IS CHOLINE THE FACTOR IN THE PANCREAS THAT PREVENTS FATTY LIVERS IN DEPANCREATIZED DOGS MAINTAINED WITH INSULIN?

BY C. ENTENMAN AND I. L. CHAIKOFF

(From the Division of Physiology, University of California Medical School, Berkeley)

(Received for publication, December 2, 1940)

It has been known for some time that the feeding of raw pancreas prevents the development of fatty livers in completely depancreatized dogs maintained with insulin. Recent developments have dealt with two phases of this problem, (1) the mechanism by which the gland eliminates the anti-fatty liver factor and (2) the substance in the gland that is effective in this respect. The fact that fatty livers result from the exclusion of the external secretion of the pancreas from the gastrointestinal tract by duct ligation (1-3), as well as the observation that the daily feeding of fresh pancreatic juice inhibits the deposition of excessive amounts of fat under these conditions (4), suggests that the release of pancreatic juice into the gastrointestinal tract under normal conditions serves to maintain a normal lipid content in the liver. The finding that choline is just as effective as the raw glandular tissue in preventing fatty livers from occurring in duct-ligated (5) and in depancreatized dogs maintained with insulin (6, 7) led to the belief that choline was the active constituent in the pancreas. Dragstedt and his coworkers, however, claim to have ruled out choline (8). As evidence for this view they point to their observation that the daily administration of 200 mg. to 1 gm. of choline for 9 to 28 days failed to cure fatty livers in completely depancreatized dogs (weighing 9.4 to 14.5 kilos) maintained with insulin. But a measurable effect of choline need not have been expected under the conditions of their experiment. It was shown elsewhere that, although the preventive action of choline can be readily demonstrated when daily feedings of it are instituted imme-
diately after pancreatectomy, its curative action is very slow, daily feedings of as much as 3 gm. daily being necessary to produce measurable effects upon livers in which large amounts of fat have already accumulated (7). Thus 26 per cent fatty acids were found in a liver of a 6.3 kilo dog despite the ingestion of 2 gm. of choline chloride daily during the 6 weeks preceding removal of its liver. Moreover, the feeding of the same amount of choline to dogs weighing 5.8 to 8.0 kilos for 11.5 to 15 weeks failed to restore the lipid content of the liver to normal. Curiously enough, the entire series of observations on choline reported by Dragstedt et al. consisted of those on four dogs, two of which died 4 to 5 weeks after pancreatectomy despite the fact that, as shown in this laboratory (9), such animals can be kept alive for years without choline supplements when maintained on a diet of lean meat and sucrose, supplemented with vitamins and salts. More recently these workers claimed to have observed effects with amounts of a pancreas fraction as small as 55 to 125 mg. (10). It must be clear, however, from the above considerations that further investigations of the rôle of choline in the total physiological action of raw pancreas are highly desirable. It is still an open question whether the lipotropic effect of raw pancreas in the depancreatized dog is the result of its choline content or whether it contains a liver factor in addition to the choline.

Although the above discussion has dealt with the fatty livers of only the completely depancreatized dog, it should be noted here that the lipotropic effects of choline and pancreas have also been compared in the case of fatty livers produced in normal rats by the feeding of a low protein-high fat diet. No agreement as to whether choline accounts for the total lipotropic effect of the pancreas has been obtained even in the rat (11-13). It appears necessary to point out again, however, that, since the mechanisms for the production or cure of fatty livers in the depancreatized dog and normal rat have not been shown to be identical, conclusions derived from one should not at present be applied to the other.

EXPERIMENTAL

Twenty-five adult dogs were used in the present study. For periods of 1 to 3 weeks before pancreatectomy, each dog was fed per day a mixture containing 30 gm. of lean meat and 6 gm. of
sucrose per kilo. Vitamins and salts were added to this mixture daily in the form of 3 cc. of cod liver oil, 5 cc. of gelen B (vitab, Type II, liquid), and 2 gm. of Cowgill’s salt mixture (14).

The standard diet fed twice daily after pancreatectomy consisted of 250 gm. of lean meat, 50 gm. of sucrose, and 5 gm. of bone ash. The vitamin supplements recorded above were added once daily. Food was administered at 8.00 a.m. and 4.00 p.m.; 8 units of insulin were injected at each time of feeding.

