THE EFFECT OF CHOLINE ON THE LIPID METABOLISM OF BLOOD AND LIVER IN THE COMPLETELY DEPANcreatIZED DOG MAINTAINED WITH INSULIN*

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Previous studies from this laboratory (1, 2) have shown that pancreas contains two factors active in the lipid metabolism of the depancreatized dog maintained with insulin: (1) a heat-labile blood factor, the ingestion of which causes a rise above normal in all blood lipid constituents, in particular cholesterol; this factor is partially or completely destroyed when pancreas is subjected to autoclaving at 20 pounds steam pressure for 30 minutes; (2) a heat-stable liver factor, the administration of which prevents the deposition of fat in the liver; this factor is not destroyed by the above heat treatment.

The curative action of lecithin on the fatty liver of the depancreatized dog receiving insulin was discovered by Hershey (3). A later study by Best and his coworkers (4) showed that its active constituent is choline, and these workers ascribe the action of pancreas on the liver to its choline content. Dragstedt and his coworkers (5), however, claim to have ruled out choline as the active factor in the pancreas. For 2 to 4 weeks they fed depancreatized dogs choline in amounts up to 700 mg. daily (the latter amount being that contained in approximately 250 gm. of pancreas) and this treatment failed to cure fatty livers. But it was found in this laboratory (2) that raw pancreas, even when fed in amounts

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as great as 250 gm. daily, does not empty the liver of abnormal amounts of fat in as short a period as 3 weeks. In three dogs that had received 250 gm. of the raw glandular tissue for 4 to 5.5 weeks after fatty livers had been established, 29, 27, and 23 per cent fatty acids were still present in mixed samples of the liver. The curative action of the raw glandular tissue is a slow process, and a period exceeding 5.5 weeks is required to empty the liver of abnormal amounts of fat once it has been deposited. As further evidence against the view that choline is the active factor in pancreas, Dragstedt et al. (5) fed liver and brain, tissues rich in lecithin, and reported failure of these to cure fatty livers. But a curative action is not to be expected from these two tissues despite their high phospholipid content. In the first place, Blatherwick et al. (6) demonstrated several years ago that fatty livers are produced in rats by the feeding of liver. This, indeed, has been amply confirmed by Beeston and Wilkinson (7). Furthermore, while brain is rich in phospholipids, it also contains cholesterol, the feeding of which to rats has been shown by many workers to produce fatty livers. An example of the type of reaction to be expected from feeding such tissues is provided by the work of Okey and her co-workers (8), in which it was shown that egg yolk, though exceptionally rich in phospholipids, nevertheless (owing to its cholesterol content) results in fatty livers when fed to rats. While no attempt is here made to associate the pancreatic liver factor with choline, it yet seems necessary to point out that a more rigid proof than that offered by Dragstedt et al. \(^1\) must be provided before their dissimilarity can be accepted.

The relation of choline to the lipid changes in the blood of completely depancreatized dogs maintained with insulin has not hitherto been investigated. The fact that the pancreatic blood factor is heat-labile suggests, however, that this factor is not choline.

\(^1\) It has previously been pointed out (9) that the method of fat estimation employed by Dragstedt et al. is open to question. Moreover, in their choline studies they record four dogs, two of which died in 4 to 5 weeks after pancreatectomy despite the addition of choline to the diet. From the fact that long survival of a large number of completely depancreatized dogs has now been demonstrated in this laboratory without the aid of choline supplements, it is difficult to accept the data offered by these workers.
EXPERIMENTAL

The preparation and maintenance of the completely depancreatized dogs used in this study have been previously described (2). Following pancreatectomy each dog received twice daily, at 8 a.m. and 4 p.m., a mixture containing 280 gm. of lean meat, 50 gm. of sucrose, and 7 gm. of bone ash. Vitamin supplements (A and D as cod liver oil;2 the B complex in the form of a concentrate3 obtained from rice bran) were added to the diet mixture twice a week. Each animal received 16 units of insulin daily, 8 units at each time of feeding. When it was desired to administer choline chloride, this was mixed with the diet a few minutes before the meal was served.

Blood and liver were taken for analyses between 8 and 9 a.m.; the dogs had received their last injection of insulin and their last meal at 4 p.m. of the previous day. This state of the animal in which it has been deprived of both food and insulin for 16 hours is here referred to as the postabsorptive state.

The liver was removed after the animal had been anesthetized with sodium amytal. A mixed sample of the whole liver was used for lipid estimation. The method of sampling the liver and the procedures employed for lipid determinations of liver and blood have been previously described (11).

