Method for the Isolation of Intact Islets of Langerhans from the Rat Pancreas

Paul E. Lacy, M.D., and Mery Kostianovsky, M.D., St. Louis

SUMMARY

A simple method for the isolation of intact islets from the normal rat pancreas is described. The method is based upon disruption of the acinar parenchyma by injecting Hanks solution into the pancreatic duct system followed by incubation of the pancreas in collagenase. Islets can be separated rapidly from this mixture by sedimentation. The isolated islets release insulin in vitro and appear normal by light and electron microscopy after incubation. A centrifugation method is also described for isolation of large quantities of islets for biochemical studies. **DIABETES *16*:35-39, January, 1967.**

The endocrine portion of the rat pancreas consists of individual islets scattered throughout the acinar parenchyma and the volume of the islets comprises only a few per cent of the entire pancreas. For this reason direct metabolic studies on pure islet tissue have been hampered and several methods have been proposed to isolate intact islets from the exocrine pancreas. Hellerström[1] reported a method for free-hand dissection of the enlarged islets that are present in the pancreas of obese hyperglycemic mice. Islets isolated by this procedure have been used for in vitro studies on enzymatic activity[2-4] and on oxygen consumption of individual islets. Keen et al.[5] ligated one of the main pancreatic ducts in the rat and two to four weeks later islets were dissected from the atrophic pancreatic tissue. A pathologic state exists in both of these procedures, spontaneous hyperglycemia is present in the animals used by Hellerström[1] and pancreatic duct obstruction with fibrosis and atrophy of the pancreas is present in the method described by Keen et al.[5] Hellerström[1] also dissected islets from the pancreas of normal mice, guinea pigs and rats. This procedure was extremely difficult in the normal rat and guinea pig since the pancreas is thick in these species and the islets are firmly attached to the adjacent tissue.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri.

Moskalewiski[6] reported recently that islets of Langerhans of normal guinea pigs could be isolated by means of incubation of the pancreas with collagenase. The islets remained intact after incubation and individual ones could be identified with a dissecting microscope and transferred with a cataract knife to an organ culture vessel. In organ culture, the beta cells responded to a high glucose concentration in the medium as evidenced by marked degranulation of beta cells. Since treatment with collagenase apparently did not damage the islets, we attempted to isolate large numbers of islets from a single rat pancreas by disrupting the acini prior to incubation with collagenase and separating the islets by centrifugation on a discontinuous sucrose gradient. Initial studies indicated that this technic was feasible.[7] The purpose of this report is to describe in detail the centrifugation method for isolating islets in large quantities from the rat pancreas and to describe a more rapid sedimentation method for isolation of islets for in vitro metabolic studies. The light and electron microscopic appearance of the isolated islets will be presented as well as some in vitro studies on insulin release.

MATERIAL AND METHODS

Animals. Male albino Wistar rats weighing 400 to 500 gm. were used. A few studies were done using adult guinea pigs (400 to 500 gm.) and one Rhesus monkey. In each instance the animals were anesthetized with Nembutal prior to the operative procedures.

Incubation of islets. The medium used for incubation of the islets was a modified Krebs bicarbonate solution[8] supplemented with sodium salts of pyruvic acid, glutamic acid and fumaric acid at concentrations of approximately 5 mm. and containing bovine plasma albumin (2 mg. per ml.).[9]

The microincubation flasks used for the islets were similar to those described by Keen et al.[10] The medium (0.5 ml.) was placed in the center well and the islets transferred by the method described under procedures. The gas phase was 95 per cent O_2 and 5 per cent CO_2 and the flasks were incubated in a Dubnoff metabolic shaker, 72 cycles per minute at 37° C.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/16/1/35/335777/16-1-35.pdf by guest on 25 April 2024

The assay method of Morgan and Lazarow[11] was used to measure insulin release in the incubating medium. Crystalline beef insulin (Eli Lilly and Company) was used as the standard and beef insulin labeled with I-131 was obtained commercially (Abbott Laboratories). The anti-insulin serum used in the assay was produced in guinea pigs immunized with crystalline beef insulin. Insulin assays were performed in duplicate on aliquots (100 μL.) of the medium removed at thirty-minute intervals. The flasks were regassed with 95 per cent O_2 and 5 per cent CO_2 (five minutes) following the removal of each of the aliquots.

