1935, v29, 1683.
25. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.* 1952, v195, 357.
26. Brown, W. R., Hansen, A. E., Burr, G. O., and McQuarrie, I., *J. Nutrition,* 1938, v16, 511.
27. Hansen, A. E., and Wiese, H. F., *Fed. Proc.,* 1946, v5, 233.

Received July 19, 1954. P.S.E.B.M., 1954, v86.

Technic for Complete Pancreatectomy in the Rat. (21261)

C. R. TREADWELL AND JOSEPH H. ROE, JR.
From the Department of Biochemistry, George Washington University School of Medicine, Washington, D. C.

In the rat the pancreas is a diffuse, noncapsulated, discontinuous organ lying in the gastro-duodenal and the gastro-splenic mesenteries. The bile duct for a considerable portion of its length is embedded in the pancreatic tissue. The size of the animal, the anatomical distribution of the pancreas, as well as its relation to the bile duct have been obstacles to complete removal of the pancreas. Several technics for partial pancreatectomy in rats have been published. The procedures of Ingle and Griffith(1) and of Foglia (2) have been widely used experimentally. In rats subjected to partial pancreatectomy the diabetes is of a relatively mild type (unless the animal is subjected to additional stress) and requires weeks or months to develop.

In studies in this laboratory on the origin and function of serum amylase, it became desirable to make observations on completely depancreatized rats. These are reported in a following paper(3). The procedure of Foglia(2) for 95% pancreatectomy had been employed previously and because of this experience it was decided to attempt to remove the additional 5% of pancreas which lies in the narrow band of mesentery between the bile duct and upper duodenum. By using adult animals, introducing some modifications in procedure, and with standardization of the pre- and post-operative care, a high percentage (70 to 90%) of survival of completely depancreatized rats could be obtained. The present communication describes the operative procedure and the diabetic state which ensues. Data which show that the gland is essentially completely removed are also included.

Materials and methods. Several strains of white rats, male and female, were used. Body weight ranged from 150 to 400 g. The animals were kept in individual metabolism cages and received the synthetic low-residue diet described below. Qualitative tests for glucose and acetone bodies were run on 24 hour urine samples. Glucose tolerance was determined as follows: after an overnight fast, an 0.1 ml sample of tail blood was taken and 3.5 g of glucose per kg of body weight injected intraperitoneally as a 10% solution. Subsequent blood samples were taken at ½, 1, 2, 3, and 5 hours. Blood sugar was determined by a combination of the methods of Somogyi(4) and Nelson(5). Tissue amylase was determined by a modification of the method of Smith and Roe(6). The low-residue diet had the following composition: 30% casein, 25% lard, 14% starch, 14% sucrose, 5% salt mixture, 10% brewer's yeast, and 2% cod liver oil.

Operative procedure. The rats were placed on the low-residue diet at least 3 days prior to operation and fasted 18 to 24 hours immediately preceding it. The low-residue diet and the fasting period insured absence of solid material in the stomach and intestine. If there was an appreciable amount of residue in the stomach or intestine there was considerably more hemorrhage during and after operation. The animal is given an intraperitoneal injection of 0.05 ml of nembutal (60 mg/ml)/100 g body weight. The abdomen is moistened with 70% alcohol and opened by a mid-line incision extending about 3.5 cm caudal-ward from the xiphoid process. The stomach is drawn out and turned upward so that the

