The fatty liver that develops in insulin-treated, depancreatized dogs and in dogs subjected to ligation of their pancreatic ducts has been the subject of considerable study (1). Soon after its discovery it was shown to be prevented by the feeding of raw pancreas (2), while later findings revealed that it could also be prevented by the feeding of pancreatic juice and certain fractions derived from raw pancreas (1).

In understanding this type of fatty liver, it is important to recognize that the condition develops in completely depancreatized dogs fed abundant amounts of choline and methionine in the form of lean meat (1, 3, 4). But the feeding of extra choline or extra methionine, in their free forms, did prevent the development of a fatty liver (4). This difference in the action of free and bound methionine suggested that, in dogs deprived of the external secretion of their pancreases, there is some disturbance in the gastrointestinal tract whereby the methionine of ingested protein is not made available for lipotropic purposes. The subsequent observation that hydrolyzed casein prevented the development of fatty livers, whereas unhydrolyzed casein had no such effect, lent further support to such a view (5).

On the basis of the above findings it was proposed as a working hypothesis that the proteolytic enzymes contained in pancreas accounted for its antifatty liver activity. Evidence for this hypothesis is presented here. It is shown that the feeding of crystalline trypsin effectively prevents the development of fatty livers in completely depancreatized dogs maintained with insulin.

EXPERIMENTAL

For several weeks before pancreatectomy, the dogs were fed a diet high in lean meat and adequate in calories, vitamins, and salts. Only those that showed a vigorous appetite for meat were selected for pancreatec-
omy. After pancreatectomy, each dog was fed twice daily a mixture containing 250 gm. of lean meat, 50 gm. of sucrose, 5 gm. of bone ash, and 1.5 gm. of salt mixture. Once daily, vitamin supplements were fed along with the morning meal. 8 units of insulin were injected at each time of feeding. For the first 3 weeks after pancreatectomy, 25 gm. of raw pancreas were added to each dietary mixture. This preliminary feeding of pancreas was adopted to insure that at the time the feeding of trypsin was begun the animal's liver was free of abnormal amounts of fat.

The assay period (Table I) began with the removal of pancreas from the animal's body.

### Table I

**Effects of Trypsin Feeding on Fatty Acid Content of Liver of Insulin-Treated Depancreatized Dogs**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Weight</th>
<th>Trypsin fed</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preoperative</td>
<td>Final</td>
<td>Per meal</td>
</tr>
</tbody>
</table>
|         | kg. | kg. | mg. | wks. | gm. | per cent.
| D646    | 9.5 | 7.2 | None | 20 | 670 | 21.5 |
| D662    | 6.0 | 5.5 | " | 24 | 580 | 13.3 |
| D751    | 7.9 | 6.5 | " | 19 | 310 | 37.5 |
| D757    | 9.2 | 8.2 | " | 12 | 720 | 17.2 |
| D621    | 12.2 | 8.0 | 10† | 20 | 470 | 2.6 |
| D624    | 12.0 | 8.3 | 10 | 20 | 483 | 3.1 |
| D753    | 8.3 | 8.5 | 10 | 18 | 402 | 2.2 |
| D754    | 10.8 | 9.2 | 10 | 18 | 488 | 2.2 |
| D755    | 11.5 | 11.5 | 10 | 18 | 430 | 2.9 |
| D758    | 8.0 | 6.3 | 10 | 18 | 210 | 2.4 |
| D748    | 10.5 | 12.0 | 5 | 21 | 403 | 2.1 |
| D750    | 7.0 | 6.0 | 5 | 21 | 402 | 1.9 |

* Each animal received two meals per day.
† The values shown in this column are the mg. of crystalline trypsin present in the amounts of the MgSO₄ mixture fed.