Histologic procedures. For light microscopy, the individual islets were fixed in Zenker formal two hours, washed in water and stained lightly with eosin in order to facilitate recognition of the tissue during dehydration and imbedding in paraffin. The histologic sections were stained with hematoxylin and eosin, aldehyde fuchsin[12] and aldehyde thionine.[13]

For electron microscopy, the isolated islets were fixed in 2 per cent gluteraldehyde diluted in Hanks solution, postfixed in 1 per cent osmium, dehydrated through a graded series of ethanol and imbedded in Dow Epoxy Resin.[14] The sections, cut on a Porter-Blum microtome using glass knives, were stained with "lead tartrate"[15] and examined in an RCA (EMU 3G) electron microscope at magnification of four to eight thousand times. The electron micrographs were enlarged photographically as desired.

PROCEDURES AND RESULTS

Sedimentation method. In order to facilitate identification of the islets in the initial studies, neutral red was injected intravascularly according to the method of Bensley.[16] After the rat was anesthetized, the abdominal aorta was cannulated below the renal arteries, the inferior vena cava was cut and approximately 60 ml. of neutral red (1:15,000 in 0.15 M NaCl) were injected into the aorta. This produced intense red staining of the islets of Langerhans. Subsequently the common bile duct was cannulated with a polyethylene catheter near the hilus of the liver. The distal end of the common bile duct was clamped adjacent to the duodenum and the acinar tissue was disrupted by injecting 7.0 ml. of Hanks solution into the common bile duct. This method of injection was used since, in the rat, the pancreas drains directly into the common bile duct through two main pancreatic ducts.[5] The body and tail of the pancreas were removed, trimmed, washed in two changes of Hanks solution. The pancreas was then placed in a freshly prepared solution of collagenase* (50 to 60 mg. dissolved in 5 ml. of Hanks solution) and cut into small pieces with scissors. The tissue was stirred in a closed weighing bottle using a magnetic stirrer and incubated twenty minutes at 37° C.

After incubation of the pancreas with collagenase, the mixture was diluted with 15 to 25 ml. of Hanks solution in a conical graduate cylinder and allowed to settle for one minute. The intact islets would settle to the bottom of the cylinder during this interval. The supernatant was removed with a syringe and needle and discarded. The sediment containing the islets was resuspended in Hanks solution and allowed to settle for thirty seconds. This procedure was repeated for a total of eight times using cold Hanks solution for the last four suspensions. If, during this procedure, the suspension appeared flocculent it was dispersed by injecting the mixture through a syringe fitted with a No. 14 needle. The sediment remaining was diluted with Hanks solution, transferred to a glass dish surrounded by an ice bath and examined with a dissecting microscope.

After the technics for separation of the islets by the sedimentation method had been established, the injection of neutral red was eliminated from the procedure. The unstained islets could be recognized easily under a dissecting microscope when viewed against a black background. They appeared as free, round or ovoid structures with a greyish-white to brownish-red color. A small glass loop, which was slightly larger than the diameter of the islets, was used to transfer individual islets. The fluid entrapped within the glass loop suspended the islets and they could be transferred easily to the medium in incubation flasks without damage to the islet cells. Approximately 75 to 100 islets could be transferred within a period of twenty to twenty-five minutes.

