

## Effects of Feeding Autoclaved Pancreas to Depancreatized and Duct-Ligated Dogs.\* (18342)

LESTER R. DRAGSTEDT, WILLIAM B. NEAL, Jr., AND GEORGIANA R. ROGERS.

*From the Department of Surgery, University of Chicago.*

Dragstedt, van Prohaska, and Harms(1) concluded as a result of experimental studies that the beneficial effect of feeding fresh pancreas to depancreatized dogs was due to the presence in pancreas of a specific substance aside from lecithin or choline, and for which they suggested the name "lipocaic," believing it to be a hormone concerned with the utilization of fat. They found that liver and brain tissue, which contained lecithin and choline in amounts roughly equivalent to that present in pancreas, were entirely ineffective. Fat-free pancreatic extracts were prepared from which most of the choline originally present in the pancreas had been removed, and these were found to be effective, while other extracts containing the fat of the pancreas and most of its choline and lecithin were ineffective. Recently, Chaikoff and Entenman(2) have come to the conclusion that the anti-fatty liver factor of pancreas is a proteolytic enzyme, which, by its digestive action in the intestinal tract, liberates the methionine of protein and possibly other lipotropic agents for more effective action. It is proposed that in the absence of pancreatic juice from the intestinal tract, a disturbance in the digestion of proteins results, which in effect deprives the body of lipotropic agents present in the diet, which fail to be absorbed under these conditions. This thesis is supported by their finding that the oral administration of fresh pancreatic juice exerts a beneficial effect in preventing the development of fatty livers in insulin-treated, depancreatized dogs and in animals

whose pancreatic ducts have been ligated. It is also supported by their observation that diets containing hydrolyzed casein were more effective in preventing fatty livers than similar diets containing equivalent amounts of unhydrolyzed casein, and their demonstration that pancreatic extracts prepared by them, and which were found to be effective in the treatment of depancreatized dogs, are heat labile. In a recent study, Montgomery, Entenman, Chaikoff and Fineberg(3) have reported that the oral administration of as little as 5 mg of crystalline trypsin to each lean meat meal fed to insulin-treated, depancreatized dogs completely prevented the development of fatty livers in these animals. Best(4) commented on the work of Palmer in his laboratory, who apparently found that the active principle in extracts of pancreas prepared by the method of Entenman and Chaikoff was trypsinogen and chymotrypsin. Best(5) has elaborated his view that the beneficial effect of pancreas feeding to depancreatized dogs can be accounted for on the basis of its content of known lipotropic agents, such as choline, methionine, or vitamin B<sub>12</sub>, which may potentiate or spare choline.

These newer developments have prompted the present report of observations begun many years ago and which have recently been confirmed and extended. They demonstrate that beef pancreas, autoclaved at 15 lb pressure for from 15 to 30 minutes, continues to exert its beneficial effect in insulin-treated, depancreatized dogs and in dogs whose pancreases have become extensively degenerated as a result of ligation of the pancreatic ducts. Liver, brain, and salivary glands similarly treated exert no such beneficial effect. The

---

\* This work has been aided by a grant from the Otho S. A. Sprague Memorial Institute.

1. Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Physiol.*, 1936, v117, 175; Van Prohaska, J., Dragstedt, L. R., and Harms, H. P., *Am. J. Physiol.*, 1936, v117, 166.

2. Chaikoff, I. L., and Entenman, C., *Advances in Enzymology*, 1948, v8, 171, Interscience Publishers, New York.

3. Montgomery, M. L., Entenman, C., Chaikoff, I. L., and Fineberg, H., *J. Biol. Chem.*, 1950, v185, 307.

4. Best, C. H., *Science*, 1946, v103, 207.

5. Best, C. H., *Fed. Proc.*, 1950, v9, 506.

TABLE I. Effect of Feeding 200 g Autoclaved Pancreas Daily to Depancreatized Dog, D977.

