PANCREATIC TRANSPLANTATIONS IN THE SPLEEN.*

By JOSEPH H. PRATT, M.D., AND FRED T. MURPHY, M.D.

(From the Laboratory of the Theory and Practice of Physic, Harvard University, Boston.)

Plates 51 and 52.

It is now acknowledged by all authorities that total removal of the pancreas produces diabetes. Minkowski found, however, that the presence of a subcutaneous transplant of a portion of the pancreas would prevent the development of diabetes provided that the blood supply of the graft was left intact.

The lower part of the pancreas in the dog lies free in the mesentery. This part of the gland has been named by Pflüger the processus uncinatus, and it is joined above to the corpus pancreatis which is in close contact with the duodenum. Into the lower end of the processus uncinatus enter good sized vessels, branches of the inferior duodenal artery and vein. Minkowski cut through the processus uncinatus in its upper part. The portion of the pancreas thus freed was drawn out of the abdomen and placed in a prepared pocket in the subcutaneous tissue. The blood vessels entering the tip of the gland were carefully preserved, and formed a vascular stalk which passed from the peritoneal cavity through the incision in the anterior wall of the abdomen to the graft. The external secretion of the gland was discharged through a fistulous opening in the skin. After such a graft had been made the removal of the intra-abdominal remains of the pancreas did not produce persistent glycosuria. Usually the urine contained sugar for twenty-four hours or less after the operation. Subsequent extirpation of the graft by the simple extraperitoneal operation of removing the graft, without injury to any other structures, produced a severe and rapidly fatal

* This investigation was made with the aid of a grant from the Proctor Fund for the Study of Chronic Disease. Received for publication, November 21, 1912.


diabetes. Minkowski's experiments were soon repeated and the results were confirmed by Hédon and Thiroloix. This experiment has formed one of the chief supports for the theory that the pancreas produces an internal secretion. It led Thiroloix to abandon his theory that the diabetes that followed extirpation of the pancreas was due to gross traumatic lesions of the nerves or nervous plexuses in the neighborhood, and to adopt the theory of an internal secretion.

Pflüger insisted that the graft experiment did not overthrow the neurogenic hypothesis, and pointed out that the nerve supply of the portion of the pancreas that formed the graft was unchanged, as the nerves that accompanied the vessels in the mesenteric stalk remained intact and held the graft in connection with the nerve centers.

When Minkowski cut this stalk, diabetes resulted although the graft was left. Pflüger explained that this might be due to severing of the nerves rather than to interference with the blood supply. "If centripetal nerves passing from the pancreas can inhibit the activity of the sugar centers of the brain and spinal cord, then the section of these inhibitory nerves would result in an increased production of sugar" (Pflüger).

Hédon in 1892 reported one experiment in which the vascular stalk of the graft was tied without diabetes resulting. This was done on July 6. During the following two days 800 cubic centimeters of urine were passed, containing 4 per cent. of sugar. The glycosuria then disappeared. On July 15 the graft was extirpated. Severe diabetes followed. In 1898 Hédon was able to report only two additional experiments out of a large series in which he tied the pedicle without diabetes resulting.8

1 Hédon, E., Compt. rend. Soc. de biol., 1892, iv, 307, 678; Arch. de physiol. norm. et path., 1892, ser. 5, iv, 617.
2 Thiroloix, J., Bull. Soc. anat. de Paris, 1892, lxvii, 483; Arch. de physiol. norm. et path., 1892, ser. 5, iv, 716.
4 Pflüger, E. F. W., loc. cit., p. 504.
6 The following abstracts of these two successful experiments are given by Pflüger. We have not had access to the original publication of Hédon.
Pancreatic Transplantations in the Spleen.

In a single experiment made in Minkowski's laboratory, Lombroso succeeded in cutting the vascular stalk of a subcutaneous graft without the production of diabetes. This was done one month after the graft was placed under the skin. During this period there were traces of sugar (less than 0.3 per cent.) in the urine. For four days after the operation there was definite glycosuria which at first amounted to 1.5 per cent., but steadily decreased. Twelve days after tying the stalk the graft was excised. Severe diabetes developed and continued until the death of the animal. The success of the experiment was undoubtedly due to a newly formed artery which was found growing into the graft from the skin. This nourished the pancreatic tissue after the original blood supply was shut off.

Martina, an assistant of Payr, made an experiment that has been widely quoted. In discussing a paper by Minkowski, Martina stated that on January 18, 1905, he removed half of the pancreas from a dog and transplanted it in the spleen, using the method that Payr had devised for transplanting the thyroid. The piece of pancreas measured eighteen centimeters in length. The operation was followed by no symptoms. On March 14 the remaining half of the pancreas was extirpated. Sugar appeared in the urine on the second day after the operation. It varied in amount from 3 to 6 per cent.; occasionally it ran as high as 9 per cent. The animal died on July 15 of suppurative peritonitis which took its origin from a fistula in the abdominal wall. At autopsy no pancreatic remains were found at the site of the pancreas. The portion of the organ

Experiment 19.—Subcutaneous transplantation, March 1. After eighteen days total extirpation of the intra-abdominal pancreas and ligature of the mesenteric pedicle of the graft. Transitory glycosuria. After three days urine was free from sugar. Graft extirpated. March 23. Severe diabetes.

Experiment 20.—Subcutaneous transplantation, March 24. After nineteen days (April 12) total extirpation of the intra-abdominal remains of the pancreas and resection of the mesenteric stalk between two ligatures.

From April 13 to 17 only traces of sugar in the urine.

April 17. Extirpation of the graft. Diabetes.


A detailed report of the work was not published owing to the death of this young investigator. The only description that exists is in the report of a meeting of the Medizinischer Verein in Greifswald.

Martina, *Deutsch. med. Wochenschr.*, 1908, xxxiv, 45.
transplanted in the spleen was "vollkommen eingeheilt." It showed central necroses, but the peripheral cells in contact with the splenic tissue were found on microscopical examination to be intact. As the transplant was entirely separated from the nerve supply of the pancreas Minkowski regarded this experiment as furnishing strong evidence against the neurogenic theory. It should be pointed out that the graft did not prevent the development of glycosuria. In fact the animal had a severe diabetes. It lived the unusually long period of four months after the extirpation of the pancreas, and this would indicate that the pancreatic tissue in the transplant was functionating, provided there was no pancreatic tissue left when the rest of the gland was removed.