By light microscopy, islets isolated by this technic appeared intact without evidence of necrosis or degeneration. The beta cells contained numerous secretory granules and they appeared similar to islets in control portions of the pancreas which had not been incubated with collagenase. The electron microscopic appearance of islets isolated by the sedimentation method is illustrated in figures 1 and 2. The ultrastructure of the beta cells appeared normal with no evidence of swelling of mitochondria, dilatation of the ergastoplasm or disruption of the plasma membranes. The alpha cells also

*Worthington Biochemical Corporation.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/16/1/35/335777/16-1-35.pdf by guest on 25 April 2024

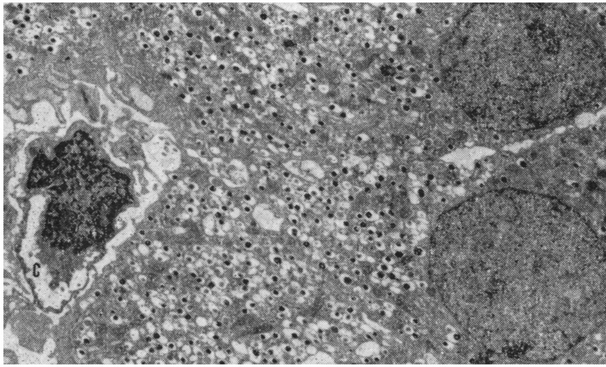

FIG. 1. Electron micrograph of beta cells of a rat islet after isolation by the sedimentation method. The cells contain a normal complement of beta granules and the organelles of the cells appear intact. The endothelial cell of a capillary (c) is also intact. Magnification approximately X 9,000.

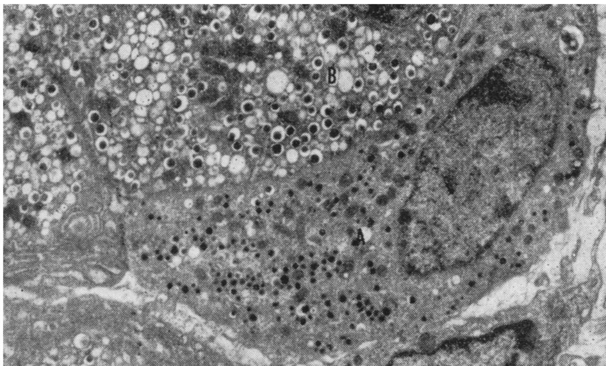

FIG. 2. Electron micrograph of an alpha cell (A) in an islet isolated by the sedimentation method. The ultrastructure of the cell appears normal with numerous alpha granules in the cytoplasm. A portion of a beta cell (B) is also present. Magnification approximately X 8,500.

appeared normal and contained distinct alpha granules in their cytoplasm (figure 2). Studies are in progress to determine whether ultrastructural changes in the beta cells stimulated in vitro are similar to these described in vivo.[17]

In vitro studies on insulin release by the isolated islets was attempted using five islets per flask and incubating different groups of the islets in modified Krebs bicarbonate solution containing either 30 or 300 mg. per cent glucose. The rate and magnitude of insulin release in vitro by islets isolated by this technic is illustrated in figure 3. In the presence of 300 mg. per 100 ml. glucose the rate of insulin release was approximately linear during the one-and-one-half-hour incubation period with a mean total production of 256 μU. during this interval. In contrast, the mean total insulin released

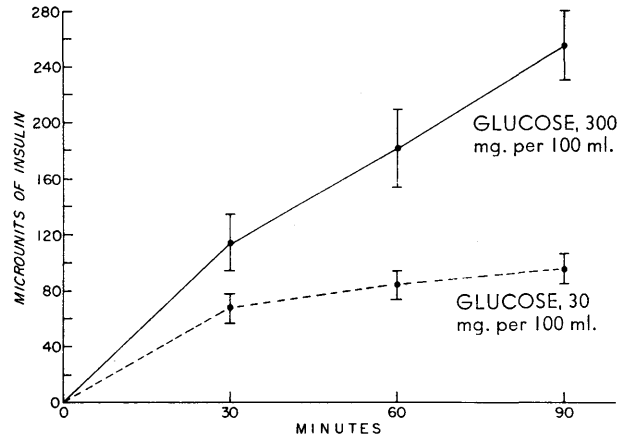

FIG. 3. Insulin release from isolated islets incubated in vitro in the presence of high and low concentrations of glucose. The points at the different time intervals for each curve are the mean values of insulin released in five incubation flasks containing five islets each and the vertical lines represent the standard error of the mean. The rate of insulin release is approximately linear in the presence of 300 mg. per 100 ml. glucose.