| Date<br>1948 | Remarks                         | Wt,<br>kg | Serum<br>lipids<br>mg % | Alkaline<br>phosphatase<br>of serum units | Liver<br>fat | Insulin<br>units |
|--------------|---------------------------------|-----------|-------------------------|-------------------------------------------|--------------|------------------|
| 4/7          | Total pancreatectomy            | 14.7      | 747                     | 8.5                                       | 0            | —                |
| 5/11         | No supplements                  | 12.4      | 504                     | 68.5                                      | +++          | 78               |
| 8/30         | " "                             | 14.2      | 536                     | 58.0                                      | +++          | 88               |
| 9/29         | " "                             | 13.1      | 340                     | 59.1                                      | ++           | 80               |
| 3/16/49      | " "                             | 10.1      | 380                     | 123.4                                     | ++           | 16               |
| 3/17         | 200 g autoclaved pancreas daily |           |                         |                                           |              |                  |
| 3/30         | " " " "                         | 11.7      | 694                     | 41.7                                      |              | 36               |
| 4/13         | " " " "                         | 12.0      | 727                     | 24.5                                      |              | 40               |
| 4/27         | " " " "                         | 13.7      | 925                     | 18.8                                      | +            | 52               |
| 5/5          | No supplements                  |           |                         |                                           |              |                  |
| 5/11         | " "                             | 13.2      | 565                     | 51.9                                      | ++           | 30               |
| 6/8          | " "                             | 11.7      | 573                     | 89.0                                      | +++          | 34               |
| 7/7          | " "                             | 12.2      | 436                     | 125.4                                     | +++          | 68               |
| 7/19         | Died                            |           |                         |                                           | 21.1%        |                  |

development of lipocaic insufficiency in depancreatized dogs, whose diabetes is controlled and glycosuria prevented by the adequate administration of insulin, is manifested by the following signs and symptoms: a progressive decrease in body weight and disappearance of body fat, a progressive increase in the alkaline phosphatase of the serum, a decrease in the amount of insulin required to prevent glycosuria, a progressive decrease in the concentration of the blood lipids, the development of fatty infiltration of the liver with impairment of liver function, and an increase in sensitivity to insulin so that hypoglycemia convulsions are difficult to prevent. Some or all of these findings appear in about 90 percent of insulin-treated depancreatized dogs and in the majority of animals whose ducts have been ligated and the pancreatic parenchyma destroyed.

Fresh pancreas, liver, brain, and salivary glands of beef were ground, spread in pans, and autoclaved at 15 pounds pressure for 15 to 30 minutes. This treatment sterilized the tissues and inactivated the enzymes present as indicated by the fact that bits of pancreas placed in tubes of sterile salt solution and incubated for several days at 37°C displayed no evidence of digestion or bacterial growth. Pancreatectomized dogs and animals whose pancreatic ducts had been ligated, divided, and omentum interposed between the pancreas and duodenum, were used to determine if feeding the autoclaved tissues could prevent the onset of lipocaic insufficiency or re-

verse it if already developed. After recovery from surgery the animals were fed daily 800 g of horse meat, 50 g bread, 25 g cane sugar, 400 cc milk, 25 g suet, 30 g bone meal, and 5 g of brewer's yeast. During the period of test 200 g of autoclaved tissue were substituted daily for an equal amount of horse meat. The animals were weighed daily and the urinary sugar determined. Regular insulin was given in amounts adequate to prevent all but a minimum glycosuria. Needle biopsies of the liver were secured at two week intervals, and on these occasions determinations were made of the total serum lipids(6), the serum alkaline phosphatase, serum proteins, hemoglobin, and red corpuscles. Four depancreatized dogs with well developed findings of lipocaic insufficiency were completely relieved by feeding 200 g autoclaved pancreas daily for 4 to 8 weeks. Two depancreatized dogs were protected from lipocaic insufficiency by feeding autoclaved pancreas for 6 and 8 weeks, and when the pancreas was discontinued the insufficiency developed. Table I illustrates the curative effect of feeding autoclaved pancreas and the return of lipocaic insufficiency when the pancreas was stopped.

Eight dogs with ligated pancreatic ducts developed lipocaic deficiency, and in all of them the deficiency was entirely relieved by feeding autoclaved pancreas. Table II illustrates the curative effect of feeding autoclaved

6. Dragstedt, L. R., Donovan, P. B., Clark, D. E., Goodpasture, W. C., and Vermeulen, C., *Am. J. Physiol.*, 1939, v127, 755.