The transplantation of pancreatic tissue into the spleen has been attempted by Alessandri, Ottolenghi, Kyrle, and Tiberti. The first three of these investigators described only degenerative changes. Their grafts underwent rapid and complete necrosis. Kyrle also failed in his attempts to keep alive direct implantations of pieces of the pancreas. He had success, however, with a two stage operation. A portion of the duodenal end of the pancreas (probably the processus uncinatus) was freed from its mesenteric attachments and inserted into the spleen. At a second operation the part of the pancreas which had been thrust into the spleen was separated from the remainder of the gland close to the intestine. Dogs as well as guinea pigs were used. The longest interval between the operation and death of the dog was forty days. Kyrle concerned himself with the histological changes in the pancreatic cells and brought forward no evidence that the graft was able to functionate.

We have studied the influence of pancreatic transplants in the spleen on the prevention of diabetes. The spleen was selected because Payr showed that transplanted bits of tissue were especially well nourished in the pulp of this organ. Direct implantations were

\*Alessandri, R., Poli clinico, 1896, iii-C., 253; 1897, iv-C., 289; cited by Ottolenghi, D., loc. cit.
\*Ottolenghi, D., Arch. ital. de biol., 1901, xxxvi, 447.
\*Kyrle, J., Arch. f. mikr. Anat., 1908, lxxii, 141.
\*Tiberti, N., Arch. ital. de biol., 1909, li, 123.
Pancreatic Transplantations in the Spleen.

made except in one experiment when a graft was supplied with a vascular stalk. Such an investigation promised to yield information which would either support or oppose the theory of an internal secretion. If diabetes did not occur, it was planned to determine the limit of tolerance for carbohydrates and to keep the animals under observation for a long period. A histological examination of the grafts was undertaken with the hope of obtaining new knowledge on the relation of the islands of Langerhans to the internal function of the pancreas.

Minkowski and Hédon had shown that subcutaneous grafts would prevent for a few weeks the occurrence of diabetes. It was not known that a graft would carry on the internal function of the pancreas for a longer time.

In the experiments with subcutaneous grafts, provision had been made by investigators for the discharge of the external secretion. In three of Minkowski's dogs\textsuperscript{17} the pancreatic fistula in the skin failed to develop. In these animals atrophy of the graft was followed by diabetes in thirty-seven days or less. Judging from this experience and that reported by Sandmeyer\textsuperscript{18} in his study of the effect of partial extirpation of the pancreas, one would expect that a graft in the spleen, where no escape for external secretion were possible, would be followed by atrophy of the graft and diabetes. Our previous observations\textsuperscript{19} had shown, however, that extreme atrophy of the pancreas and complete occlusion of all the pancreatic ducts with retained secretion of the pancreatic juice can occur without diabetes supervening. One dog lived thirty-four

\textsuperscript{17} Minkowski, O., \textit{Arch. f. exper. Path. u. Pharmakol.,} loc. cit., pp. 129-134.
\textsuperscript{18} Experiment 17.—Graft 8 cm. long placed under the skin, June 24. The graft swelled at first, later became small and hard. Extirpation of intra-abdominal pancreas, July 15. Glycosuria developed, July 22.
\textsuperscript{19} Experiment 18.—Subcutaneous graft 5 cm. long, February 17. Extirpation of pancreas, March 7. Diabetes followed. Death, March 14. No trace of graft found.

\textsuperscript{19} Experiment 19.—Subcutaneous graft 3.5 cm. long, March 3. After two weeks the graft was so small as to be scarcely palpable. Pancreatectomy, April 6. Diabetes developed, April 9.
\textsuperscript{12} Sandmeyer, W., \textit{Ztschr. f. Biol.,} 1895, xxxi, 12.
months after permanent occlusion of all the pancreatic ducts, and did not develop glycosuria.\textsuperscript{20}

In the present study dogs and cats were used. Tissues were fixed in Zenker's fluid or 10 per cent. formalin, imbedded in paraffin, and stained with eosin and methylene-blue, or with hematoxylin and eosin.

**DIRECT IMPLANTATION OF PANCREATIC TISSUE IN THE SPLEEN.**

**Experiment 1.**—Female dog; weight, 5,300 gm.  
November 8. Operation. Animal had not been fed for twenty-four hours. Ether narcosis. Incision in median line of abdomen. A tight ligature was placed about the processus uncinatus and a transverse section made just distal to the point of constriction. The terminal portion of the processus uncinatus, about 5 cm. long, 2 cm. wide, and 2 to 3 mm. thick, was removed after tying the vessels connected with it. An incision 4 cm. long was made in the lower end of the spleen, and with a sharp knife a pocket was made in the substance of the organ. The peritoneal coat of the excised portion of the pancreas was partly removed. The graft was then buried in the spleen. The wound in the spleen was closed with interrupted sutures. The oozing quickly stopped. The hemorrhage produced a tumefaction about 3 cm. in size beneath the surface of the spleen near the incision.

November 9. Urine voided since the operation, 52 c.c. Specific gravity, 1.058. No sugar. Urine slightly levorotary. The dog is lying quietly in the cage, and took 150 c.c. of warm milk this morning.

November 10. The dog rose to its feet as the cage was approached. 250 c.c. of milk were given. Urine, 250 c.c. Specific gravity, 1.025. No reduction of Fehling's solution.

November 11. The dog is bright and active.  
Symptoms of distemper developed on November 15 and the animal died on November 27.

**Autopsy.**—A small, fairly well nourished dog. Pus exuded from a stitch abscess at the site of the operation wound in the skin. There were small foci of suppuration in the abdominal wall. None of the abscesses were connected with the peritoneal cavity.

The peritoneum was smooth and glistening. The spleen was of normal size and natural appearance externally. Firm adhesions united the omentum to the spleen. The pancreatic transplant was represented by a mass of rather soft, grayish white tissue imbedded in the substance of the spleen. It was separated from the splenic substance by a grayish opaque wall 1 mm. thick. The graft was only slightly adherent to the wall, and on sectioning the organ it fell away leaving a small cavity lined by a white membrane. The splenic tissue about the graft appeared natural. There were no areas of hemorrhage in the spleen.