anterior surface lies on the thorax. This movement usually brings out the spleen. If not, it is exposed by drawing out the mesentery between it and the stomach. The gastrosplenic mesentery contains about ½ of the pancreas. The transverse colon is pushed in a caudal direction. This exposes the origin of the pancreatic and splenic blood vessels and the attachment of the mesentery to the posterior abdominal wall. Exposed organs and the fingers of the operator are kept moist with sterile saline. Small swabs made by twisting a small piece of cotton about the end of a toothpick are employed for removal of the pancreas. The mesentery is held on the ball of the index finger and the pancreas rolled out of the mesentery in small pieces by gentle rubbing of the exposed moist surface. Removal of the pancreas in this area is begun by rubbing upwards and outwards on the mesentery from its point of attachment to the posterior wall of the abdomen. This is continued upward to the spleen and centrally to the greater curvature of the stomach. Care must be taken to preserve the splenic blood vessels. However, the rubbing may be done directly on the vessels as they rest on the index finger without danger of serious hemorrhage. Particular attention is paid to complete removal of pancreatic tissue about the plexus of vessels leading into the hilum of the spleen and that immediately adjacent to the stomach. Rubbing is carried downward along the greater curvature of the stomach to the pyloric end with particular attention to the gastro-duodenal and gastro-epiploic arteries. To insure complete removal of all pancreatic tissue most of the fat is also rubbed out so that all that remains are the larger blood vessels and fragments of mesentery. Small blood vessels leading into the pancreatic tissue may be disrupted without severe hemorrhage. The spleen is now returned to its normal position in the abdomen. The first loop of the small intestine is then exposed by exerting traction on the duodenum at its origin from the stomach. The remainder of the pancreas is located in the mesentery within this duodenal loop of intestine. After the loop has been exposed the rat is rotated so that its head points to the operator's right. By gentle pressure with the aid of a swab the loop is freed from its attachment to the transverse colon. Considerable care must be taken here because during the separation the portal vein is exposed and may be easily ruptured. At this point the course of the bile duct should be determined. By holding up the loop of duodenum the bile duct may be seen appearing near the pylorus of the stomach and passing obliquely through the mesentery until it enters the duodenum at about 1/3 the length of the loop from the stomach. The 5% of the pancreas situated between the bile duct and duodenum is removed first. This is done with gentle rubbing, starting at the point of attachment to the stomach and working downward and parallel to the bile duct. Then the pancreas is removed from the rest of the mesentery within the loop, starting along the line of the portal vein and working upward and outward. Usually all of the pancreas can not be conveniently removed while working from only one side of this mesentery, so after the pancreas on one side has been removed the position of the rat is reversed and the opposite side of the mesentery is rubbed to remove the remainder of the pancreas. Care must be taken to remove all pancreas which lies against the inner curvature of the duodenal loop. The organs are replaced and the animal closed in 2 steps. Peritoneal and muscle walls are closed together with a continuous suture. The cutaneous layer is closed with an interrupted stitch or with metal skin clamps. Immediately after the incision is closed, the rat is given two 4 ml injections of isotonic saline subcutaneously in the dorsal region and 0.01 ml of penicillin (300000 units/ml) subcutaneously in the lateral aspect of the thigh. Twenty to 30 minutes are required for the entire procedure. Some small blood vessels are ruptured or destroyed during the operation. However, at autopsy in animals surviving for several days or longer there has been no evidence of failure in blood supply to any organs. While it is important to avoid damage to the bile duct this structure can withstand considerable gentle rubbing with a swab if supported on the index finger. For the most rapid removal of the pancreas the surface should be kept moist with saline and dry swabs used. We use 20-30 swabs/operation.

Following operation the animal is kept at

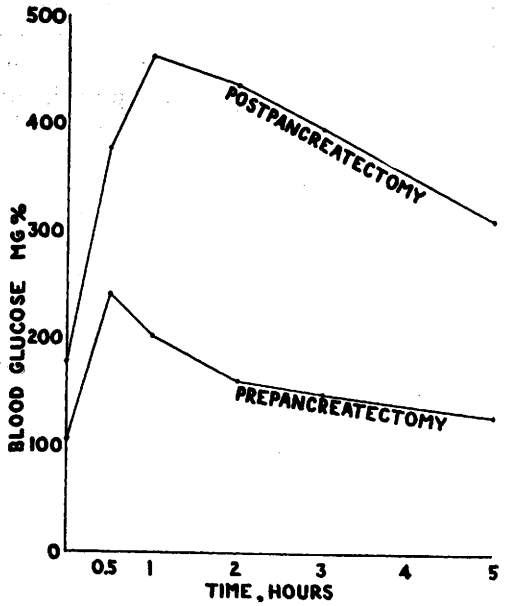

FIG. 1. Glucose tolerance curves before and after pancreatectomy. Each point, avg of 9 rats.

about 35°C (for 72 hours) and given no food or water for 24 hours. After this period the animal is given saline to drink for 48 hours. The low residue diet is started in the afternoon of the second day post-operative. The post-operative fast allows the intestine to recover from trauma caused by handling during the operation. At present we obtain 6 or 7 operative survivors out of each group of 8 rats. Deaths are due either to severe hemorrhage, usually the portal vein, in which case the animal dies within 24 hours, or to rupture of the bile duct or biliary obstruction. In the latter case death usually occurs after several days.

