Studies on Insulin Secreted by Isolated Islets of the Monkey, *Macaca radiata radiata*

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SUMMARY

The effects of various experimental conditions during the isolation of monkey islets by the collagenase method on the insulinogenic response of the isolated islets to glucose have been studied and compared with rat islets isolated under similar conditions. The monkey islets gave a normal response for at least 120 min. The results are compared with available studies on primate islets. DIABETES 28:865–869, September 1979.

The isolation of pancreatic islets by collagenase digestion and their subsequent incubation in saline media could introduce a number of possible distortions in their secretory properties. Nevertheless, this method offers so many advantages that its development has been a landmark in the study of islet function.[1] This method has been applied successfully and widely to a number of small laboratory animals.[2,3] The application of this method to large animals would not only make it possible to study the response of isolated islets in such species but could also provide large numbers of islets from a single animal for experimental studies or other procedures, such as transplantation, in which a large yield is an advantage.[4,5] In this report, we present the successful application of the collagenase method to the isolation of monkey islets and the effect of a number of experimental variations on the secretion of insulin by islets isolated in this way.

MATERIALS AND METHODS

CHEMICALS

All chemicals used were of analytic grade. Bovine albumin was bought from Nutritional Biochemical Corporation, Cleveland, and collagenase (CLS) from Worthington Biochemical Corporation, Freehold, New Jersey.

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Insulin antiserum was produced in adult guinea pigs against crystalline bovine insulin (lot 79532) according to Wright et al.[6] Standard for all the assays was crystalline pork insulin (lot 615-D63-10). Both were gifts from Eli Lilly and Company, Indianapolis.

ANIMALS

Indian bonnet monkeys (*Macaca radiata radiata*) of both sexes, weighing between 1.5 and 5.0 kg, were obtained from local suppliers and kept in animal cages under standard conditions for at least 10 days before use. They were fed a standard, mixed diet of natural foods. Male, white albino rats (200 to 300 g) were from the inbred colony in this laboratory and were fed a commercial food (Hindustan Lever).

ISLET ISOLATION

Animals not previously fasted were anesthetized with Nembutal (30 mg/kg i.p.). Rat islets were isolated according to Lacy and Kostianovsky,[1] except that Krebs' bicarbonate (KRB) with low calcium[7] was used for both isolation and incubation of the islets. Attempts to disrupt the monkey pancreas by inflation with fluid through the duct or by infiltration were unsuccessful due to the fibrous content and compact structure of the adult primate pancreas.[8–10] About 3 g of the tail portion of the pancreas was excised quickly and rinsed in the medium. Since this tissue proved difficult to mince quickly with scissors, the pancreas was first sliced with an improvised tissue slicer having eight parallel razor blades about 1 mm apart. The slices were further minced with a pair of fine scissors under the medium. After removal of obvious adipose tissue or connective tissue bits, the mince was allowed to settle in a round-bottomed centrifuge tube (18 × 120 mm) and the supernatant and excess tissue were removed by suction. Fifteen milligrams of collagenase (CLS) was added to 3 ml of the mince. The gas phase in the tube was displaced by a mixture of 95% O_2 and 5% CO_2. The stoppered tube was then agitated under water (37 °C) with an improvised mechanical shaker, allowing 12 cm longitudinal displacement of the tube at a rate of 200/min. The