**Microscopical Examination.**—Sections through the spleen at the site of the

Pancreatic Transplantations in the Spleen.

graft showed a large area of necrotic tissue surrounded by a zone of pigmented cells. Beyond this was a wall of widely meshed connective tissue. No pancreatic tissue was found.

Experiment 2.—Male dog; weight, 5,000 gm.

November 10. No food for twenty-four hours. Operation under ether narcosis. A portion of the processus uncinatus of the pancreas measuring 5 cm. in length was excised and imbedded in the spleen. After closing the incision in the spleen the omentum was drawn over the wound and sutured to the spleen.

November 12. Rapid recovery from the operation. Urine, 310 c.c. Specific gravity, 1.012. Sugar, 0.55 per cent.; amount, 1.7 gm.

November 14. Urine free from sugar. The dog was sent to the dog farm where it died of distemper on December 6.

Autopsy.—A fairly well nourished dog. Aside from a small subcutaneous abscess the operation wound was completely healed. The peritoneal cavity was dry, the serosa pale and glistening. The omentum was firmly adherent both to the anterior and posterior surface of the spleen. The lower third of the spleen was swollen, measuring 1.5 cm. in thickness, while the remainder was only half this size. The pancreatic graft was represented by a soft, pale yellow body of almost circular outline and measuring from 2 mm. to 1 cm. in width. On the anterior surface the graft was joined to the omental tissue. The spleen was firm and pale red on section.

Microscopical Examination.—The site of the graft was occupied by structureless necrotic material. Between this and the splenic tissue was a zone of granulation tissue containing in its meshes many endothelial cells, some of which contained blood pigment.

Experiment 3.—Male dog; weight, 9,100 gm.

November 29. Operation under ether. The processus uncinatus of the pancreas was tied off between ligatures and the peritoneal covering partially removed. The pancreatic tissue was sliced into strips 6 cm. long, 1 cm. wide, and 0.5 cm. thick. One of these was transplanted into the lower pole of the spleen, and a second piece, with peritoneum attached to one side, was buried in the upper part of the spleen. The incisions in the spleen were closed with interrupted fine linen sutures. The opening in the upper pole of the spleen was longer than usual, making a trough rather than a pocket. The omentum was drawn over incisions and stitched in position. In this operation a dull knife was used. Some tearing of the splenic tissue and excessive hemorrhage occurred. The dog recovered from the operation, but was soon attacked with distemper, and was killed on December 12.

Autopsy.—The body was warm. Rigor mortis was not present. The operation wound had healed. The upper end of the spleen was adherent to the parietal peritoneum, the lower end to the small intestine and its mesentery. The lower part of the spleen was not thickened. The graft was represented by a bit of soft white material about 1 cm. long and 1 to 2 mm. thick. Occupying the site of the upper graft was a mass of fairly firm pinkish tissue 1 cm. in diameter. It was closely adherent to the surrounding splenic substance and reached to the surface of the organ.

Microscopical Examination.—Sections through the spleen showed that the
grafts had been entirely destroyed and converted into necrotic material containing many chromatin fragments and polynuclear leucocytes. Young connective tissue separated the necrotic mass from the splenic tissue.

Experiment 4.—Female dog. November 20. Operation under ether anesthesia. The processus uncinatus was tied off between ligatures. A piece of this, 7 cm. long, 1 cm. wide, and 0.5 cm. thick, was so cut as to be free from peritoneal covering, and was planted in the lower lobe of the spleen. A deep pocket was made and the graft was fixed in place without tension or pressure. The line of incision was covered with omentum.

The dog made a good recovery. As distemper subsequently developed it was chloroformed on December 12.

Autopsy.—The body was warm. The operation wound had healed. The spleen was 10.5 cm. in length. The upper part was thin and narrow, 2 cm. wide, and 6.5 cm. long; the lower part bulbous, 4.5 cm. wide, and 4 cm. long. On section of this swollen part of the spleen a soft red disintegrated substance exuded. There was a zone of grayish red tissue 4 mm. thick surrounding this softened material.

Microscopical Examination.—A bit of pancreatic tissue 1 mm. long and about 0.1 mm. wide was found. It was composed of acini (figure 1). No islands of Langerhans were present. Near this were a few isolated acini imbedded in connective tissue. Most of the acini took the nuclear stain well. There was no leucocytic or lymphocytic infiltration about the pancreatic cells. Separated from this graft by a fibrous wall 1 mm. thick was a large mass of necrotic tissue. Sections from other parts of the pancreatic transplant, showed only necrotic material and young connective tissue.

Thirteen days after transplanting a piece of pancreas into the spleen, normal acini were found. But out of a graft measuring 7 by 1 by 0.5 centimeters only a tiny nodule 1 by 0.1 millimeters remained.

Experiment 5.—Male dog; weight, 9,400 gm.

April 6. First operation, under morphin and ether. The processus uncinatus of the pancreas was sectioned between two ligatures at a point 6 cm. from the distal end. The omentum was placed between the divided portions.

April 13. Second operation. The dog was given morphin subcutaneously before etherization. In the anterior abdominal wall in the vicinity of the first incision was a large amount of chronic inflammatory tissue. The pancreas was of increased consistence and greatly swollen. The terminal portion of the processus uncinatus was closely bound to the remainder of the gland from which it had been separated at the first operation, and the omental tissue which had been placed between the two had almost disappeared. The separation of the two portions was easily made and no connections were found except on the external surface of the gland. The isolated part of the processus uncinatus had undergone considerable atrophy. It was of firm consistence and the surface was granular. It measured 3 cm. in length, 2 cm. in width, and 1.5 cm. in thickness. On section no duct was visible and no hemorrhage or fat necrosis was found. An incision was made in the lower part of the spleen and several thin transverse
sections of the atrophied pancreas were placed in a deep pocket which was prepared in the usual manner. A few grafts were also buried in the upper end of the spleen. The two incisions in the spleen were closed with silk and were left uncovered by omentum. On attempting to separate the corpus pancreatis from the duodenum, the serosa and a portion of the muscular coat were torn away from the wall of the duodenum which was abnormally friable. Owing to the inflamed state of the pancreas the attempt to separate this organ from the gut was abandoned. Several hours after the operation the dog was walking about the cage and in good condition.

April 14. At 9.30 A. M. the dog was found lying on its side and would make no attempt to rise. The animal died half an hour later (eighteen hours after the operation).