Post-operative observations. Rats, depancreatized by the above technic, have been observed for periods up to 90 days. The animals appear healthy, eat well, and gain in weight on either the low residue diet or a standard laboratory chow (after 7 days) without administration of insulin. In about 80% of the animals a marked protracted glucosuria appears in 1-3 days after return to the diet. The remainder exhibit an intermittent glucosuria. Polyuria (15-50 ml/24 hr) is also present. Acetonuria has not been observed in animals on the low-residue diet or laboratory chow. Fig. 1 shows the average glucose tolerance curves of 9 representative animals before and 12 to 22 days after pancreatectomy. All animals, regardless of degree of glucosuria, gave a typical diabetic response after pancreatectomy. Representative animals, 60 to 90 days post-operatively, were sacrificed and block sections of the area normally containing the pancreas prepared. Histological evaluation of sections from an animal, giving the typical diabetic response described above, revealed some remnants of pancreatic tissue in which the acinar cells were less compact than normal; the granules were indistinct and diffuse with a decrease in staining properties. No islet cells were found. Sections from an animal, in which the operation was considered unsatisfactory because of the mildness of the diabetes, contained remnants with essentially normal acinar cells and a few small islet cells.* In evaluating the technic for total pancreatectomy in the rat it was felt that because of the diffuse nature of the gland some procedure in addition to histological examination should be employed. The procedure adopted was to remove from representative animals all tissue in areas which the pancreas normally occupies and analyze this tissue for its amylase content. Rat pancreas has more than 1000 times as high a content of this enzyme as any other tissue (with the possible exception of salivary glands) so that the presence of any pancreatic tissue in the area would be evidenced by a relatively high amylase content. Parallel analyses on comparable tissues from unoperated animals gave a basis for calculating the percentage of the gland remaining in operated animals. Anylase levels obtained were: for 9 unoperated animals, 25, 799 units with S. D. of $\pm$ 6,849 and for 9 operated animals 53.7 $\pm$ 35.8 units per g of fresh tissue. Assuming that all amylase activity in this area in operated animals was due to pancreatic acinar cells, at least 99.8 of the pancreas had been removed.

Summary. A technic, giving a high survival rate, for essentially complete removal of the pancreas in rats is described. The use of a

* The sections were evaluated by F. N. Miller and I. R. Telford, George Washington University Medical School.

low residue diet and standardized pre- and post-operative care appear to be mainly responsible for the high survival rate.

1. Ingle, D. J., and Griffith, J. Q., *The Rat in Laboratory Investigation,* 2nd ed., J. B. Lippincott Co., Philadelphia, 1949.

2. Foglia, V. G., *Rev. Soc. Argent. Biol.,* 1945, v21, 360.

3. Roe, J. H., Jr., Smith, B. W., and Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., in press.

4. Somogyi, M., *J. Biol. Chem.,* 1945, v160, 69.

5. Nelson, N., *ibid.,* 1944, v153, 375.

6. Smith, B. W., and Roe, J. H., *ibid.,* 1949, v179, 53.

Received July 12, 1954. P.S.E.B.M., 1954, v86.

Reduction of Pain-Conditioned Anxiety by Analgesic Doses of Morphine in Rats. (21262)

HARRIS E. HILL, RICHARD E. BELLEVILLE, AND ABRAHAM WIKLER.

From the National Institute of Mental Health, NIMH Addiction Research Center, Public Health Service Hospital, Lexington, Ky.

Previous investigations(1,2) have demonstrated that an analgesic dose of morphine (15 mg) reduces the behavior-distrupting effects of anticipation of pain in man. The present study was designed to test the hypothesis that morphine also reduces anxiety associated with anticipation of pain in animals. Confirmation of this hypothesis may lead to the development of an analgesic testing method in animals which differs in principle from previously reported technics(3-6).

In accordance with this objective, it was considered essential that the method meet the following criteria: a) The response must be easily quantified, b) Induced changes in the response should be based upon principles similar to those underlying the human work, and c) The response change must be sensitive to graded doses of opiates. In addition, the method should, if possible, meet a requirement proposed by Wikler(7): It should distinguish between analgesic, hypnotic, and paralytic effects of drugs. The present paper is a presentation of the methodology employed with rats and of the results obtained on a first experiment on morphine.

Methods and materials. The conditioned operant or instrumental response of bar-pressing for food rewards under food deprivation was studied in a modified Skinner Box(8). Partial or complete cessation of bar-pressing, considered to be conditioned anxiety or inhibition, was produced by pairing a tone with electric shock. The degree of restoration of bar-pressing followed subcutaneous administration of graded doses of morphine provided measures of the reduction of the inhibition. Correspondence between the drug dose and the % of restoration of the previous response rate was the pertinent datum. The subjects were 12 male albino rats approximately 4 months of age when the experiment was begun. However, the N's as shown in Table 1 for the various test days are not equal since an animal was omitted from the experiment when its bar-pressing behavior was severely impaired by the morphine injections. To produce a high and fairly constant rate of response which would survive the conditioning procedure and drug injections, the animals were maintained at approximately 70% of satiation weight; they were weighed daily and fed individual amounts of Purina laboratory chow. Water was always available in the home cage but not in the experimental apparatus. The Skinner Box was modified in several respects. It was somewhat smaller than the original design. A conditioning chamber (8 x 7 x 10 inches), the floor of which was a barred shocking grid, was mounted in a sound proof box (14 x 14 x 18 inches); the bar and other accessories were similar to those described by Skinner. Circulation of air was insured by the action of a small vacuum pump.