Liver Lipids

Nine completely depancreatized dogs maintained with insulin received choline supplements at various intervals following excision of the gland (Table I). In a single animal (Dog D-27) the daily administration of 2.5 gm. of choline chloride was begun immediately after pancreatectomy4 and was continued for 11 weeks. At

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1 The standardized cod liver oil used in this study was kindly furnished by Mead Johnson and Company.

2 The rice bran concentrate was kindly furnished by Vitab Products, Inc., Emeryville, California. This concentrate contained 55 international units of vitamin B1 and 10 modified Bourquin-Sherman units of vitamin G (flavin) per cc. The same concentrate has also been shown to be a good source of both rat and chick antidermatitis factors (10).

4 Although animals may suffer a slight impairment in appetite for a short interval immediately following the operation, Dog D-27 showed no such effect, vigorously ingesting all diet mixtures including the choline supplements fed after pancreatectomy, which were begun on the day following the operation.
**Table I**

Curative and Preventive Action of Choline upon Fatty Livers of Completely Depancreatized Dogs Maintained with Insulin

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight (lbs)</th>
<th>Period depancreatized (wk)</th>
<th>Period receiving choline (wk)</th>
<th>Liver Weight (gm)</th>
<th>Cholesterol Total (per cent of body weight)</th>
<th>Free (per cent)</th>
<th>Ester (per cent)</th>
<th>Total lipid (per cent of total)</th>
<th>Total fatty acids (per cent)</th>
<th>Phospholipid (per cent)</th>
<th>Residual fatty acids (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-27</td>
<td>10.5</td>
<td>11</td>
<td>11*</td>
<td>320</td>
<td>0.26</td>
<td>0.24</td>
<td>0.02</td>
<td>3.84</td>
<td>2.50</td>
<td>2.35</td>
<td>2.26</td>
</tr>
<tr>
<td>D-15</td>
<td>7.0</td>
<td>10</td>
<td>Last 3†</td>
<td>375</td>
<td>0.25</td>
<td>0.21</td>
<td>0.04</td>
<td>22.2</td>
<td>19.6</td>
<td>2.14</td>
<td>20.7</td>
</tr>
<tr>
<td>D-21</td>
<td>6.3</td>
<td>17</td>
<td>“ 6‡</td>
<td>545</td>
<td>0.36</td>
<td>0.16</td>
<td>0.20</td>
<td>25.7</td>
<td>23.6</td>
<td>1.61</td>
<td>24.5</td>
</tr>
<tr>
<td>D-23</td>
<td>6.8</td>
<td>18</td>
<td>“ 6‡</td>
<td>315</td>
<td>0.52</td>
<td>0.19</td>
<td>0.33</td>
<td>14.2</td>
<td>12.4</td>
<td>1.93</td>
<td>12.7</td>
</tr>
<tr>
<td>D-48</td>
<td>8.7</td>
<td>26.5</td>
<td>“ 11.5‡</td>
<td>490</td>
<td>0.43</td>
<td>0.25</td>
<td>0.18</td>
<td>42</td>
<td>7.07</td>
<td>5.66</td>
<td>1.40</td>
</tr>
<tr>
<td>D-49</td>
<td>7.2</td>
<td>26</td>
<td>“ 11.5‡</td>
<td>365</td>
<td>0.30</td>
<td>0.18</td>
<td>0.12</td>
<td>40</td>
<td>7.00</td>
<td>5.70</td>
<td>1.49</td>
</tr>
<tr>
<td>D-77</td>
<td>8.0</td>
<td>36.5</td>
<td>“ 15‡</td>
<td>365</td>
<td>0.30</td>
<td>0.18</td>
<td>0.12</td>
<td>40</td>
<td>6.92</td>
<td>5.35</td>
<td>1.49</td>
</tr>
<tr>
<td>D-83</td>
<td>8.5</td>
<td>36</td>
<td>“ 15‡</td>
<td>395</td>
<td>0.43</td>
<td>0.25</td>
<td>0.10</td>
<td>29</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>D-81</td>
<td>5.8</td>
<td>35</td>
<td>“ 15‡</td>
<td>400</td>
<td>0.35</td>
<td>0.25</td>
<td>0.10</td>
<td>29</td>
<td>15.1</td>
<td>13.3</td>
<td>1.83</td>
</tr>
</tbody>
</table>

* 2.5 gm. of choline chloride daily.
† 3.0 gm. of choline chloride daily.
‡ 2.0 gm. of choline chloride daily.
the end of this time the animal was sacrificed for lipid analysis of
the liver. The liver showed no enlargement. Total fatty acids
were present to the extent of 2.5 per cent. In a large series of
normal animals maintained in this laboratory the livers contained
up to 2.7 per cent fatty acids. The other lipid constituents in the
liver of Dog D-27, namely cholesterol and phospholipids, were also
present in normal amounts.