1 This amount of salt mixture contained 600 mg. of NaCl, 450 mg. of Mg citrate, 200 mg. of KH₂PO₄, 130 mg. of CaHPO₄·2H₂O, 30 mg. of ferrie citrate, 0.1 mg. of KI, and 115 mg. of KCl.
2 10 cc. of Galen "B" and 3 cc. of a fish oil were used. Each cc. of the former contained 0.15 mg. of thiamine, 0.15 mg. of riboflavin, 2.0 mg. of niacin and niacinamide, 0.15 mg. of pyridoxine, 0.4 mg. of pantothenic acid, 7.2 mg. of inositol, and 0.001 mg. of biotin. Each cc. of the fish oil contained 400 A. O. A. C. chick units of vitamin D and 1000 U. S. P. units of vitamin A.
the diet, and lasted for 18 to 20 weeks. The trypsin used was Armour's crystalline preparation containing 50 per cent MgSO₄. The dry mixture was added to the diet just before feeding. Six dogs received daily 10 mg. of crystalline trypsin; i.e., 20 mg. of the mixture with each meal. Two other dogs received 10 mg. of the trypsin mixture with each meal.

Four dogs served as controls; the dietary treatment of these four dogs was identical with that of the other eight, except that they received no trypsin.

At the end of the assay period, the dogs were anesthetized with nembutal and their livers were excised. Each whole liver was thoroughly ground and mixed, and a sample of the mixture was taken for determination of total fatty acids (7).

Results

In order to assess the antifatty liver activity of trypsin, trypsin was fed twice daily for 18 to 20 weeks. The reasons for extending the assay period to this length of time, as well as the validity of the assay procedure used here, have been discussed elsewhere (6).

The results shown in Table I clearly demonstrate the effectiveness of trypsin, added to the diet, in preventing the accumulation of fat in the liver of the insulin-treated, depancreatized dog. The fatty acid contents of the livers of the eight dogs that received 5 or 10 mg. of trypsin per meal did not exceed 3.1 per cent. The livers of the four control dogs were uniformly fatty, and contained from 13 to 37 per cent fatty acids.

The possibility that trypsin might have an effect on lipide metabolism was also recognized recently by Canepa et al. (8). These investigators fed this enzyme to insulin-treated, depancreatized dogs for 1 week and found no response in the blood lipides. This extremely short feeding period precluded, of course, a precise determination of the antifatty liver activity of this substance.

DISCUSSION

Lean meat or casein, even when fed in very large amounts, fails to prevent the development of fatty livers in insulin-treated, depancreatized dogs. Although the usual daily ration contained 500 gm. of lean meat, fatty livers have also been observed in dogs that were fed as much as 1120 gm. of lean meat per day (3). The most striking example of the failure of protein to exert its normal lipotropic effect was observed in a study in which depancreatized dogs were fed, daily, 500 gm. of lean meat plus 80 gm. of casein (5); the livers of three dogs fed this diet contained 17 to 22 per cent fatty acids.

But when admixed with trypsin, lean meat does exert its normal lipo-
tropic effect in the insulin-treated, depancreatized dog. It was reported earlier that the feeding of 5 cc. of pancreatic juice along with the lean meat diet also prevented fatty livers in these dogs and it would appear that the trypsic activity of this juice may account for its antifatty liver property. These observations are, of course, consistent with the view that ascribes the development of fatty livers in the insulin-treated, depancreatized dog to a loss of the external secretion of its pancreas.

The results of the present investigation illustrate an important aspect of the lipotropic action of proteins; namely, its dependence upon the digestive capacity within the small intestine. In this sense, trypsin may be regarded as an intrinsic antifatty liver factor.

It should not be inferred, from the results of this study, that trypsin is the only intestinal enzyme concerned with antifatty liver activity. We have recently observed that the feeding of crude papain also prevents fatty livers in completely depancreatized dogs injected with insulin. Since chymotrypsin and carboxypeptidase may be concerned in the liberation of methionine from proteins, they also should be considered as possible antifatty liver agents. These possibilities are being explored in this laboratory.

SUMMARY

1. Trypsin has been identified as a possible intrinsic antifatty liver factor in the dog. The addition of as little as 5 mg. of trypsin to each lean meat meal fed insulin-treated, depancreatized dogs completely prevented the development of fatty livers.

2. The dependence of lipotropic action of proteins upon the digestive capacity of the intestine is discussed.

BIBLIOGRAPHY

ANTIFATTY LIVER ACTIVITY OF CRYSTALLINE TRYPsin IN INSULIN-TREATED DEPANCREATIZED DOGS

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