from islets incubated in medium containing 30 mg. per 100 ml. glucose was only 95 μU. which was significantly less ($p < .001$) than with 300 mg. per 100 ml. glucose. Light microscopic studies of islets incubated in vitro for one and one-half hours indicated a normal morphologic appearance. With electron microscopy, the mitochondria, beta granules, ergastoplasm, Golgi complex, plasma membranes and nuclear structure of beta cells in the incubated islets appeared intact (figure 4) with no evidence of degeneration. A few alpha cells have been examined with electron microscopy after incubation and these cells also appear intact with many secretory granules in their cytoplasm.

Centrifugation method. The first portion of this procedure was the same as used in the sedimentation method: The rat was anesthetized; the common bile duct cannulated; the acinar tissue was disrupted by the injection of 7.0 ml. of Hanks solution into the common bile duct; the body and tail of the pancreas were cut into small pieces and incubated with collagenase. The mixture was diluted with Hanks solution, centrifuged two and one-half minutes, 250 rpm and the supernatant was discarded. The tissue was resuspended in cold Hanks solution and layered on the surface of four discontinuous sucrose gradients. The concentrations of sucrose used for the different layers were 1.4, 1.6, 1.7 and 1.8 molar; 8 to 10 ml. in each layer. The gradients were centrifuged 2,600 rpm, fifteen minutes at 4° C. Isolated islets were present on the upper surfaces of the 1.6 and 1.7 molar layers and approximately 200 to

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/16/1/35/335777/16-1-35.pdf by guest on 25 April 2024

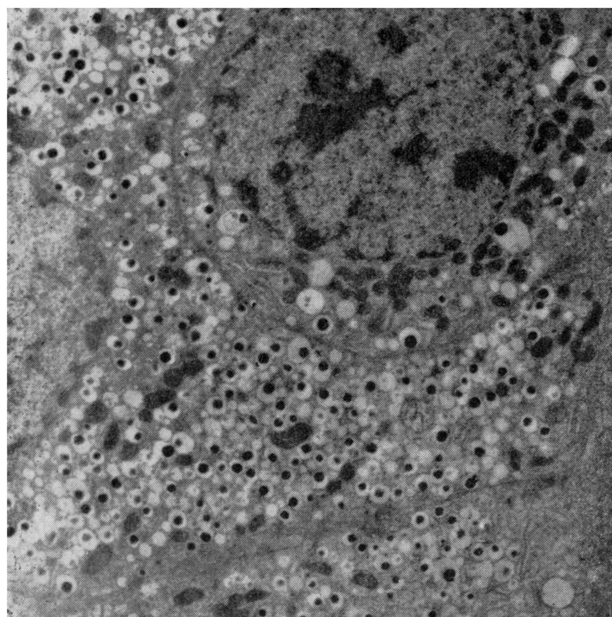

FIG. 4. Electron micrograph of portions of beta cells in an islet incubated 1.5 hrs. in vitro in a medium containing 30 mg. per 100 ml. glucose. The mitochondria, beta granules, ergastoplasm and plasma membranes appear normal. Magnification approximately X13,500.

300 islets could be isolated from a single rat pancreas by this procedure. It was estimated that the islets comprised approximately 80 to 90 per cent of the elements present on the 1.6 molar layer and 40 to 50 per cent of the structures present on the 1.7 molar layer. If the acinar tissue was not disrupted by injection of Hanks solution into the ductal system of the pancreas, then it was impossible to isolate the islets by centrifugation. This same procedure was used for separation of islets in the guinea pig and one monkey pancreas. In these species, the acinar tissue was disrupted by direct cannulation of the pancreatic duct and injection of Hanks solution. The islets separated on the same layers of the sucrose gradient as occurred with the rat pancreas.