Autopsy.—The serosa of the small intestines was dull and lusterless and was covered with elevated lines and nodosities which were apparently produced by irregular contractions of the muscular coat of the intestine. There were about 50 c.c. of bloody fluid free in the peritoneal cavity and considerable hemorrhage about the wound in the abdominal wall and in the mesentery near the pancreas and duodenum. The pancreas was swollen, the surface smooth, and the consistence firmer than normally. The gland surrounded one half of the duodenum. There were no foci of hemorrhage or fat necroses. The duodenum was of normal color and its wall was apparently not inflamed. The spleen was slightly larger than at the time of operation. Scattered over its plum colored surface were a number of darker nodular elevations averaging 1 cm. in size. These marked the direction of the burrows made in the spleen and on section were found to be composed of blood. The two incisions in the spleen were closed by sutures and there was no evidence of any escape of blood from them. The grafts of pancreas placed in the spleen the day before had disappeared. In their place were a few small shreds apparently of clotted blood. The splenic pulp surrounding the pockets appeared normal. The heart, lungs, liver, and kidneys were normal.

Microscopical Examination.—Spleen. The pancreatic transplants had been converted into structureless necrotic material containing fragmented nuclei and hemorrhagic areas; there was no infiltration with polymuclear leucocytes. Here and there between the necrotic material and the splenic tissue were scanty remains of pancreatic tissue. One of these measured 2 mm. in length. The acini in some places stained well and were of normal appearance. In other sections the acini were so distorted that they resembled giant cells.

Experiment 6.—Female dog, strongly built and well nourished; weight, 8,800 gm. April 13. Operation under ether anesthesia. A burrow was made with scissors in both the upper and lower ends of the spleen, and four or five thin transverse sections of the partially atrophied pancreas of the dog used in experiment 5, which had been removed thirty minutes previously and kept in moist gauze, were inserted into the lower pocket in the spleen, and three or four sections into the upper pocket.

The pancreas was separated from the duodenum. Two good sized ducts were found and tied. In making the dissection a large branch of the pancreatico-duodenal artery was cut. The omentum was drawn between the pancreas and the duodenum.
April 14. This morning the dog was walking about the cage and did not seem sick. When seen at 12 o'clock it was apparently in good condition. At 2 P. M. the animal was found dead and rigor mortis was already present (twenty-one hours after the operation).

Autopsy.—There was no evidence of infection about the fresh abdominal wound. The peritoneal cavity was dry and the serosa smooth and glistening. The stomach was moderately distended. There were some petechiae on its serous coat. The pancreas was soft, but not swollen. There were no hemorrhages into the pancreas, and no fat necrosis. The ducts were not dilated. The spleen was swollen. It measured 13 by 5 by 1.5 cm. Several hemorrhagic areas had produced nodular elevations of the surface. The organ was carefully sectioned, but of the pancreatic grafts placed in the spleen the day before the dog's death, not one could be found. The channels in the spleen made at the operation contained only dark red, soft, homogeneous material, apparently blood and splenic pulp. The heart was distended with blood. The lungs were pale and flaccid. The liver was normal in size and appearance and no focal necroses were seen. The kidneys were normal.

Microscopical Examination.—On section of the spleen not a trace of pancreatic tissue could be found. Degeneration of the acini and a few small areas of fat necrosis were found in the pancreas.

The results in the last two experiments are remarkable. While the grafts of normal pancreas used in the other dogs did not produce any symptoms, the two dogs which had grafts from a pancreas which was the seat of a mild acute pancreatitis died within twenty-four hours. The dog (experiment 5) which had acute pancreatitis, as revealed by the operation, seemed perfectly well prior to the implantation of the pancreatic tissue in the spleen. Yet the transfer of a bit of its own pancreas to the spleen caused speedier death than in the case of the normal dog. This dog (experiment 6) seemed well until less than two hours before its death. Some poisons such as the diphtheria toxin act in this way. A rabbit injected with a lethal dose of diphtheria toxin may not appear sick until less than an hour before death occurs. The implantation of a normal pancreas in the peritoneum causes the death of the animal, but von Bergmann and Guleke found that a preliminary injection of trypsin conferred immunity. On the other hand, our dog, the subject of mild inflammation of the pancreas, appeared very susceptible to the toxic action of the pancreatic grafts, more so in fact than the normal dog. This is a subject that is worthy of further investigation, for it promises to throw new light on the

Pancreatic Transplantations in the Spleen.

toxic action of the pancreas and the cause of death in acute pancreatitis.

Experiment 7.—Male dog; weight, 8,000 gm.

January 12. Received meat, 300 gm., and glucose, 115 gm.

January 21. Urine, 80 c.c. (acid); specific gravity, 1.036. No sugar.

February 11. Received meat, 300 gm., and glucose, 125 gm.

February 12. Urine, 175 c.c.; specific gravity, 1.050. Sugar, 0.3 per cent.; amount, 0.53 gm.

February 16. Weight, 7,600 gm.

First operation. Ether narcosis. The pancreas was large and of normal appearance. The corpus pancreatis measured 7 by 2.5 cm.; the processus uncinatus, 10 by 2.5 cm.; the processus lienalis, 10 by 2.5 cm. A large pancreatic duct was found on the ventral surface of the corpus pancreatis, midway between the upper and lower boundary of this portion of the pancreas. It was directly behind a branch of the pancreaticoduodenal vein. About 3 cm. above this was a second duct nearly as large. Both were cut between double silk ligatures and the intestinal ends buried in the wall of the duodenum. The pancreas was separated from the duodenum, and the omentum was placed between the two. A portion of the processus uncinatus, measuring 7 cm. in length and 2 to 3 cm. in width, was removed by cutting between double ligatures. This portion was cut into thin longitudinal strips with the blade of a safety razor. Some of these pieces were placed in a pocket made by separating the peritoneum from the upper part of the right rectus muscle near the operation wound. A large deep incision was made in the spleen. A piece of gauze was placed in this wound and time allowed to permit clotting of the blood. A sheet of pancreatic tissue 3 cm. long, 1 cm. wide, and 1 to 2 mm. thick, was pushed into the pocket. There was still some oozing of blood when the grafts were buried in the spleen. The incision in the spleen was closed with sutures and covered with omentum.

Recovery from the operation was rapid.

