is also given in Table III.

The response of the plasma fatty acids of normal and treated dogs (12th week) to glucose feeding is illustrated in Table IV. The animals were fasted for 3 days before being used, and 3 gm. of glucose per kilo of body weight were given in solution after a fasting blood sample had been taken. The dogs were bled again at 1.5 and 3 hours after the sugar feeding.

Since only three normal animals were used, individual data as well as averages are given.

**DISCUSSION**

At the end of 2 weeks treatment the plasma of the treated animals had a distinct yellow tint, and the average weight loss was 8.3 per cent of the original body weight. From Table II it will be seen that at this time the fasting plasma values of total fatty acids and cholesterol had undergone a marked decrease. Liver damage from carbon tetrachloride poisoning therefore produces the same effect on the fasting plasma fatty acids and cholesterol as does that brought about by the Eek fistula. In addition, there was a definite decrease in the iodine number of the treated dogs as compared to that of the normal animals. These changes were relatively abrupt, occurring in the first 2 weeks of treatment, before the animals showed any marked clinical evidence of injury.

In spite of this evidence of an impaired fat metabolism, the dogs with early (2 weeks treatment) liver injury showed an essentially normal lipemia curve following the feeding of linseed oil (iodine number, 150). The rise in fatty acids was within the normal range, and the expected rise in iodine number occurred. The response at the end of 4 weeks treatment cannot be considered abnormal. By the 11th week, however, while the plasma fatty acids rose as usual following the fat meal, the iodine number was
found to undergo little change. At this time the plasma had a
deep yellow color, the weight loss averaged 18.9 per cent, and the
animals were very thin and showed a loss of appetite and activity.
The fat depots were entirely depleted.

The following explanation is offered as a partial interpret,
ation of the above observations. The desaturation of fatty acids shown
by Schoenheimer and Rittenberg (15) to occur in the animal body
is brought about by the liver, at least to a large extent. This
process is interrupted by poisoning with carbon tetrachloride,
with an immediate drop in unsaturated fatty acid and phospholipid
production (Tables II and III). After several months of such
treatment, the unsaturated acid supply of the tissues is exhausted
by the process of wear and tear. Subsequently, in accordance
with Sinclair's observations (16) on the rate of turnover of tissue
phospholipids, absorbed unsaturated fatty acids are removed from
the blood stream by the tissues too rapidly to allow a rise in the
iodine number of the plasma fatty acids. Furthermore, liver
damage could thus prevent the preferential removal and desatura-
tion of saturated acids from the blood, or the displacement and
release of unsaturated acids, when stimulated to do so by the in-
gestion of glucose (Table IV).

The cause of the decrease in blood fatty acids and the fat stores
of the body, and the question of fat absorption as influenced by
liver damage remain to be investigated.

SUMMARY

1. Dogs in which liver injury has been produced by carbon tetrachloride poisoning show a reduction below normal of the fasting value of total plasma fatty acids and cholesterol. This is in accord with observations on the Eck fistula dog.

2. The iodine number of the total plasma fatty acids (fasting) of dogs treated with carbon tetrachloride is less than that of normal controls.

3. In dogs, after 10 weeks treatment with carbon tetrachloride, the amount and iodine number of the plasma phospholipid fatty acids are reduced.

4. After 4 weeks treatment with carbon tetrachloride, the response to linseed oil feeding is normal in amount and iodine number change. At the end of 3 months treatment, while the response is normal in amount, no change in iodine number occurs.
5. The ingestion of glucose by normal dogs leads to a decrease in the amount and an increase in the iodine number of the plasma fatty acids. Dogs treated with carbon tetrachloride for 3 months show the usual decrease in amount, but no change in the iodine number.

6. A partial interpretation of these findings in the light of the rôle of the liver in fat metabolism is presented.

BIBLIOGRAPHY

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