In the present investigation various amounts of choline and of two pancreas fractions (AR and KAR) were examined with respect to their effects in inhibiting fat deposition in the livers of depancreatized dogs. The feedings of these substances were not begun immediately after pancreatectomy; they were begun when the animals had fully recovered from the operation, as shown by complete healing of the wound and by the acquisition of a vigorous appetite. During the interval between the operation and the commencement of the administrations of choline, Fraction AR, and Fraction KAR, each depancreatized dog received 125 gm. of raw pancreas twice daily in addition to the standard mixture listed above. This precaution was believed to insure the presence of a normal lipid content in the liver (15) at the time that choline, Fraction AR, and Fraction KAR treatments were started.

Livers were removed for analysis between 8.00 and 9.00 a.m. after the animals had been anesthetized with an intravenous injection of nembutal. At this time the dogs were in the postabsorptive state, having received their last meal and injection of insulin at about 4.00 p.m. of the previous day. The whole liver was thoroughly ground and the lipids determined from 10 gm. samples by the oxidative procedures described elsewhere (16). Free cholesterol was determined in an acetone solution after the phospholipid had been precipitated.

Minimum Dose of Choline Necessary to Prevent Fatty Livers

It must be apparent by now that no decision concerning the relation of choline to the action of pancreas upon the livers of the

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1 The standardized cod liver oil was kindly furnished by Mead Johnson and Company; the vitamin B concentrate by Vitab Products, Inc., Emeryville, California.

2 The insulin used in this study was generously supplied by Eli Lilly and Company.
completely depancreatized dog receiving insulin can be made until the minimum effective dose of choline is known. The effects of various amounts of choline upon the lipid content of the livers of eighteen depancreatized dogs were therefore determined; the results are recorded in Table I. The administrations of choline were not begun until intervals of 1 to 8 weeks had elapsed after excision of the pancreas. As noted above, the lipid content of

**Table I**

*On Minimum Amount of Choline Required to Prevent Fatty Livers*

The liver values are expressed as per cent of wet weight of tissue.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight</th>
<th>Period of observation*</th>
<th>Choline</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Propreptive</td>
<td>Choline added</td>
<td>Final</td>
<td>Receiving pancreas</td>
</tr>
<tr>
<td></td>
<td>kg.</td>
<td>kg.</td>
<td>kg.</td>
<td>wks</td>
</tr>
<tr>
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<td>6.5</td>
<td>7.6</td>
<td>26</td>
</tr>
<tr>
<td>D-235</td>
<td>6.6</td>
<td>6.6</td>
<td>5.3</td>
<td>23</td>
</tr>
<tr>
<td>D-240</td>
<td>9.8</td>
<td>9.8</td>
<td>7.1</td>
<td>20</td>
</tr>
<tr>
<td>D-242</td>
<td>9.0</td>
<td>8.3</td>
<td>5.0</td>
<td>20</td>
</tr>
<tr>
<td>D-186</td>
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<td>10.4</td>
<td>9.5</td>
<td>23</td>
</tr>
<tr>
<td>D-215</td>
<td>8.8</td>
<td>8.4</td>
<td>5.3</td>
<td>22</td>
</tr>
<tr>
<td>D-221</td>
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<td>7.7</td>
<td>6.7</td>
<td>19</td>
</tr>
<tr>
<td>D-220</td>
<td>6.0</td>
<td>7.0</td>
<td>0.0</td>
<td>23</td>
</tr>
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<td>11.2</td>
<td>8.8</td>
<td>22</td>
</tr>
<tr>
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<td>11.0</td>
<td>6.7</td>
<td>6</td>
</tr>
<tr>
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<td>9.8</td>
<td>7.2</td>
<td>6</td>
</tr>
<tr>
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<td>7.5</td>
<td>5.0</td>
<td>24</td>
</tr>
<tr>
<td>D-227</td>
<td>9.5</td>
<td>9.3</td>
<td>7.4</td>
<td>28</td>
</tr>
<tr>
<td>D-151</td>
<td>10.5</td>
<td>10.0</td>
<td>7.6</td>
<td>28</td>
</tr>
<tr>
<td>D-156</td>
<td>11.3</td>
<td>10.0</td>
<td>8.0</td>
<td>13</td>
</tr>
<tr>
<td>D-145</td>
<td>7.5</td>
<td>7.5</td>
<td>8.0</td>
<td>22</td>
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<td>D-154</td>
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<td>11.7</td>
<td>7.0</td>
<td>27</td>
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<tr>
<td>D-164</td>
<td>10.3</td>
<td>10.0</td>
<td>3.8</td>
<td>13</td>
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* Each dog after pancreatectomy received twice daily 250 gm. of meat, 50 gm. of sucrose, 5 gm. of bone ash, and 125 gm. of raw pancreas. Vitamin supplements were given twice weekly. When the dogs regained their appetites, the raw pancreas was eliminated from their diets and the amounts of choline specified above added to their evening meals.
the liver was normal at the time choline treatments were introduced (7).