March 1. Weight, 6,400 gm.

March 6. Received meat, 300 gm., and glucose, 100 gm.

March 7. Urine, 400 c.c.; specific gravity, 1.020. No reduction of Fehling's solution. The dog has a ravenous appetite, but seems weak. The stools have been numerous and of large calibre. They contain a great number of muscle fibers.

March 8. Weight, 5,800 gm. The dog is now unable to jump into the cage. Received meat, 300 gm., and glucose, 125 gm.

March 9. 11 A. M. No urine voided since yesterday.

March 10. 230 c.c. of urine beneath cage; specific gravity, 1.033. No sugar. Received meat, 300 gm., and glucose, 125 gm.

March 11. Urine, 245 c.c.; specific gravity, 1.035; slight trace of albumin. Sugar, 0.2 per cent.; amount, 0.64 gm.

March 12. Urine, 265 c.c. No sugar.

March 13. Weight, 6,000 gm.

March 20. Weight, 5,900 gm. Received meat, 500 gm., and glucose, 100 gm.

Five hours later 100 c.c. of urine were collected. This contained 4.8 per cent. of sugar.
March 21. Urine voided since yesterday afternoon is free from sugar.

On April 26 the feeding of raw pancreas was begun and continued until May 19.

May 5. Received meat, 300 gm., 3 pancreases, and glucose, 100 gm.

May 6. Urine, 140 c.c. Sugar, 0.6 per cent.

May 23. Weight, 5,900 gm.

August 18. Weight, 5,200 gm. Received meat, 300 gm., and glucose, 100 gm.


August 22. Received meat, 300 gm., and glucose, 50 gm.

August 23. Urine, 180 c.c.; specific gravity, 1.035. A trace of sugar was present. Slight reduction of Fehling's solution on standing.

August 24. Received meat, 300 gm., and glucose, 45 gm.


September 11. Second operation. Morphin-ether anesthesia. The omentum, liver, and upper part of the small intestine, including the duodenum, were found bound together by adhesions. Over the lower third of the spleen the omentum was adherent. After separating the adhesions, the duodenum was brought outside the abdomen, and the region of the pancreas was exposed. The pancreas was represented by a small dense mass attached to the wall of the duodenum 2 to 3 cm. below the pylorus. It was grayish white on section and was apparently composed of scar tissue. Its outlines were indistinct as it merged into the adhesions about the omentum, intestine, and retroperitoneal tissues. Attempts to remove it were abandoned after cutting a large branch of the pancreaticoduodenal artery. The spleen was removed.

September 12. Weight, 4,700 gm. The dog was lively and drank water freely. At 11 A. M. the amount of urine voided since the operation on the day before (eighteen hours) was 85 c.c. It was free from sugar; specific gravity, 1.023.

September 18. Weight, 4,600 gm. The dog was weak. Received meat, 300 gm., and glucose, 40 gm. Vomited a few minutes later.

September 20. Received meat, 300 gm., and glucose, 30 gm. The dog did not eat all the mixture.

September 21. Urine, 70 c.c. It reduced Fehling's solution. 0.2 per cent. sugar was present. The dog ate very little of the 300 gm. of meat and 300 c.c. of milk given today.

September 22. Animal found dead in the cage in the morning.

Autopsy.—Body of a greatly emaciated dog; weight, 4,100 gm. The operation wound was not fully healed. Some thin pus exuded. The anterior abdominal wall was thickened in the region of the incision, measuring 1 to 2 cm. in thickness. It consisted of a grayish white tissue, probably necrotic. It had a worm-eaten appearance due to communicating areas of softening. The inflammation extended through the entire abdominal wall. The edge of the liver, pyloric end of stomach, and the duodenum were firmly adherent to the abdominal wall. The serosa of this portion of the peritoneal cavity was injected and in places was covered with shreds of fibrin. The pancreas was represented by a nodule measuring 2 cm. in length, and 1 cm. from above downwards. On section it was firm and grayish white, and soft in texture, as if composed of connective tissue. Scattered through it were yellowish opaque areas about 1 to 2 mm. in size.
Pancreatic Transplantations in the Spleen.

Microscopical Examination.—Serial sections of the spleen through the site of the graft revealed no pancreatic tissue. Many sections of portions of the anterior abdominal wall were examined, but they showed no remains of the pancreatic graft.

On August 25 the dog was able to take forty-five grams of glucose without the production of glycosuria. The spleen was removed on September 11. On September 20 sugar appeared in the urine when less than thirty grams of glucose were taken. This reduction of tolerance may have been produced in various ways. It was due possibly to the removal of functioning pancreatic tissue in the spleen, although no pancreatic cells were recognized on microscopical examination. It is more probable that the lowered limit of assimilation was due to injury of the atrophied pancreas during the operation, and to interference with its blood supply. Another possible cause was the poor general condition of the animal. Hence, no definite conclusion can be drawn from this experiment in regard to the function of the pancreatic grafts. It does, however, confirm in a striking manner the claim of Pratt and Spooner\(^2\) that the occlusion of all the pancreatic ducts produces extreme atrophy of the pancreas and lowered tolerance for glucose.

Experiment 8.—Cat.
March 8, operation under ether narcosis. The pancreas seemed unusually soft. The processus uncinatus, measuring about 5 cm. in length and 1 cm. in width, was cut into pieces, each about 1 cm. long and 3 to 4 mm. thick. The spleen was long and thin. A pocket 4 to 5 cm. deep was made in the lower half of the spleen and in this the pieces of pancreas were placed. There was very little bleeding during this procedure. The incision in the spleen was closed with two silk ligatures. The body of the pancreas was then separated from the duodenum in the usual way.
March 9. The cat lies quietly in the cage and seems sick.
March 10. The cat was found dead this morning.

Autopsy.—The serosa was smooth and glistening. The corpus pancreatis and the processus lienalis were firm and edematous. Areas of fat necrosis 1 to 2 mm. in size and of yellowish white color were present on the mesenteric and omental tissue near the pancreas and on the pancreas itself. The duodenum was firm, pale, and swollen. No hemorrhagic areas were seen, and there was no inflammation about the spleen. The lower half was twice the thickness and twice the width of the upper half of the organ. An elevated hemorrhagic area extended upwards 3 cm. from the incision in the lower part of the spleen. There had been no bleeding into the peritoneal sac. On section of the spleen the pancreatic graft, 5 to 7 mm. wide, was surrounded by splenic tissue with only a thin dark line of hemorrhage between the two.