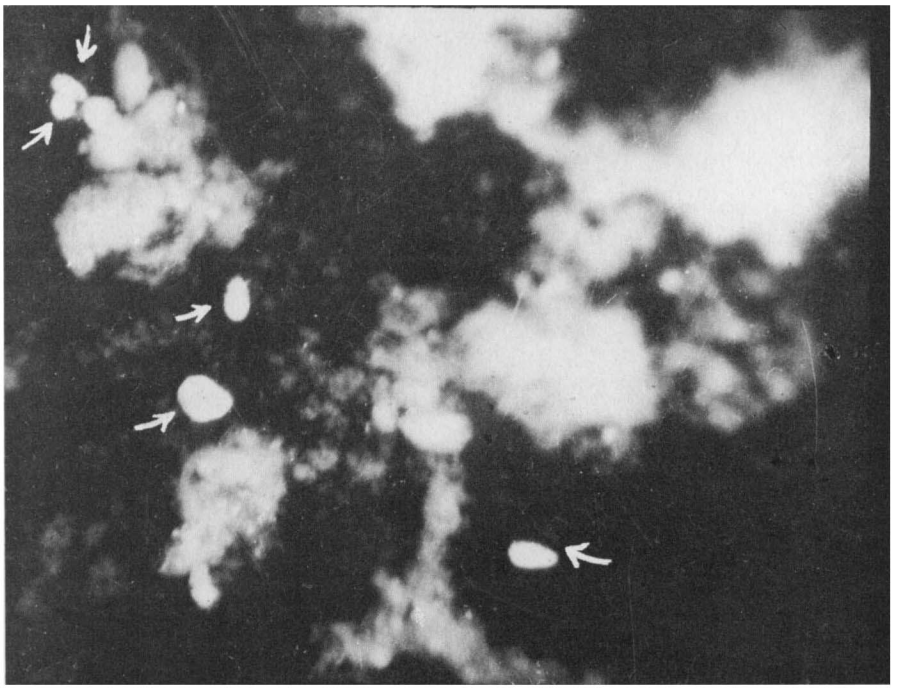


FIGURE 1. Appearance of pancreatic tissue digest under the dissecting microscope (×10) just before manual harvesting of the islets. The distinction between the islets (indicated by arrows) and the acinar tissue is obvious.

optimal duration of collagenase digestion, as assessed by the yield of islets, varied from 7.5 to 10 min in preliminary experiments according to the potency of the different batches of the enzyme preparation. Digestion was then quickly depressed by dilution with 15 ml of medium. The centrifuge tube was rapidly brought up to 520 *g* in a swinging bucket and stopped again as quickly as possible. The viscous supernatant was discarded. The sediment was diluted with another 15 ml of medium and sedimented again in the same way. The sediment was washed out into a Petri dish with a blackened bottom. Under a dissecting microscope (×10), the islets were readily identified as dense, discrete, white particles having a clear oval or round shape, whereas the pieces of acinar tissue were less dense, with hazy margins, and were irregular in shape or size (Figure 1). The islets (about 200 to 300 μm in diameter) were picked up by suction into a finely tipped capillary tube under the dissecting microscope. The average yield was about 80 to 100 islets. In the initial experiments to study the effect of conditions of isolation, the islets were isolated either in KRB or in Hanks' medium,[11] care being taken to keep the medium thoroughly oxygenated at every step. Both media were used either at room temperature (about 27 °C) or at 5 °C. Afterwards the pancreas and the islets were always handled at 5 °C in Krebs' bicarbonate medium, except during digestion, preincubation, and incubation.

INCUBATION PROCEDURE

In the initial experiments to determine the effects of different incubation media, either Krebs' bicarbonate or Hanks was used. In the later studies, the medium was always the former. Incubation media always contained bovine albumin (0.5 g/100 ml). Five or ten islets were transferred to a culture tube (12 × 75 mm). After removing the medium as completely as possible, exactly 1 ml of medium containing 50 mg% D-glucose was quickly added. After oxygenation and stoppering, the tubes were kept in water bath at 37 °C, with constant mechanical agitation of the water, for a variable period of time, during which insulin secretion was *not* measured (preincubation period). (The preincubation media always contained 50 mg% glucose.) The medium was then removed as completely as possible and discarded. Fresh medium (1 ml) containing either 50 or 300 mg% of glucose was added. The tubes were incubated again, after oxygenation and stoppering, for variable periods of time, during which insulin secretion was estimated (incubation period). At the end of the incubation period, the medium was completely removed and assayed for insulin after suitable dilution with the same medium.

ASSAY

The method of Wright et al.[12] was used, all samples being assayed in triplicate.

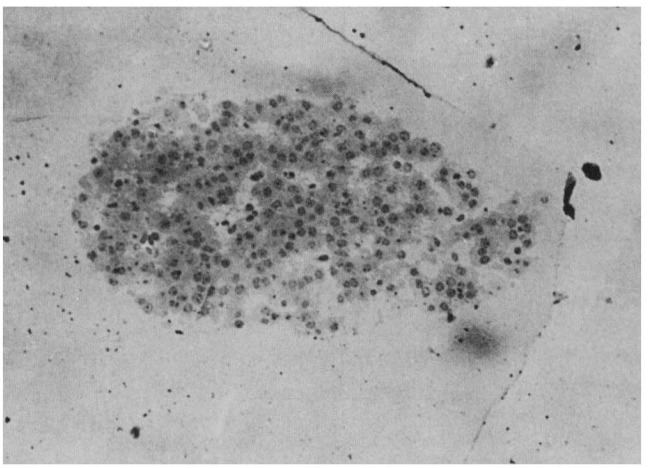


FIGURE 2. Microscopic appearance of typical isolated monkey islet after staining with hematoxylin and eosin. The islet tissue is free of exocrine cells.