In eight dogs the administration of choline chloride was begun
at various intervals after pancreatectomy. These may be grouped
as follows:

1. The administration of 3 gm. of choline chloride daily for 3
weeks, begun at an interval of 7 weeks after pancreatectomy,
failed to empty the liver of its fat.

2. In two dogs the feeding of 2 gm. daily was begun 11 and 12
weeks after pancreatectomy and continued for 6 weeks. Despite
this treatment 25.7 and 14.2 per cent of total lipids were still
present in the livers.

3. The livers of Dogs D-48 and D-49 were analyzed 26.5 and 26
weeks after pancreatectomy, during the last 11.5 weeks of which
they received daily 2 gm. of choline chloride. The fatty acid
content of these livers was reduced to 5.7 per cent. In a study of
the time of onset of fatty livers in depancreatized dogs, fatty acids
in excess of 14 per cent were found at intervals of 20 to 36 weeks
after pancreatectomy (2).

4. Three dogs received 2 gm. of choline chloride daily for the
last 15 weeks of their 35 to 36.5 week period of maintenance after
pancreatectomy. Although the lipid content of their livers was
reduced below that found at this time interval after pancreatec-
tomy in animals not receiving choline supplements (2), it should
be noted that the lipid content of the livers of Dogs D-77, D-81,
and D-82 was still above normal. The lowest lipid value was
found in the liver of Dog D-77, in which 5.4 per cent of fatty
acids was present.

**Blood Lipids**

*Feeding of Choline at Various Intervals after Pancreatectomy*—In
a single dog (No. D-27) the administration of 2.5 gm. of choline
chloride was begun the day following pancreatectomy and con-

— A progress report containing other data has appeared (12).
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Continued daily for 11 weeks (Table II). The preoperative levels for free and ester cholesterol were respectively 127 and 31 mg. per cent, whereas 11 weeks after choline administration these two constitu-

| Dog No. | Weight Period after pancreactomy | Period, mg. per cent of total | mg. per 100 cc. | mg. per 100 cc. | mg. per 100 cc. | Cholesterol Total fatty acids Phospholipid Total lipid Residual fatty acids |
|---------|---------------------------------|-------------------------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| D-27    | 8.8                             | Preoperative                  | 158             | 127             | 31              | 20             | 360            | 301            | 518            | 127            |
|         | 8.8                             | 5                             | 159             | 114             | 45              | 28             | 429            | 348            | 588            | 163            |
|         | 9.7                             | 8.5                           | 159             | 123             | 36              | 23             | 410            | 392            | 569            | 121            |
|         | 10.5                            | 11                            | 147             | 100             | 47              | 32             | 335            | 335            | 482            | 77             |
| D-15    | 8.5                             | Preoperative                  | 150             | 118             | 32              | 21             | 284            | 276            | 389            | 109            |
|         | 7.5                             | 7                             | 105             | 104             | 1               | 1              | 284            | 276            | 389            | 109            |
|         | 7.0                             | 10                            | 153             | 125             | 28              | 18             | 399            | 358            | 552            | 139            |
| D-21    | 7.7                             | Preoperative                  | 131             | 103             | 28              | 21             | 328            | 316            | 459            | 96             |
|         | 6.0                             | 11                            | 182             | 133             | 0               | 0              | 347            | 282            | 479            | 168            |
|         | 6.3                             | 17                             | 142             | 124             | 18              | 13             | 386            | 284            | 528            | 183            |
| D-48    | 10.1                            | Preoperative                  | 155             | 108             | 47              | 30             | 361            | 334            | 516            | 103            |
|         |                                 |                               | 110             | 110             | 0               | 0              | 234            | 227            | 344            | 82             |
|         | 11.5†                           |                               | 166             | 131             | 31              | 21             | 428            | 405            | 594            | 130            |
| D-49    | 6.9                             | 14.5                          | 123             | 100             | 23              | 19             | 250            | 271            | 373            | 62             |
|         | 7.2                             | 26                             | 153             | 131             | 22              | 14             | 450            | 393            | 603            | 171            |
| D-81    | 4.9                             | 20                             | 129             | 106             | 23              | 18             | 250            | 242            | 379            | 71             |
|         | 6.0                             | 31                             | 142             | 110             | 32              | 23             | 368            | 378            | 478            | 88             |
|         | 5.8                             | 35                             | 148             | 111             | 37              | 25             | 243            | 216            | 345            | 92             |
| D-82    | 7.7                             | 20                             | 102             | 91              | 11              | 11             | 243            | 216            | 345            | 92             |
|         | 8.5                             | 31                             | 123             | 112             | 11              | 9              | 420            | 373            | 563            | 162            |
|         | 8.5                             | 35                             | 135             | 107             | 28              | 20             |                    |                |                |                |