By light microscopy the islets isolated by this technic appeared intact without evidence of degeneration or necrosis. In vitro studies on insulin release by the isolated islets in the presence of high and low glucose concentrations indicated a lack of uniform response. Apparently some damage to the islets had occurred during the process of separation, thus making it impossible to use the centrifugation technic for in vitro metabolic studies on the isolated islets. However, this technic may be useful for separation of beta granules from the islets, since by light and electron microscopy the degree of beta granulation appeared normal.

DISCUSSION

The present studies indicate that intact islets can be isolated easily from the normal rat pancreas by the sedimentation method; the isolated islets will release insulin in vitro in response to high levels of glucose in the incubating medium; the ultrastructure of islets incubated in vitro appears normal. This technic makes it feasible to accomplish in vitro metabolic studies on isolated islets of the rat pancreas without the previous difficulties of free-hand dissection[1] or the utilization of pathologic material for isolation of islets.[1,5]

The centrifugation method is not satisfactory for in vitro metabolic studies on insulin biosynthesis. Possibly the hyperosmolar sucroses may have damaged the islets or the centrifugation and prolonged interval required for their separation may have initiated cellular damage which was not evident by light microscopy. However, this method could be used for separation of large numbers of islets for other investigations. Information is needed on the biochemical composition of alpha and beta granules in the mammalian pancreas. The centrifugation method could be used as the first step in this procedure by isolating a large number of intact islets for subsequent ultracentrifugation and separation of the organelles since this would decrease the contamination of the preparation by zymogen granules and organelles of the exocrine pancreas. Islets isolated by this technic may also be useful for production of antibodies to endogenous insulin and glucagon.

The sedimentation method provides a rapid and simple procedure for isolating islets from the rat pancreas for in vitro studies on insulin biosynthesis. Studies in progress indicate that the insulin release in vitro in the presence of high concentrations of glucose can be blocked with mannoheptulose and the rate of insulin release will be affected by other known stimulators of insulin secretion. The electron microscopic studies indicate that the organelles of the beta cells are intact with no evidence of degeneration either immediately following isolation or after incubation in vitro for a period of one and one-half hours. Since the isolated islets appear morphologically and functionally intact with respect to insulin secretion, it should be possible to accomplish correlative studies on the changes in these two parameters following the addition in vitro of certain agents which may affect insulin formation, storage and release.

ACKNOWLEDGMENT

This investigation was supported in part by U.S. Public Health Service grants 5TI-GM897, AM-01226

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/16/1/35/335777/16-1-35.pdf by guest on 25 April 2024

and AM-03373.

The authors wish to acknowledge the excellent technical assistance of Mr. Donald Young and Mrs. Joan Fink in these investigations.

REFERENCES

[1] Hellerström, C.: A method for the microdissection of intact pancreatic islets of mammals. Acta Endocr. *45*:122-31, 1964.

[2] Hellerström, C., and Hellman, B.: Quantitative studies on isolated pancreatic islets of mammals. Acta Endocr. *42*:615-24, 1963.

[3] Hellman, B.: Fluorometric assays of glutamic-pyruvic transaminase activity in microdissected pancreatic islets from obese hyperglycemic mice. Acta Physiol. Scand. *65*:357-63, 1965.

[4] Hellerström, C.: Oxygen consumption of isolated pancreatic islets of mice studied with the cartesian-diver microgasometer. Biochem. J. *98*:7c-9c, 1966.

[5] Keen, H., Sells, R., and Jarrett, R. J.: A method for the study of the metabolism of isolated mammalian islets of Langerhans and some preliminary results. Diabetologia *1*:28-32, 1965.