TABLE 1
Insulin secreted in response to glucose in monkey islets isolated under different experimental conditions

Isolation medium	Isolation temperature	Basic incubation medium	Number of experiments	Rate of Insulin Secretion* (ng/h/islet)	
				Low glucose (50 mg%)	High glucose (300 mg%)
KRB†	5°C	KRB	10	0.64 ± 0.12‡	2.22 ± 0.29
KRB	27°C	KRB	9	0.09 ± 0.05	2.70 ± 0.48
Hanks	5°C	KRB	4	0.15 ± 0.02	2.34 ± 0.08
Hanks	27°C	Hanks	8	0.10 ± 0.01	2.07 ± 0.26

Islets were preincubated in low glucose media for 30 min. The insulin secretion was estimated during subsequent incubation for 60 min in the same media but containing either 50 or 300 mg% glucose.

* Values are mean ± SEM.

† Krebs' bicarbonate medium.

‡ In these early experiments, the incubation medium had been diluted before assay and the actual assay values were at the lower limits of sensitiveness of the assay.

RESULTS

Purity of islets. Twenty islets each were isolated from five monkeys successively and as quickly as possible. The pool of 100 isolated islets was transferred to phosphate-buffered (pH 7.2) glutaraldehyde fixative and embedded in Araldite resin. Sections 1 μm in thickness were stained with eosin and hematoxylin. Examination under light microscope did not reveal any acinar tissue adherent to the islets or in separate pieces. This not only indicated the accuracy of islet identification under the dissecting microscope, but it also showed that the isolated islets were completely free of acinar tissue (Figure 2).

Effect of different incubation media and isolation temperature. To determine the optimal experimental conditions, islets were isolated and incubated under the different conditions shown in Table 1. It is clear that the response to glucose was not influenced by the temperature at which the islets were isolated or whether KRB or Hanks' medium was used.

Effect of duration of preincubation on insulin secretion. Using KRB as the basal medium and isolating the islets at 5 °C, we investigated whether variations in the duration of preincubation in low glucose medium affected the rate of insulin secretion during the subsequent 60 min of incubation in high glucose medium (300 mg%). It is clear from Table 2 that the time interval of preincubation of the islets did not change their subsequent insulin secretory response to glucose. To obtain a basal state in the islets, some period of preincubation was desirable; 30 min was chosen as the standard duration of preincubation in all the subsequent experiments.

Comparison with isolated rat islets. Since the insulin secretory response of the isolated monkey islets to glucose in all the above experiments appeared lower than in most isolated rat islet preparations, the experimental conditions were verified by estimating the secretory response of rat islets isolated under exactly identical conditions (i.e., basal medium KRB, isolation at 5 °C, preincubation for 30 min in low glucose medium, and incubation in low or high glucose medium for 60 min). In five paired experiments in rat islets, the insulin secretion rate was 0.12 ± 0.02 (SEM) ng/h/islet in low glucose medium (50 mg%) and 5.56 ± 0.43 (SEM) ng/h/islet in high glucose medium (300 mg%). This response of rat islets was comparable with that in numerous other studies on isolated rat islets,[13,14] though it was more than twice the response of our monkey islets.

Time course of insulin secretion during incubation. The insulin output of monkey islets incubated for periods varying from 15 to 60 min after a standard preincubation of 30 min is shown in Table 3 and Figure 3. It is clear that the rate of insulin secretion is fairly constant throughout this period, though there is a slight, progressive increase in the rates estimated from longer periods of incubation.

DISCUSSION

The yield of islets from the monkey by the present technique is about the same as that reported by Lacy and Kostianovsky[1] for rat. Scharp et al.[4,5] have reported an improved isolation technique incorporating a filtration system during digestion and giving a higher yield in monkeys.

Either KRB or Hanks has found favor with different workers as the preferred medium in previous studies on isolated islets. The present comparative study on the effect of using either medium, under otherwise similar conditions, suggests that both media are equally satisfactory. Similarly, it appears that islets behave comparably, irrespective of whether care was taken to lower their oxygen requirement during the isolation procedure by hypothermia. However, on theoretic grounds, it would seem preferable to carry out the isolation procedure at low temperature, as has been done in the present studies.

TABLE 2
Effect of variations in the duration of preincubation on the secretion of insulin during a subsequent incubation of 60 min.
(For experimental conditions, see text.)

