THE ISOLATION AND STUDY OF MAMMALIAN ISLETS OF LANGERHANS

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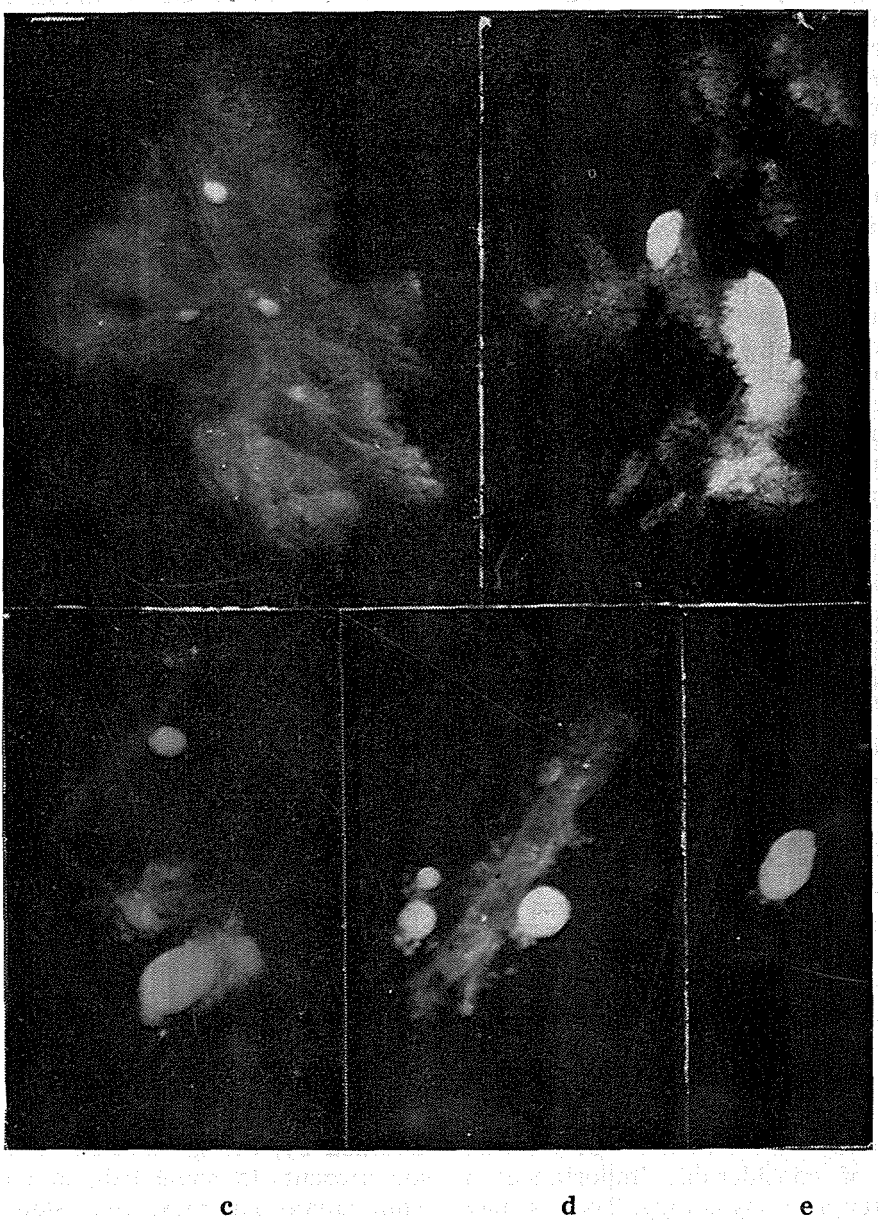
The study of metabolism of isolated tissues is of considerable importance in biochemistry and physiology. Tissues may be studied as homogenates or slices, as cell cultured *in vitro*, or whole organs may be perfused. Pancreatic tissue, however, presents a particular problem. A homogenate of mammalian pancreas, for example, is representative of the acinar portion of the organ, but it is not very representative of the islets of Langerhans which constitute perhaps 1-2 percent of the pancreas. One can never be sure that any effect observed which is ascribable to the islets, is not either mediated through, or modified by, the great bulk of acinar tissue present. In some fish, in contrast to mammalian pancreas, the islet tissue is segregated as a single body (the principal islet) from the diffuse acinar tissue, and several investigators have found fish a good source of islet cells for this reason (see Lazarow, 1963).