Microscopical Examination.—This showed that the pancreatic tissue implanted in the spleen had almost completely disappeared. What had been mistaken for the graft on the gross examination was necrotic material. This was infiltrated with blood. Surrounding a portion of this hemorrhagic area was a thin rim of pancreatic cells. In one section a bit of pancreas 2 by 2 mm. was found. The acini were more or less fused together so that their outline and that of the component cells were indistinct. In places the acinar structure was entirely lost. Nothing was seen that could be identified as an island of Langerhans.

Experiment 9.—Male cat, well nourished.

March 13. Operation under ether narcosis. The large pancreatic duct, entering just below the common bile duct, was dissected out and cut between double ligatures. A second smaller duct was found 1 cm. below the first duct. It was ligated in a similar manner.

The corpus pancreatis was freed from the duodenum, sparing no connecting vessels that passed between the upper half of the corpus pancreatis and the intestine. The processus lienalis was removed. It was 15 cm. long and 1 cm. wide. A long narrow pocket was made in the spleen after making an incision 1 cm. long near its lower pole. The lower half of the spleen was burrowed with scissors upwards for a distance of about 5 cm. and downwards 2 cm. With forceps a strip of pancreas 3 cm. long and 3 cm. wide was taken from the splenic end of the pancreas and inserted into the upper pocket, and a second strip 1.5 cm. long was pushed into the lower pocket. The opening in the spleen was closed with two linen sutures, and no omentum was attached to it.

March 21. The cat was found dead this morning. It had not been well since the operation. Yesterday it refused food and appeared weak and apathetic.

Autopsy.—There was serosanguinolent fluid in the subcutaneous tissue in the vicinity of the incision, but no evidence of pyogenic infection. The peritoneal cavity was dry, and the serosa smooth. The duodenum and pancreas were bound quite firmly to the omentum and mesentery. The wall of the duodenum was intact. The corpus pancreatis was very firm and considerably swollen. It was separated from the duodenum by thickened omentum. This portion of the pancreas was about 1.5 cm. in width, and 3 to 4 mm. thick. The surrounding fat tissue was firmer than normally. In color and consistence it resembled the pancreatic tissue so closely that the outlines of the pancreas could not easily be made out. On the mesentery and omentum near the body of the pancreas were a dozen or more areas of fat necrosis 1 to 3 mm. in size, and about the ligated end of the main pancreatic duct the tissues were softened over an area about 5 mm. in size. The spleen was 9 cm. long. The larger pancreatic graft was represented by a firm yellowish white mass, 5 mm. in diameter and about 2 cm. long, lying in close contact with the splenic tissue which was deep red and firm. The lower pocket was filled with a blood clot.

Microscopical Examination.—The graft in the spleen was necrotic and structureless except for a thin broken line of pancreatic epithelium between the necrotic mass and the splenic tissue. The epithelial cells occurred singly and in small groups which resembled giant cells. No definite acini were seen. Other sections through the site of the grafts showed hemorrhagic and necrotic areas, but no pancreatic cells.
SUMMARY OF THE EXPERIMENTS ON THE IMPLANTATION OF PANCREATIC TISSUE IN THE SPLEEN.

Pancreatic transplants to the spleen underwent rapid autolysis. In five of the nine animals examined eighteen hours to 218 days after the transplantation, no pancreatic tissue was demonstrable. In one dog well preserved acini were found thirteen days after the transplantation. In the other three experiments in which pancreatic cells were present in the spleen, the interval between the operation and the death of the animal varied from eighteen hours to eight days.

Experiment 1.—Dog. Nineteen days after transplantation no pancreatic cells were found.

Experiment 2.—Dog. Twenty-six days after transplantation no pancreatic cells were found.

Experiment 3.—Dog. Thirteen days after transplantation no pancreatic cells were found.

Experiment 4.—Dog. Thirteen days after transplantation a bit of pancreatic tissue measuring 1 by 0.1 millimeters was found. It consisted of acini surrounded by connective tissue. No islands of Langerhans were seen (figure 1).

Experiment 5.—Dog. Eighteen hours after transplantation there was mild inflammation (autoplastic transplant). Only scanty remains of pancreatic tissue were found.

Experiment 6.—Dog. Twenty-one hours after transplantation there was mild inflammation (homoplastic transplant). No pancreatic cells were found.

Experiment 7.—Dog. Two hundred and eighteen days after transplantation there were no pancreatic remains.

Experiment 8.—Cat. Two days after transplantation a thin zone of pancreatic tissue was found. It was composed of acini.

Experiment 9.—Cat. Eight days after transplantation there was a thin layer of pancreatic cells between the spleen and the necrotic material.

PANCREATIC TRANSPLANTS IN THE SPLEEN WITH THE BLOOD SUPPLY TEMPORARILY PRESERVED BY MEANS OF A MESENTERIC STALK.

Experiment 10.—Male dog; weight, 13 kilos.
January 16. Received meat, 300 gm., and glucose, 100 gm.
January 17. Urine, 250 c.c.; specific gravity, 1.026. No sugar.
January 20. Received meat, 300 gm., and glucose, 115 gm.
February 10. Received meat, 300 gm., and glucose, 125 gm.
February 11. Urine, 185 c.c.; specific gravity, 1.052. Sugar, 0.3 per cent.; amount, 0.49 gm.
February 13. First operation. Weight, 11,100 gm. Morphin was given subcutaneously prior to the operation. The processus uncinatus was separated from the remainder of the pancreas at a point 5 cm. from the end by cutting between double ligatures. An incision was made in the mesial surface of the spleen and a large pocket was prepared. Into this was introduced the large pancreatic graft which measured 5 by 3 cm. During the manipulation a considerable amount of blood was lost. Finally all the pancreatic tissue except about 1 cm. of the terminal portion was within the spleen. The mesenteric stalk attached to the end of the processus uncinatus which contained an artery and vein, both of good size, was preserved. The graft was secured in place by sutures and the omentum was drawn around the vascular pedicle.

The dog made a good recovery.