Duration of preincubation (min)	Glucose concentration in incubation medium (mg%)	Rate of insulin secretion* (ng/h/islet)
30	50	0.12 ± 0.01
15	300	2.13 ± 0.08
30	300	2.10 ± 0.10
45	300	2.05 ± 0.07
60	300	2.09 ± 0.15

* Mean of four experiments ± SEM.

TABLE 3
Rate of insulin secretion in monkey islets during incubations of varying duration

Conc. of glucose (mg%)	Duration of incubation (min)	Rate of insulin secretion* (ng/h/islet)
50	60	0.16 ± 0.02
300	15	1.94 ± 0.07
300	30	2.02 ± 0.03
300	45	2.11 ± 0.07
300	60	2.31 ± 0.08

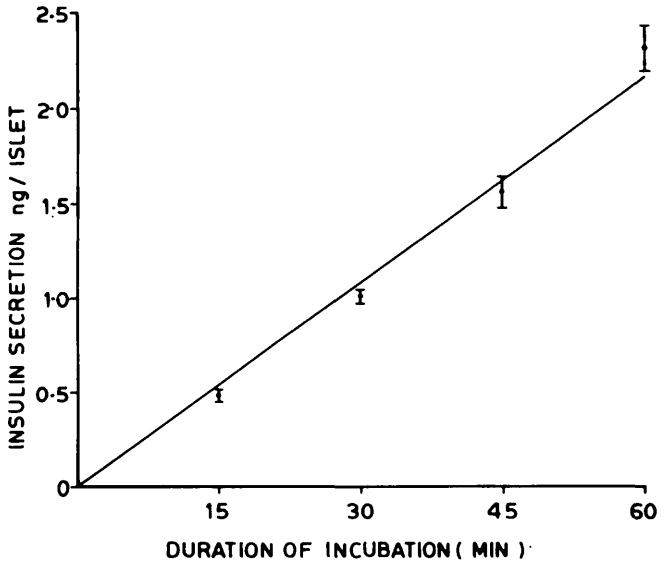
* Mean of ten experiments ± SEM.
Preincubation of all islets was for 30 min in low glucose medium. Islets were then incubated for varying durations and in different media, as shown.

Since there was little difference in the secretory rates between islets studied for a total of 45 min and 120 min, we concluded that islets isolated by the present procedures are viable for at least 120 min.

The steady time course of insulin secretion, depicted in Figure 3, indicates that this preparation is suitable for secretory studies where the experimental design requires different combinations of incubation periods over a range of 15 to 60 min. Of course, in all these estimates, the initial peak and the subsequent prolonged phase of insulin secretion induced by glucose are being averaged.

The major peculiarity observed in the present study on monkey islets is that the amount of insulin released in response to high glucose is distinctly lower than that observed in islets isolated from the small, laboratory animals.[13,14] This cannot be caused by the isolation or incubation procedures, since, under identical conditions, the rat islets showed the expected rates of insulin secretion. Strict quantitative comparisons are not justified, however, since both the rat and monkey insulin concentrations were assayed against standards of porcine insulin. The rate of insulin released by monkey islets in the present study, though only about half that in the rat islets, was comparable with that reported by Scharp et al.[5] for isolated monkey islets.

FIGURE 3. Rate of secretion in monkey islets during incubations of varying duration. Values are mean ± SEM of ten experiments. In each experiment, islets from the same animal were incubated for all the four periods. For details of experimental conditions, see text. The continuous line is a straight line joining zero and the experimental points.



In the first, reported study on human islets isolated in a similar way, Ashcroft, Bassett, and Randle[15] observed insulin secretion rates of about 1.1 ng/h/islet in high glucose medium (300 mg%); this was about three and a half times their basal value in low glucose medium (60 mg%). Similarly Lundgren et al.[16] had reported insulin secretion rates of about 1 ng/h/human islet in high glucose medium (16.7 mM), which was about twice their basal value in low glucose medium (3.3 mM). In other studies reported on isolated human islets,[9,17] the precise magnitude of the insulinogenic response to glucose is not available, though they observed the biphasic response to glucose.

Wilson and Martin[18] observed up to an eightfold increase in plasma insulin concentrations in monkeys when the plasma glucose values were raised from about 50 mg/dl to 225 mg/dl. In our studies, except for the early values in the first row in Table 1 (see footnote to Table 1), the insulin secretion at low glucose levels never exceeded 0.17 ng/h/islet. On exposure to high glucose, the insulin secretion always exceeded 1.9 ng/h/islet. Thus the relative increase in insulin secretion in response to glucose was never less than about tenfold, which compares well with the sixfold increase in human islets,[19] suggesting that the secretory pattern of these islets was normal, at least in regard to the response to glucose.