In the last two or three years several groups of workers have found it possible to isolate individual islets of Langerhans from mammalian pancreas, and by the use of micro-methods, to study their metabolism. It is the purpose of the present article to review some of the methods used by



Legend to Fig. I

Fig. 1. Micro-dissection of mouse pancreatic islets. (a) A thin piece of pancreas of a normal mouse. The islets are recognised as white spots disseminated along the ducts and blood vessels. (b) After teasing the tissue into smaller pieces, the islets are seen as spherical or ovoid bodies partly surrounded by acinar cells. (c) (d) Removal of surrounding acini reveals intimate connections between islets and blood vessels. (e) A single islet has been isolated by cutting off its connections with the blood vessel. A few acinar cells remain at the lower pole. (Photograph kindly supplied by Dr. C. Hellerström).

myself and others, and to indicate some of the results obtained.

There are two methods by which mammalian islets of Langerhans can be isolated: (1) micro-dissection, and (2) selective enzyme treatment to loosen or remove much of the acinar tissue. These two methods will be considered separtely, although it is sometimes advantageous to use both methods, that is, to digest with an enzyme prior to micro-dissection.

Micro-dissection

A method has been developed whereby fresh tissue is frozen solid, sections are cut with a microtome and are lyophilised; individual islets are then micro-dissected from the surrounding acinar tissues (Lowry, 1953; Lacy, 1962; Dixit, Lowe, and Lazarow, 1962; Kotoulas, Morrison, and Recant, 1965; Kotoulas and Recant, 1966). Such pieces of tissue have been used to study co-enzyme levels and certain enzyme systems believed to be connected with the systhesis of insulin. The enzymes of the tissue do not appear to be destroyed by this treatment, though the tissue can no longer be regarded as living. The implication must be that tissue prepared in this way is valuable material for enzyme studies, but cannot claim to represent tissue in its normal dynamic state.

Hellerström (1964) on the other hand, has reported encouraging results from micro-dissection of whole fresh pancreas *(see Fig. 1)*. Watchmaker's forceps are used to pull out intact islets from the surrounding acinar tissue. The acinar tissue may be cut away with sharpened hypodermic needles, and the whole process is performed under the dissection microscope at 12-50 × magnification. The case with which dissection is accomplished depends very much on the animal used. Mouse pancreas is the best material since the organ is thin and has comparatively large islets which stand out as whitish bodies against a pinkish acinar tissue. The pancreas of the congenitally obese-hyperglycaemic mouse has also been used as a source of islet tissue. The pancreas (and the islets) in such animals is larger than in normal mice, and the islets are therefore correspondingly more easy to remove. Furthermore, Beta-cells comprise more than 90% of the cells in these islets. The metabolism of such islets is thus of particular interest, but until the specific defect causing the obesity and hyperglycaemia is better understood, it is perhaps wise not to consider the material identical with normal islets. Rat, guinea-pig, and rabbit pancreas may also be micro-dissected, but the process is made more difficult by several factors. These factors are the relatively greater thickness of the acinar tissue in which the islets are embedded, the small size and irregular shape of the islets, and the thinness of the capsule surrounding the islets. In the rat, removal of islets by micro-dissection is reported to be facilitated by ligation of the upper pancreatic duct 2-4 weeks before dissection (Keen, Sells, and Jarrett, 1965).

Attempts to reduce the bulk of mouse pancreas by drug treatment as opposed to ligaturing have so far met with little success. Dunnigan, Gagnon, and Berlinguet (1964) reported that an amino acid analogue, 1-aminocyclopentane carboxylic acid (ACPC), caused rapid atrophy of the acinar portion of rat pancreas without affecting the islets. We found (Wood and Smith, unpublished work) that in mice, the dose of this drug sufficient to cause a considerable amount of acinar atrophy was in the region of the lethal dose. In a group of six mice which were injected with ACPC in five daily doses of 0.25 mg. per gram body weight, all were very sick, and one of them died before the termination of the experiment. The amount of proteolytic enzyme activity present in homogenates of pancreas after activation of the zymogens was measured to obtain an indication of the amount of acinar tissue present. Although the total proteolytic enzyme activity was reduced to 20 percent of that of the pancreases of untreated controls, the pancreatic weights did not fall in the same proportion. The pancreases of the treated animals were consequently only marginally easier to micro-dissect.

Digestion with Enzymes

Micro-dissection has certain limitations. It is difficult to obtain more than 20-40 islets within 30 minutes of killing the animal. Furthermore, only with mouse and rat pancreas is it a reasonably easy task. To obtain (a) larger amounts of tissue, and (b) islets from other species, digestion of the pancreas with enzyme appears to be the best method. In order to obtain islets of Langerhans for culture *in vitro*, Moskalewski (1965) treated guinea pig pancreas with bacterial collagenase. In a preliminary report, Kostianovsky and Lacy (1966) described how 200-300 islets could be obtained from normal rat pancreas by digesting with collagenase. The acinar parenchyma is initially disrupted by injecting physiological medium into the pancreatic duct, and after digestion, the islets are obtained by sedimentation or centrifugation (see also Lacy and Kostianovsky, 1967). A modification of this method was used by Howell and Taylor (1966) for the isolation of rabbit islets.