*2.5 gm. of choline chloride daily.
†3.0 gm. of choline chloride daily.
‡2.0 gm. of choline chloride daily.

ents were present to the extent of 100 and 47 mg. per 100 cc. of blood. The preoperative and final blood samples did not differ significantly in their phospholipid or total fatty acid contents.

It was previously shown that a lowered lipid level can be pro-
duced after pancreatectomy by feeding diets containing no raw pancreas (13). In order to observe its effect upon this condition, the administration of choline was begun in six dogs at various intervals after pancreatectomy when a lowered blood lipid level had been established.

In Dogs D-15, D-21, and D-48, the administration of choline restored the blood lipids to the preoperative level. 7 weeks after Dog D-15 was depencratized, total and ester cholesterol fell from preoperative values of 150 and 32 mg. to 105 and 1 mg. per 100 cc. of blood. The administration of 3 gm. of choline daily was begun at this time and continued for 3 weeks thereafter. At the end of this period the blood of Dog D-15 contained 153 and 28 mg. per cent of total and esterified cholesterol respectively. Similar changes in cholesterol esters occurred in Dogs D-21 and D-48. In these two it may be further observed that, although total fatty acids and total lipids dropped after pancreatectomy, both constituents showed a rise to values close to preoperative as a result of choline feeding.

Although cholesterol esters rose somewhat after choline administration in the three dogs mentioned above, it should be observed
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that a rise above the normal or preoperative level did not occur. Further evidence of the inability of choline to effect a rise in blood cholesterol above normal is shown in Dogs D-49, D-81, and D-82. In Dog D-49, 20 mg. per cent of cholesterol esters were still present in the blood at an interval of 14.5 weeks after pancreatectomy, and although total cholesterol rose from 123 to 153 mg. per cent as a result of the ingestion of 2 gm. of choline chloride daily for 11.5 weeks, cholesterol esters showed practically no change. The cholesterol levels in the blood of Dogs D-81 and D-82 also rose above the values found at the time choline feeding was begun, but the lipid values attained after 15 weeks of choline ingestion were still within normal range.

Substitution of Choline for Raw Pancreas—Further evidence of the dissimilarity of choline and raw pancreas appears in Table III, which shows the effects of choline upon a high blood lipid level produced by the feeding of raw pancreas. Dog D-55 received raw pancreas for 142 days after pancreatectomy, and by the end of this interval total cholesterol had risen from 172 to 236 mg. per cent, while cholesterol esters had practically doubled, attaining a value of 100 mg. per 100 cc. of blood. 20 days after choline had been substituted for the raw glandular tissue, the blood lipids returned to approximately the preoperative level. It is interesting to note, however, that despite the fact that choline administration was continued for 10 days more, none of the blood lipid constituents fell below the normal level. Raw pancreas was now substituted for choline, and by the end of 9 days total cholesterol had increased by 64 mg. per cent and cholesterol esters by 49 mg.

DISCUSSION

Choline in large amounts influences the deposition of fat in the livers of depancreatized dogs maintained with insulin. But its curative action upon those livers in which a marked fatty infiltration was already present is slow, and a feeding interval longer than 3 weeks is necessary to effect a measurable change even when so large an amount as 0.4 gm. per kilo of body weight is being administered. The extent of the delay in this curative action is well brought out by the fact that 26 per cent of total lipids remained in the liver of a 6.3 kilo dog (Table I) despite the ingestion of 2 gm. of choline chloride daily during the 6 weeks preceding the examination of the liver. Indeed, the feeding of the same amount
to dogs weighing between 5.8 and 8.0 kilos for a period of 15 weeks failed to restore the liver lipids to normal levels. A somewhat similar phenomenon was observed in the action of pancreas upon the fatty livers of depancreatized dogs (2). While the daily administration of pancreas begun immediately after pancreatectomy readily prevented the deposition of abnormal amounts of fat, its curative action upon livers once they had accumulated large amounts of fat was slow, and a period longer than 6 weeks was required to demonstrate clearly a measurable decrease in liver lipids by means of the ingestion of 250 gm. of the glandular tissue daily.