With few exceptions, choline was fed for 15 to 22 weeks. It was previously shown that this period is required for fatty livers to develop consistently in depancreatized dogs maintained with insulin (15).

The effect of 75 mg. of choline was tested in a single dog, No. D-183. This amount of choline was fed daily for 21 weeks; at the end of this period the dog's liver contained 17 per cent fatty acids. Three dogs received 100 mg. of choline daily for 16 to 17 weeks; their livers contained 11 to 17 per cent fatty acids.

Complete prevention of fatty livers was observed when doses of 36 mg. or more choline per kilo of body weight were employed. Six dogs received choline in amounts varying from 36 to 43 mg. per kilo. Whether examined at the end of 4 weeks or at the end of 21 weeks after the daily administrations of this amount of choline had been begun, the lipid content of the liver was normal or quite close to normal. Thus the total fatty acid content of the livers of five of the six dogs did not exceed 2.6 per cent. In a single dog, No. D-163, the total fatty acid content of the liver was 6.4 per cent. In seven other animals the dose of choline varied from 54 to 107 mg. per kilo daily. These doses were also effective in inhibiting the deposition of excessive amounts of lipid in the liver.

**Effect of Small Amounts of Pancreas Fractions upon Lipid Content of Liver**

In the experiments described below, the whole gland was not used. A fraction of pancreas designated as Fraction AR was tested. It was prepared as follows: The raw glandular tissue obtained fresh from the slaughter-house was ground and thoroughly shaken with 2 volumes of acetone for 1 hour. The residue was extracted for a second time with the same amount of acetone and then pressed to remove as much of the solvent as possible. The residue was then dried in warm air, ground to a fine powder, and finally extracted with ethyl ether in a Soxhlet apparatus for 8 hours. This residue has been designated Fraction AR. It was stored at -18° until used. 1 gm. of this pancreas fraction corresponds to approximately 5.5 gm. of the original glandular tissue.
Table II shows that supplementing the standard diet with as small an amount of Fraction AR as 1 gm. per day is sufficient to prevent completely the development of fatty livers. In all three dogs the livers were analyzed for lipids after administration of Fraction AR had been continued for a full 20 weeks.

In another group of dogs, the effect of heat on the anti-fatty liver action of Fraction AR was examined. Fraction KAR was employed, which was prepared by subjecting Fraction AR to 20 pounds of steam pressure in an autoclave for 30 minutes. The daily administration of Fraction KAR in amounts equal to 4 times the dose found effective for Fraction AR failed to inhibit the development of fatty livers. These results show quite conclusively that the anti-fatty liver substance in Fraction AR is completely destroyed by heat.