Collagenase seems to be the only enzyme that can be used in these isolation procedures. In order to obtain intact islets by enzyme digestion, the enzyme chosen must break up the acinar tissue into single cells or small clumps of cells, but must leave intact the delicate capsule surrounding the islets. We have investigated (Wood and Smith, unpublished) several proteolytic enzymes under a variety of conditions. Trypsin and Chymotrypsin appear to have very little effect on mouse pancreas, whereas papain and several bacterial proteinases caused a great deal of digestion but were not selective, i.e. the islet as well as the acinar tissue was destroyed. The extent of digestion with collagenase depended on the rate of shaking or stirring during incubation as well as on the concentration of collagenase. On a shaker 30-60 minutes digestion at 37°C was required, but the time could be as short as 5-10 minutes if the mixture was stirred rapidly. It was found best to use a flat-bottomed test tube of 3 cm. diameter which contained a magnetic stirrer slightly less than 3 cm. in length. If the period of digestion was prolanged unduly under these conditions, the islets themselves were eventually disrupted. It was therefore necessary to take samples of the digest at intervals and to examine them under the dissection microscope in order to judge when sufficient digestion has taken place.

It was advantageous to use different digestion procedures with the pancreases from different species. With mouse pancreas, a gentle collagenase digestion could be used to weaken the acinar tissue after which micro-dissection became very easy. On the other hand, for rabbit and guinea-pig pancreas, a vigorous digestion gave the best results. After washing in cold medium the islets were allowed to sediment, and then, under the dissection microscope, were picked out with a fine pipette. Several hundred islets could be obtained in about 30 minutes by this method. The treatment of pancreas prior to digestion is important. As described by Howell and Taylor (1966), the pancreas is distended by local injection of buffered medium through a fine needle at several sites at random. This procedure appears to separate the lobes of acinar tissue and makes the pancreas more susceptible to the action of collagenase. After this treatment the organ is cut into small pieces and is incubated with collagenase.

With a little practice it is not difficult to identify the individual, freed islets in a digestion mixture. However, if there is any doubt, a sample of the digest may be treated with dithizone solution. This compound stains islet tissue brick red, acinar tissue faintly orange, and adipose cells deep green. It is interesting that the islets of the guinea pig could not be stained by this method: this presumably reflects either the amount, or the state, of zinc in these islets, in contrast to mouse, rat, and rabbit islets (*cf.* Maske, 1957).

Islet Tissue Metabolism

There is abundant evidence that islets obtained by these methods are in a state which closely resembles their state *in vivo*. They survive the tissue culture (Moskalewski, 1965); they respire normally (Hel-

lerström, 1966); they sythesise insulin, and the insulin synthesis is stimulated by glucose (Howell and Taylor, 1966; Martin and Gagliardino, 1967). Islets obtained from hypophysectomised rats show impaired insulin synthesis unless the rats are given growth hormone (Martine and Gagliardino, 1967). Certain problems arise when dealing with such small amounts of tissue as a few islets. A single islet may have a dry weight of between 1 and 10 ug., and so micro-methods must be used to follow the metabolism of isolated islets. Hellerström (1966) used a Cartesian diver microbalance to measure the respiration of a single islet. Other workers have employed radio-isotope methods because of their sensivity and accuracy.

A single pancreatic islet is a fragile structure, and a light touch with a sharp needle is enough to rupture the containing membrane and release the cells. Pipettes used for transferring islets into incubation vessels must therefore have no sharp edges. Alternatively islets may be transferred with watchmaker's forceps. During micro-dissection it is advisable to leave the islets attached to small pieces of capilary by which they can be handled. If a homogenate of islet tissue is required, the micro-homogenisor described by Eichner (1966) should prove valuable. A loop of fine wire is driven inside a capilary tube by means of a dental drill. In this apparatus, it is claimed, a very few cells can be homogenised without loss of material.

Finally some consideration needs to be given to the problem of weighing islets or of finding their protein content. One can pick out islets for a given experiment so that they are all of approximately the same size. Nevertheless it is clearly desirable to be able to express results in terms of dry weight or protein content. Islets can be weighed on a quartz fibre ultramicrobalance after they have been dried on a piece of platinum foil, and this is probably the only reliable method for measuring the size of islets, though it is a destructive method, in that after drying, the islets are useless for other purposes.

It is clear that isolated islets of Langerhans obtained by the methods described are amenable to study by suitable micro-methods. Moreover, it is encouraging that the results obtained so far are in accord with our earlier knowledge of endocrine pancreatic function. A future problem may be that even isolated islets do not contain a single cell type. Though the largest proportion of cells in islets are beta-cells, alpha-cells and other cell types are also present. It is possible that future techniques may provide us with pure preparation of beta-cells.l

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