TABLE II. Effect of Feeding Autoclaved Pancreas and Autoclaved Salivary Glands to Dog D-77 with Ligated Pancreatic Ducts.

| Date    | Remarks                                | Wt, kg | Serum lipids mg % | Serum alkaline phosphatase units | Liver fat | Insulin units |
|---------|----------------------------------------|--------|-------------------|----------------------------------|-----------|---------------|
| 3/25/49 | Ligation of pancreatic ducts           | 10.9   | 730               | 8.4                              | 0         | 0             |
| 3/26    | 200 g raw pancreas daily               |        |                   |                                  |           |               |
| 5/11    | " " "                                  | 10.9   | 750               | 6.4                              | 0         | 2             |
| 5/12    | No supplement fed                      |        |                   |                                  |           |               |
| 5/25    | " " "                                  | 10.1   | 505               | 14.9                             |           | 0             |
| 6/22    | " " "                                  | 9.2    | 313               | 43.4                             | ++        | 0             |
| 7/20    | " " "                                  | 7.4    | 315               | 66.8                             | +++       | 0             |
| 7/23    | 10 g defatted pancreas powder daily    |        |                   |                                  |           |               |
| 8/17    | " " " " "                              | 8.2    | 660               | 4.0                              | +++       | 0             |
| 10/5    | " " " " "                              | 9.0    | 702               | 4.5                              | ++        | 0             |
| 11/12   | No supplements                         |        |                   |                                  |           |               |
| 11/29   | " "                                    | 8.8    | 535               | 10.0                             | ++        | 0             |
| 1/ 5/50 | " "                                    | 7.1    | 220               | 14.5                             | +++       | 0             |
| 1/31    | " "                                    | 6.3    | 137               | 28.1                             | ++++      | 0             |
| 2/10    | 200 g autoclaved pancreas daily        |        |                   |                                  |           |               |
| 2/28    | " " " " "                              | 6.2    | 702               | 4.7                              | ++++      | 0             |
| 3/28    | " " " " "                              | 8.0    | 873               | 6.9                              | ++++      | 4             |
| 4/25    | " " " " "                              | 9.4    | 753               | 7.5                              | +         | 8             |
| 5/31    | " " " " "                              | 10.2   | 778               | 5.4                              | ++        | 2             |
| 7/18    | " " " " "                              | 10.5   | 905               | 1.2                              | 0         | 2             |
| 7/26    | 200 g autoclaved salivary glands daily |        |                   |                                  |           |               |
| 8/ 1    | " " " " "                              | 10.4   | 670               | 6.7                              | 0         | 2             |
| 8/14    | " " " " "                              | 9.8    | 337               | 4.6                              |           | 0             |
| 8/28    | " " " " "                              | 9.4    | 410               | 3.6                              | +++       | 0             |
| 9/25    | " " " " "                              | 8.9    | 238               | 17.8                             | +++       | 0             |
| 10/ 9   | " " " " "                              | 8.4    | 270               | 17.3                             | +++       | 0             |

TABLE III. Effect of Feeding 200 g Autoclaved Salivary Glands and Autoclaved Liver to Depancreatized Dog No. D-104.

| Date    | Remarks                          | Wt, kg | Serum lipids mg % | Serum alkaline phosphatase units | Liver fat | Insulin units |
|---------|----------------------------------|--------|-------------------|----------------------------------|-----------|---------------|
| 6/ 3/49 | Pancreatectomy                   | 19.8   | 790               | 2.8                              | 0         | 0             |
| 6/ 8    | 200 g raw pancreas daily         | 19.7   | 925               | 5.6                              | 0         | 30            |
| 6/22    | " " " "                          | 19.0   | 988               | 16.3                             | 0         | 74            |
| 6/29    | 200 g autoclaved salivary glands |        |                   |                                  |           |               |
| 7/ 7    | " " " "                          | 18.0   | 840               | 32.9                             | 0         | 60            |
| 8/ 3    | " " " "                          | 16.9   | 780               | 16.6                             | 0         | 44            |
| 8/17    | " " " "                          | 16.7   | 663               | 38.1                             | 0         | 58            |
| 8/31    | " " " "                          | 15.9   | 565               | 40.9                             | +++       | 42            |
| 10/ 5   | " " " "                          | 15.4   | 548               | 44.9                             | ++        | 78            |
| 1/31/50 | " " " "                          | 15.5   | 507               | 16.4                             | ++        | 64            |
| 7/18    | " " " "                          | 15.7   | 770               | 10.7                             | ++        | 48            |
| 7/26    | 200 g autoclaved liver daily     |        |                   |                                  |           |               |
| 8/14    | " " " "                          | 15.2   | 720               | 12.0                             | ++        | 48            |
| 8/28    | " " " "                          | 15.3   | 545               | 24.0                             | +++       | 52            |
| 9/11    | " " " "                          | 15.0   | 463               | 29.5                             | +++       | 52            |

pancreas to one of these animals and the return of lipocaic deficiency when autoclaved salivary gland was substituted for the pancreas.