**TABLE II**

*Effect of Daily Ingestion of Pancreas Fractions on Liver Lipids*

The liver values are expressed as per cent of wet tissue.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight Preoperative kg.</th>
<th>Weight Final kg.</th>
<th>Period of observation weeks</th>
<th>Pancreas fraction</th>
<th>Type</th>
<th>Period fed weeks</th>
<th>Amount fed gm.</th>
<th>Weight gm.</th>
<th>Cholesterol per cent</th>
<th>Free per cent</th>
<th>Total fatty acids per cent</th>
<th>Phospholipid per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-231</td>
<td>6.7</td>
<td>6.0</td>
<td>24</td>
<td>AR</td>
<td>20</td>
<td>1</td>
<td>400</td>
<td>0.21</td>
<td>0.13</td>
<td>2.26</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>D-233</td>
<td>5.9</td>
<td>6.7</td>
<td>24</td>
<td>AR</td>
<td>20</td>
<td>1</td>
<td>370</td>
<td>0.23</td>
<td>0.16</td>
<td>1.65</td>
<td>2.11</td>
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<td>D-238</td>
<td>8.0</td>
<td>7.4</td>
<td>23</td>
<td>AR</td>
<td>20</td>
<td>1</td>
<td>450</td>
<td>0.17</td>
<td>0.18</td>
<td>3.78</td>
<td>2.18</td>
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<tr>
<td>D-264</td>
<td>13.0</td>
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<td>22</td>
<td>AR</td>
<td>17</td>
<td>4</td>
<td>744</td>
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<td>0.14</td>
<td>31.80</td>
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<td>AR</td>
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<td>565</td>
<td>0.30</td>
<td>0.19</td>
<td>23.80</td>
<td>1.32</td>
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<td>4</td>
<td>283</td>
<td>0.44</td>
<td>0.35</td>
<td>18.20</td>
<td>3.46</td>
<td></td>
</tr>
</tbody>
</table>

* Each dog after pancreatectomy received twice daily 250 gm. of meat, 50 gm. of sucrose, 5 gm. of bone ash, and 125 gm. of raw pancreas. Vitamin supplements were given twice weekly. When the dogs regained their appetites, the raw pancreas was eliminated from their diets and the amounts of Fractions AR and KAR specified above added to their evening meals. For the meaning of Fractions AR and KAR see the text.
DISCUSSION

The present investigation shows that 36 mg. of choline per kilo per day or 300 mg. for an 8.4 kilo dog are sufficient to inhibit completely the deposition of abnormal amounts of lipids in the livers of depancreatized dogs maintained with insulin. This amount of choline was found effective in keeping the fatty acid contents of the liver at 3 to 4 per cent levels even when the period of observation was extended to 5 months. Although this may not be the smallest effective dose of choline, it is nevertheless definitely established here that a daily dose as small as 15 mg. per kilo per day will not prevent the accumulation of excessive amounts of fat in the liver.

In earlier studies the feeding of 250 gm. of raw pancreas daily was adopted as a safe procedure for preventing fatty livers in depancreatized dogs (15). The same amount was employed here in the interval between the operation and the introduction of the administration of choline or pancreas fraction. This quantity of raw pancreas contains about 575 mg. of choline (17). It is now clear that, in the case of the ingestion of such quantities of raw pancreas, its choline content is sufficient to account for the observed lipotropic effect. Its choline content may also explain the preventive as well as curative action previously obtained with 250 gm. of autoclaved pancreas (15). Choline is, in all probability, the heat-stable factor referred to elsewhere (15).

The observation that the daily ingestion of 1 gm. of Fraction AR will completely prevent fatty livers in depancreatized dogs shows that the choline present in this pancreatic fraction is not its active agent. At most, 1 gm. of Fraction AR could contain 13 mg. of choline;³ as noted above, 100 mg. of choline did not keep the liver normal in its fat content (Table I). The destruction of the active principle in Fraction AR by heat, as shown by the experiments in which Fraction KAR was fed, also suggests that the choline present in Fraction AR is not involved. It should be stressed, however, that while the lipotropic action of 1 gm. of Fraction AR

³ This estimate is based on the choline content of whole raw pancreas (17). The actual choline content of Fraction AR is probably less than 13 mg. per gm., for some of the choline is removed by the acetone-ether treatment used in its preparation.
cannot be ascribed to its choline content, the possibility has not been ruled out that additional amounts of choline are made available to the organism by the presence of Fraction AR in the gastrointestinal tract.

Assistance in this investigation was furnished in part by the Works Progress Administration (Official Project No. 65-1-08-62, Unit A6).

SUMMARY

The lipotropic effects of choline and pancreas were compared in the completely depancreatized dog maintained with insulin.

1. It is shown that 35 mg. of choline per kilo are sufficient for complete prevention of fatty livers.
2. The daily feeding of a fraction derived from 5.5 gm. of raw pancreas completely inhibited the development of fatty livers even when fed for as long as 5 months.
3. The choline content of the pancreas fraction did not account for its lipotropic effect. The possibility that choline is made available to the organism by the presence of the fraction in the gastrointestinal tract has not been ruled out.
4. The anti-fatty liver factor of the pancreas is heat-labile.

BIBLIOGRAPHY

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