Thus the monkey islets isolated in the present study gave as good or a better response to glucose as in available studies on isolated primate islets. In addition, it would appear that the basal level of secretion in a low glucose medium was lower than that observed by other workers. A more detailed exploration of the secretory response of these isolated islets to other stimuli is in progress.

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REFERENCES

[1] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35–39, 1967.
[2] Montague, W., and Taylor, K. W.: Pentitols and insulin release by isolated rat islets of Langerhans. Biochem. J. *109*:333–39, 1968.
[3] Coll-Gracia, E., and Gill, J. R.: Insulin release by isolated pancreatic islets of mouse incubated in vitro. Diabetologia *5*:61–66, 1969.
[4] Scharp, D. W., Murphy, J. J., Newton, W. T., Ballinger, W. F., and Lacy, P. E.: Application of an improved isolation technique for islet transplantation in primates and rats. Transplant. Proc. *7*:739–41, 1975.
[5] Scharp, D. W., Murphy, J. J., Newton, W. T., Ballinger, W. F., and Lacy, P. E.: Transplantation of islets of Langerhans in diabetic rhesus monkeys. Surgery *77*:100–05, 1975.
[6] Wright, P. H., Makulu, D. R., and Posey, I. J.: Guinea pig anti-insulin serum. Adjuvant effect of H. pertussis vaccine. Diabetes *17*:513–516, 1968.
[7] Krebs, H. A., and Henseleit, K.: Untersuchungen über die Hernstoffbildung im Tierkorper. Hoppe-Seylers Z. Physiol. Chem. *210*:33–66, 1932.
[8] Jonasson, O., Reynolds, W. A., Snyder, G., and Hoversten, G.: Experimental and clinical therapy of diabetes by transplantation. Transplant. Proc. *9*:223–32, 1977.
[9] Najarian, T. S., Sutherland, D. E. R., and Steffes, M. W.: Isolation of human islets of Langerhans for transplantation. Transplant. Proc. *7*:611–13, 1975.

[10] Ferguson, J., Allsopp, R. H., Taylor, R. M. R.,and Johnston, I. D. A.: Isolation of viable human pancreatic islets. World J. Surg. *1*:69–77, 1977.

[11] Hanks, J. H., and Wallance, R. E.: Relation of oxygen and temperature in the preservation of tissue by refrigeration. Proc. Soc. Exp. Biol. Med. *71*:196–97, 1949.

[12] Wright, P. H., Makulu, D. R., Vichick, D., and Sussman, K. E.: Insulin immunoassay by back-titration: some characteristics of the technique and the insulin precipitant action of alcohol. Diabetes *20*:33–45, 1971.

[13] Malaisse-Lagae, F., and Malaisse, W. J.: Stimulus secretion coupling of glucose induced insulin release. III. Uptake of calcium by isolated islets of Langerhans. Endocrinology *88*:72–80, 1971.

[14] Malaisse, W. J., Malaisse-Lagae, F., Baird, K., and Lacy, P. E.: A hypothetical model for the stimulus secretion coupling of glucose induced insulin release. Proc. VII Congr. Int. Diab. Fed. (ICS 231), Rodrigues and Vallance Owen, Eds. 1971, pp. 443–59.

[15] Ashcroft, S. J. H., Bassett, J. M., and Randle, P. J.: Isolation of human pancreatic islets capable of releasing insulin and metabolising glucose in vitro. Lancet *1*:888–89, 1971.

[16] Lundgren, G., Andersson, A., Borg, H., Buschand, K., Groth, C. G., Gunnarsson, R., Hellerstrom, C., Petersson, B., and Ostman, J.: Structural and functional integrity of isolated human islets of Langerhans maintained in tissue culture for 1–3 weeks. Transplant. Proc. *9*:237–40, 1977.

[17] Ballinger, W. F., and Lacy, P. E.: Transplantation of intact pancreatic islets in rats. Surgery *72*:175–86, 1972.

[18] Wilson, R. B., and Martin, J. M.: Plasma insulin concentration in dogs and monkeys after zylitol, glucose or tolbutamide infusion. Diabetes *19*:17–22, 1970.

[19] Jahr, H., Lippert, H., Fuhrmann, K., Besch, W., Lorenz, D., and Zuhlke, H.: Preparation and (Pro) insulin biosynthesis and secretion of isolated human pancreatic islets. Diabetologia *15*:242–43, 1978.