Autoclaved salivary gland was fed to 7 depancreatized dogs without any effect on lipocaic deficiency in six and a questionable beneficial effect on the seventh. It was fed to

2 dogs with ligated pancreatic ducts with lipocaic insufficiency but had no beneficial effect. Table III illustrates the ineffectiveness of autoclaved salivary gland and liver in preventing the development of lipocaic insufficiency in a depancreatized dog.

Autoclaved liver was fed to 4 depancreatized dogs with a negative effect in 2 and a

questionable effect in 2. Autoclaved brain was fed to 4 depancreatized dogs without beneficial effect in 2 and questionable effect in 2.

It should be emphasized that the onset of lipocaic deficiency in depancreatized dogs varies greatly in different animals so that preventive experiments are less valuable than curative.

The demonstration in these experiments that the oral administration of autoclaved pancreas is effective in preventing and relieving the syndrome of lipocaic insufficiency in insulin treated depancreatized and duct ligated dogs indicates that the active principle, lipocaic, is relatively heat resistant and is accordingly not trypsin or any of the known pancreatic enzymes. Furthermore, the observation that liver, brain, and salivary gland similarly treated are ineffective indicates that the active principle in pancreas is not one of the known lipotropic agents, such as choline, methionine, or inositol, or a combination of them, since these substances are apparently widespread in animal tissues and are present in liver, brain, and salivary glands in amounts roughly equivalent to that in pancreas. The conclusion seems inevitable that lipocaic is a specific substance present in pancreas but not in other organs, and which is effective on oral administration in relieving the residual deficiency in insulin treated depancreatized dogs. It was largely this conclusion which led us to suggest that lipocaic is a pancreatic hormone although it was appreciated that its mode of action was unknown.

In the first paper(1) it was suggested that lipocaic played a role in the transport and further utilization of fat and that choline might play some important intermediary role in this function. Both parathyroid extracts and large doses of calcium lactate have been found to be effective in relieving parathyroid deficiency. Perhaps in a somewhat analogous manner lipocaic and large doses of choline (2 to 2.5 g per day) are effective in relieving the deficiency in depancreatized

dogs. It can be postulated that in normal animals the supply of lipotropic materials in the diet suffices for normal fat utilization in the liver when aided or potentiated by lipocaic from the pancreas. In the absence of lipocaic the amount of lipotropic material in the usual diet is inadequate and fatty infiltration of the liver results. The work of Chaikoff and his associates indicates that failure of pancreatic juice to reach the intestines makes the supply of lipotropic material in the food less available. Pancreatectomy thus operates in 2 ways to produce the disturbance: namely through the removal of lipocaic and through a reduction in the amount of available lipotropic material. The oral administration of fresh pancreas likewise operates in two ways by providing both lipocaic and pancreatic enzymes. This is supported by the observation we have made many times that, although effective, autoclaved pancreas is not as good as a similar quantity of the fresh gland. When diets containing minimum amounts of fat are fed, as in the experiments of Chaikoff and Entenman, restoration of pancreatic digestion alone apparently may suffice for a time at least to correct the deficiency. However, in our experience, when diets rich in fat were given, even large amounts of active pancreatic juice were ineffective.

*Conclusions.* 1. The daily oral administration of 200 g of beef pancreas autoclaved at 15 lb for 15 to 30 minutes was found to be effective in relieving and in preventing fatty infiltration of the liver and other manifestations of lipocaic deficiency in 6 depancreatized and 8 dogs with ligated pancreatic ducts. 2. Autoclaved liver, brain, and salivary glands were found to be ineffective under similar conditions. 3. There is a specific heat resistant substance in pancreas, lipocaic, not found in other tissues, which is effective on oral administration in relieving the residual deficiency in insulin treated depancreatized dogs.

Received November 8, 1950. P.S.E.B.M., 1950, v75.