March 7. Second operation. Weight, 9,100 gm. Morphin-ether anesthesia. On opening the abdomen a large mass was found behind the coils of intestine covered with mesentery. This was an abscess occupying the lower part of the spleen. It was opened and there escaped about 30 c.c. of thin pinkish puriform material which had a pleasant odor resembling that of heliotrope. A portion of the pancreatic graft, about 2 cm. in size, projected from the spleen. Its outline could not clearly be made out as it was nearly surrounded by mesentery. The stalk supplying the graft was torn and the vessels were tied. There was no inflammation of the pancreas or duodenum. The stump of the processus uncinatus was healed and free from adhesions to surrounding structures. The duodenum was stripped clean of pancreatic tissue. Two ducts were found and cut between double ligatures. The pancreaticoduodenal artery and vein were spared. The pancreas was removed. The extirpated pancreatic tissue weighed 19 gm.

March 8. The dog is active. Water was given in the afternoon. At 10 A. M. there were 110 c.c. of urine under cage; specific gravity, 1.046. It does not reduce Fehling’s solution.

March 10. Urine, 305 c.c. No sugar. Received meat, 300 gm., and milk, 300 c.c.
March 11. No sugar in urine. Received meat, 400 gm., and glucose, 75 gm.
March 12. Urine, 160 c.c. Sugar, 1 per cent.; amount, 2.08 gm.
March 13. Urine, 240 c.c.; specific gravity, 1.055. No sugar. Received meat, 500 gm., and glucose, 50 gm.

March 14. Urine, 230 c.c.; specific gravity, 1.050. Sugar, 0.6 per cent.; amount, 1.38 gm. Received meat, 500 gm., and milk, 300 c.c.
March 15. Weight, 7,900 gm. Urine, 410 c.c.; specific gravity, 1.035. No reduction of copper. Received meat, 500 gm., and glucose, 30 gm.
March 16. Urine, 220 c.c.; specific gravity, 1.050. No sugar. Received meat, 300 gm., and glucose, 60 gm.
Pancreatic Transplantations in the Spleen.

March 17. Urine, 190 c.c.; specific gravity, 1.054. Sugar, 0.5 per cent.; amount, 0.95 gm.
March 20. Weight, 8.125 gm.
March 23. Weight, 7.730 gm. An absorption experiment of three days' duration ended today. The dog is weak, but does not seem sick.
March 25. At 11 A.M. the dog received meat, 500 gm., and milk, 300 c.c. At 12.30 P.M. it was found lying in the cage, and was unable to stand when placed on its feet. The animal ate 3 pancreases greedily; none were fed previously to this dog. At 5 P.M. 80 c.c. of urine were collected. It was free from albumin and sugar. When taken out of the cage the dog lay on the floor unable to walk or crawl. It was given 3 more pancreases.
March 26. When seen this morning the dog was standing up in the cage. Received 3 pancreases, meat, 500 gm., and milk, 300 c.c.
March 27. Weight, 7.500 gm. Received meat, 300 gm., 3 pancreases, and glucose, 30 gm.
March 28. Urine, 95 c.c.; specific gravity, 1.056. Sugar, 0.15 per cent.; amount, 0.14 gm. Received meat, 300 gm., 3 pancreases, and glucose, 25 gm.
March 29. Urine, 150 c.c.; specific gravity, 1.050. No sugar.
March 31. Weight, 7.600 gm.
April 4. Weight, 7.150 gm. Received meat, 300 gm., 3 pancreases, and glucose, 100 gm.
April 5. Urine, 225 c.c.; specific gravity, 1.070. Sugar, 6.2 per cent.; amount, 13.95 gm.
April 10. Weight, 7.400 gm. The stools are fewer in number and less bulky. The dog has received daily 3 pancreases. He is now stronger and can jump out of his cage and alight on his feet. Received meat, 300 gm., 3 pancreases, and glucose, 40 gm.
April 11. Urine, 65 c.c.; specific gravity, 1.053. No reduction of Fehling's solution.
April 12. Received at 10 A.M. meat, 300 gm., 3 pancreases, and glucose, 50 gm. At 4.45 P.M. 110 c.c. of urine had been voided; specific gravity, 1.046. Sugar, 3.1 per cent.; amount, 3.41 gm.
April 14. Weight, 7.900 gm. Received meat, 300 gm., 3 pancreases, and glucose, 100 gm.
April 15. Urine, 210 c.c.; specific gravity, 1.062. Sugar, 2.8 per cent.; amount, 5.88 gm.
May 1. Weight, 7.600 gm. The dog vomits almost daily. Beginning today he will receive 6 pancreases daily.
May 24. Received meat, 300 gm., bone ash, 50 gm., and glucose, 30 gm.
May 27. Urine, 200 c.c.; specific gravity, 1.039. Sugar, 1.2 per cent.; amount, 2.4 gm. 200 c.c. more of urine were collected at 6 P.M. This was free from sugar.
June 2. Weight, 7.300 gm.
August 17. Weight, 6,900 gm. The dog seems fairly strong. During the past two months and a half he has been given pancreases almost daily. Received meat, 300 gm., 5 pancreases, and glucose, 50 gm.
August 18. Urine, 260 c.c.; specific gravity, 1.034. Sugar, 3.5 per cent.; amount, 9.1 gm.
Joseph H. Pratt and Fred T. Murphy.

August 19. Received meat, 300 gm., 5 pancreases, and glucose, 30 gm.
August 20. Urine, 190 c.c.; specific gravity, 1.036. Sugar, 0.85 per cent.;
amount, 1.61 gm.
August 20. Received meat, 300 gm., 5 pancreases, and milk, 300 c.c.
August 21. Urine, 400 c.c.; specific gravity, 1.040. Sugar, 0.85 per cent.;
amount, 3.4 gm.
August 21. Received meat, 300 gm., 5 pancreases.
August 22. Urine, 400 c.c.; specific gravity, 1.035. No sugar.
August 22. Received meat, 300 gm., 5 pancreases, and glucose, 15 gm.
August 23. Urine, 300 c.c.; specific gravity, 1.035. No sugar.
August 24. Received meat, 300 gm., 5 pancreases, and glucose, 25 gm.
August 25. Urine, 145 c.c.; specific gravity, 1.050. Sugar, 2.5 per cent.;
amount, 3.62 gm.
August 25. Received meat, 300 gm., and 5 pancreases.
August 26. Urine, 360 c.c. No sugar.