[6] Moskalewski, S.: Isolation and culture of the islets of Langerhans of the guinea pig. Gen. Comp. Endocr. *5*:342-53, 1965.

[7] Kostianovsky, M., and Lacy, P. E.: A method for the isolation of intact islets of Langerhans from the mammalian pancreas. Fed. Proc. *25*:377, 1966.

[8] Renold, A. E., Martin, D. B., Dagenais, Y. M., Steinke, J., Nickerson, R., and Sheps, M. C.: Measurement of small quantities of insulin-like activity using rat adipose tissue. J. Clin. Invest. *39*:1487-98, 1960.

[9] Coore, H. G., and Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. *93*:66-78, 1964.

[10] Keen, H., Field, J. B., and Pastan, I. H.: A simple method for in vitro metabolic studies using small volumes of tissue and medium. Metabolism *12*:143-47, 1963.

[11] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system. Diabetes *12*:115-26, 1963.

[12] Gomori, G.: Aldehyde fuchsin: a new stain for elastic tissue. Amer. J. Clin. Path. *20*:665, 1950.

[13] Paget, G. E.: Aldehyde-thionin: a stain having similar properties to aldehyde fuchsin. Stain Techn. *34*:223-26, 1959.

[14] Lockwood, W. R.: A reliable and easily sectioned epoxy embedding medium. Anat. Rec. *150*:129-40, 1964.

[15] Millonig, G.: A modified procedure for lead staining of thin sections. J. Biophys. Biochem. Cytol. *11*:736, 1961.

[16] Bensley, R. R.: Studies on the pancreas of the guinea pig. Amer. J. Anat. *12*:297, 1911-12.

[17] Lacy, P. E.: Electron microscopy of the beta cell of the pancreas. Amer. J. Med. *31*:851-59, 1961.

Effects of Ethanol on Plasma Lipids in Man

The fatty liver seen in chronic alcoholism has, in the past, been attributed to a deficiency of lipotropic substances in the diet of such patients. Recently, however, the direct effects of ethanol upon fat storage in the liver of animals (C. S. Lieber and C. S. Davidson, *Amer. J. Med. 33*:319, 1962), and the utilization of free fatty acids of the plasma (*Nutrition Reviews 21*: 9, 1963) have suggested that the effects upon lipid metabolism may be more important in the development of a fatty liver than previously suspected. Although the picture is by no means complete, further evidence is presented by D. P. Jones, M. S. Losowsky, Davidson and Lieber (*J. Lab. Clin. Med.* 62:675, 1963) who included plasma triglyceride analyses in their current experiments.

These authors studied eight alcoholic patients given oral or intravenous doses of ethanol for periods of six to eight hours while at bed rest, and continued the investigation with one subject for a week. The selected dosage level of alcohol produced moderate intoxication. Serum lipids were followed closely. In the acute experiments, the average plasma triglyceride level rose to 69 mg. per 100 ml. as compared to 17 mg. per 100 ml. during a control period, while the free fatty acid levels fell to 51 per cent (0.28 mEq. per liter) of its original value. Cholesterol, phospholipid, hematocrit, and glucose levels of the blood remained unchanged, as did direct and total bilirubin, albumin, total protein, alkaline phosphatase, amylase activity, and bromsulfalein retention.

The rise in triglyceride and fall in free fatty acids of the plasma were not seen when equicaloric amounts of carbohydrate or fat were administered. It is postulated that the plasma triglycerides are derived from the liver and become elevated during ethanol administration because of an increased hepatic release, a decreased peripheral utilization, or both. The fall in plasma free fatty acids reported here and the change in arteriovenous difference across the forearm and the liver, which Lieber and associates have previously reported (see *Nutrition Reviews*, loc. cit.), combine to suggest that there is a decrease in release of the fatty acids from peripheral and hepatic depots after ethanol.

From *Nutrition Reviews,* Vol. 22, No. 2, February 1964, pp. 40-41.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/16/1/35/335777/16-1-35.pdf by guest on 25 April 2024