Significance of Choline Content of Stock Diet—The stock diet employed in the present investigation contained meat, sucrose, bone ash, and vitamin supplements and is obviously not free from choline. Each animal receives approximately 400 mg. of choline daily from this dietary mixture. Yet this amount does not prevent the deposition of large amounts of lipids in the livers of depancreatized dogs. In a study of the time of onset of these fatty changes (2), it was shown that 31 per cent of total lipids may be present in the livers of animals that have received this stock diet for 20 weeks after pancreatectomy. Since by far the largest amount of choline in the stock diet is contained in the lecithin of the meat, it is interesting to record here that in a single depancreatized dog (No. D-85) weighing 9 kilos the ingestion of 1120 gm. of meat daily failed to empty the liver of its abnormal amounts of fat (2). This animal was fed the stock diet containing 560 gm. of lean meat daily for the first 20 weeks after pancreatectomy in order to establish a fatty liver. For the next 15 weeks it received 1120 gm. of lean meat in addition to the other dietary constituents. A mixed sample of the whole liver removed at the end of the 15 week period contained 27 per cent of fatty acids. It is estimated that, during the time in which the animal received 1120 gm. of meat, it ingested approximately 800 mg. of choline daily. This apparent inactivity might be explained in various ways. It is by no means unlikely that, in the absence of pancreatic enzymes, the choline combined in the lecithin of the meat is not so readily available to the animal as when free choline is fed.

Relation of Choline to Pancreatic Liver Factor—In view of the activity of choline when fed as the chloride, due consideration must be given to the choline content of the ingested pancreas in
any attempt to determine the factor (or factors) in this glandular
tissue that influences the lipid content of the liver of depancrea-
tized dogs. In this laboratory it has been found that the daily
ingestion of 250 gm. of pancreas prevents the deposition of abnor-
mal amounts of fat in the livers of such animals (2). This amount
of pancreas provides about 600 to 700 mg. of choline daily. If
choline is the only active factor in the pancreas, it should be pos-
sible to show: (1) that the physiological action upon the liver by a
given sample of pancreas can be equated with that of the choline
which can be isolated from it; (2) that the activity of any pan-
creatic fraction\(^6\) is related to its choline content and that the re-
moval of choline from such a fraction results in loss of activity.
Thus far no such evidence has been provided, and in its absence
we must suspend judgment as to whether pancreatic action upon
the liver is due solely to its choline content or whether pancreas
contains a liver factor in addition to the choline.

Relation of Choline to Pancreatic Blood Factor—The present study
shows quite clearly that the factor in pancreas that raises the blood
lipids of the depancreatized dog above normal is not choline. But
it should be noted that, though failing to effect such a rise, choline,
when added to the diet in large amounts, nevertheless prevented
the fall in blood lipids below normal, which occurs after pancrea-
tectomy in dogs receiving the stock diet of this laboratory. A
somewhat similar interrelation between the lipid metabolism of
blood and liver was observed in the action of autoclaved pancreas
on the depancreatized dog treated with insulin (2). This auto-
claved tissue prevents as well as cures fat deposition in the liver,
and there is associated with this action the maintenance of a
normal blood lipid level. It would seem, therefore, that the fall
below normal observed in the blood lipid constituents of depan-
creatized dogs kept alive with insulin is related to the production of
a fatty liver, since choline and autoclaved pancreas, both of which
prevent abnormal amounts of fat from being deposited in the
liver, also prevent the fall in blood lipids. While, on the one

\(^6\) Although choline influences the fatty livers of rats fed a high fat, low
protein diet as well as those of depancreatized dogs maintained on a low
fat, high protein diet, the evidence available at present does not permit the
conclusion that the mechanism of production and cure of these two types of
fatty livers is the same. Hence conclusions derived from experiments on
rats cannot be applied at present to depancreatized dogs.
hand, the fall in blood lipids is probably associated with the accumulation of fat in the liver, on the other hand the rise above normal observed after raw pancreas ingestion is not due to the liver's being emptied of its large amount of fat, since a rise in the blood lipids can be produced by raw pancreas immediately after pancreatectomy; i.e., before abnormal amounts of fat have accumulated in the liver.

SUMMARY

1. The preventive and curative actions of choline upon the fatty livers of completely depancreatized dogs maintained with insulin were investigated. While it is shown that choline in large amounts influences the deposition of liver fat, its curative action is slow, and daily feeding for a long time is required to produce measurable effects on livers in which large amounts of fat have accumulated.

2. The significance of choline in the action of pancreas upon the liver lipids of completely depancreatized dogs is discussed.

3. It is shown that choline does not raise the blood lipids in depancreatized dogs above the normal level. The pancreatic blood lipid factor is therefore not choline.

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