September 10. Dog lying in cage. The animal has been ill for five days and
has vomited much of his food. Today he was quite powerless, in fact unable to
lift his head. He was chloroformed.

Autopsy.—The body was that of a greatly emaciated dog. Weight, 5,710 gm.
The omentum was free from fat. The retroperitoneal tissues were stained black
(old hemorrhage). In the median line of the peritoneal cavity there was an egg-
shaped mass 4.5 by 3.5 cm. This proved to be an abscess cavity which occupied
the lower portion of the spleen. A cone-shaped projection of firm grayish white
tissue, with its base formed by the wall of the abscess, extended into the spleen
for a distance of 2 cm. (figure 2). Its maximum diameter was 5 mm. This
nodule received its blood supply from the splenic tissue or from the thick fibrous
wall of the abscess. The abscess cavity did not connect with the intestine,
although it was adherent anteriorly to the jejunum and posteriorly to the large
intestine. No pancreatic remains were found at the site of the pancreas.

Microscopical Examination.—The cone-shaped mass extending from the ab-
ssess into the spleen was found to consist of dense fibrous tissue. In the center
of this were the remains of the transplant of pancreatic tissue. They con-
sisted of acini scattered over an area about 5 mm. in width and less than 1
cm. in length. Except in a few places where the acini were in close contact,
they were separated by connective tissue rich in cells (figure 3). Some of the
acini were well preserved and contained a definite lumen. The nuclei were
vesicular and situated in the periphery of the cells. The protoplasm was fairly
abundant and many of the cells were granular. Some vacuoles were seen. Sec-
tions stained with phosphotungstic hematoxylin showed blue staining granules
(figure 4) in some of the acinous cells (zymogen granules). Scattered through-
out the connective tissue surrounding the pancreatic remains were nerve fibers
and occasional ganglion cells. There was no necrotic tissue and no infiltration
with polymorph nuclear leucocytes in the vicinity of the pancreatic graft. Between
the splenic tissue and the pancreatic remains was a wall of dense fibrous tissue 2 to
3 mm. thick. The largest amount of pancreatic tissue found in any one section
measured about 4 by 2 mm. No islands of Langerhans were found in any part
of the graft.
Pancreatic Transplantations in the Spleen.

This dog lived for 187 days with the internal function of the pancreas maintained by a graft in the spleen entirely separated from its original vascular supply and nervous connections. The interval between the date of the transplantation and the death of the animal was 209 days. Although the tolerance for carbohydrates sank to a low point, persistent glycosuria did not develop. Inanition due to the absence of pancreatic secretion from the intestine was the cause of death. The feeding of fresh pancreas supplied this lack in part and apparently prevented the death of the animal in the third week following pancreatectomy. The limit of tolerance to glucose rose for a short time after the feeding of pancreas was begun, but was at a fairly constant low level from May 24 to August 20. The pancreatic remains at death as determined by serial sections were less than one centimeter in size. The larger part of this small nodule was composed of fibrous tissue. Acini were found, and some of the cells contained well preserved granules staining blue with phosphotungstic hematoxylin and identical in appearance with the zymogen granules of the normal pancreas. As these granules do not occur in the cells of the islands of Langerhans their presence furnishes additional evidence that the pancreatic cells in which they occurred in the graft were acinar cells. No islands of Langerhans were found in any of the sections. If Ribbert's statement is true that only cells that can functionate will live when transplanted, then the presence of normal appearing acinar epithelium in this graft 209 days after the pancreatic tissue was transplanted into the spleen is evidence that the acinar cells can carry on the internal function of the pancreas. This experiment does not disprove the neurogenic theory of diabetes, but it presents additional evidence against it, and in favor of the theory of an internal secretion.

The prevention of diabetes for more than six months by means of the small nodule of sclerosed pancreatic tissue in the spleen makes the accuracy of Martina's observations improbable. If his graft in the spleen remained active, why did sugar appear in the urine two days after the remainder of the pancreas was removed and persist in large amount, 3 to 6 per cent., until the death of the animal? Inasmuch as the animal lived four months with this degree of glyco-
Joseph H. Pratt and Fred T. Murphy.

suria it is probable that some pancreatic juice was entering the intestine, secreted from remains of pancreatic tissue so small that they were overlooked at the autopsy. Among the dogs in which we have shut out all the pancreatic secretion from the intestine, marked disturbance of absorption attended with great loss of weight, as shown in this experiment, has always occurred.23

Minkowski's dogs with pancreatic subcutaneous grafts did not lose weight after the intra-abdominal pancreatic tissue was removed. This is shown in the following abstracts made from the protocols published in his paper.24

The fact that these dogs held their weight after removal of the pancreas seems to be conclusive evidence in the light of our findings that even as skilled an operator as Minkowski left some secreting pancreatic tissue attached to the intestine.

CONCLUSION.

Pancreatic tissue implanted in the spleen and separated from its original vascular and nervous connections can live and functionate for months. A small nodule of pancreatic tissue composed of acini without demonstrable islands of Langerhans prevented the development of diabetes. Death occurred 187 days after the extirpation of the pancreas.

EXPLANATION OF PLATES.

PLATE 51.

FIG. 1. Pancreatic acini found in the spleen thirteen days after direct implantation of a portion of the processus uncinatus of the pancreas. A distinct lumen is present in some of the acini.


Fig. 2. Spleen sectioned transversely and both ends folded back. The direction of the incision is indicated by the dotted line. Fibrous tissue is seen in the center of each cut surface. Near the middle of the fibrous nodule groups of pancreatic acini are found (figure 3). There was an interval of 209 days between the transplantation of pancreatic tissue and the death of the dog. No other pancreatic tissue was found in the body. Diabetes did not develop.

Plate 52.

Fig. 3. Pancreatic tissue in the spleen 209 days after transplantation. The section is a portion of the fibrous nodule shown in figure 2. There are definite acini but no islands of Langerhans.

Fig. 4. Section of the pancreatic graft shown in figure 2 stained with phosphotungstic hematoxylin. The isolated dots and the masses of fused granules in the protoplasm of the cells are identical in appearance and staining reaction with the zymogen granules of the normal pancreas.
(Pratt and Murphy: Pancreatic Transplantations.)
FIG. 3.

FIG. 4.

(Pratt and Murphy: Pancreatic Transplantations.)