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Potassium Ions and the Secretion of Insulin by Islets of Langerhans Incubated *in vitro*

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1. A method was devised for the isolation of islets of Langerhans from rabbit pancreas by collagenase digestion in order to study the influx and efflux of K^+ in islets during insulin secretion. 2. Glucose-induced insulin release was accompanied by an increased rate of uptake of $^{42}K^+$ by the islets of Langerhans, though this was not the case for secretion in response to tolbutamide. Ouabain significantly inhibited the uptake of $^{42}K^+$ by islet tissue. 3. No significant increase in the rate of efflux of $^{42}K^+$ was demonstrated during active insulin secretion. 4. Slices of rabbit pancreas were incubated in media of different K^+ content, and rates of insulin release were determined. Alteration of the K^+ concentration of the medium between 3 and 8mM had no effect on the rate of insulin release by pancreas slices. However, decrease of the K^+ concentration to 1mM resulted in inhibition of secretion in response to both glucose and to tolbutamide. Conversely, an increase in K^+ concentration increased rates of insulin release in response to both these stimuli. 5. It is concluded that, though unphysiological concentrations of K^+ may influence the secretion of insulin, fluxes of K^+ in the islets do not appear to be important in the initiation of insulin secretion.

Impaired glucose tolerance may accompany K^+ depletion in experimental animals (Gardner, Talbot, Cook, Berman & Concepcion, 1950; Fuhrman, 1951), as well as in man (Sagild, Andersen & Andreasen, 1961; Conn, 1965), and this may result from a diminished secretion of insulin (Spergel, Schmidt, Stern & Bleicher, 1967; Mondon, Burton, Grodsky & Ishida, 1967). In addition, the administration of K^+ may increase concentrations of circulating insulin (Spergel, Bleicher, Goldberg, Adesman & Goldner, 1967).

It therefore seemed possible that alterations of serum K^+ concentrations might directly affect the secretion of insulin. While the present work was in progress, it was reported that changes in K^+ concentration may alter insulin secretion from perfused pancreas, or isolated pancreas *in vitro* (Grodsky & Bennett, 1966; Milner & Hales, 1967*a*). Analogous effects of increasing K^+ concentrations on release of hormones from other tissues, including the adrenal medulla (Vogt, 1952) and neurohypophysis (Douglas & Poisner, 1964; Dicker, 1966), have also been observed. It was therefore decided to investigate further the way in which K^+ might influence rates of insulin secretion by mechanisms that may be common to gland cells of several types. This was attempted by a study of the movement of K^+ in and out of islet cells in different phases of their secretory activity.

METHODS

Materials. D-Glucose of A.R. grade was obtained from Hopkin and Williams Ltd., Chadwell Heath, Essex. Bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex. Tolbutamide was provided by Hoescht G.m.b.H., Frankfurt, W. Germany. Inorganic salts and other reagents were of A.R. grade, or of the purest grade available. Ouabain (Strophanthin G) was obtained from British Drug Houses Ltd., Poole, Dorset. Collagenase (form II) was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks. DL-[4,5-$^3H_2$]Leucine (specific radioactivity 24·3c/m-mole) and ^{125}I-labelled insulin (specific radioactivity approx. 50μc/μg.) were from The Radiochemical Centre, Amersham, Bucks. $^{42}K^+$ was obtained as an iso-osmotic solution of ^{42}KCl in distilled water (specific radioactivity 5–10mc/g. of K at the time of delivery) from The Radiochemical Centre, Amersham, Bucks.

Animals. Male rabbits of a New Zealand White strain weighing 2–3kg. were deprived of food overnight and were given 5% glucose in water to drink before slaughter.

Incubation media. A bicarbonate-buffered salt solution was used (Gey & Gey, 1936). It was gassed with O_2+CO_2 (95:5) immediately before use. In experiments in which the K^+ concentration was altered from 5mM, this was accomplished by the addition or omission of KCl; the concentration of NaCl in the media was also adjusted to maintain a constant overall osmolarity of the incubation media in all experiments.

Media containing $^{42}K^+$ were prepared by the addition of KCl solution (11·5mg./ml.) to give a final concentration of

K^+ in the medium of 5 mM and specific radioactivity approx. 40 μc/m-mole.

Insulin assay. The insulin content of incubation media and of extracts of islet tissue was determined by radioimmunoassay (Hales & Randle, 1963). Solutions of crystalline ox insulin were used as standards, as in previous experiments when rabbit insulin was assayed (Coore & Randle, 1964; Howell & Taylor, 1967).

Determination of radioactivity. Radioactivity of ^{125}I-labelled insulin used in the immunoassay was determined in a liquid-scintillation spectrometer (Beckman Instruments Ltd., Glenrothes, Fife).

Radioactivity of ^{42}K was determined in a crystal-scintillation counter (Nuclear Enterprises Ltd., Edinburgh). The isotope was counted with an efficiency of approx. 20%. Samples were counted for at least 60 min. each or until a total of 4000 counts had accumulated, and the radioactivity above background was corrected for decay to the time of counting of the first sample.

Determination of K^+. The K^+ content of media and of tissue extracts was measured with a flame photometer (Evans Electroselenium Ltd., Harlow, Essex) calibrated to give a maximum deflexion with a standard solution containing 100 μM-K^+ in ion-free water.

Incubation of pancreas slices. Rabbits were killed instantaneously by the intravenous injection of sodium hexobarbitone (Nembutal; Abbott Laboratories Ltd., Queenborough, Kent) (100 mg./kg. body wt.) and the pancreas was immediately removed into ice-cold medium containing glucose (0·5 mg./ml.) in a Petri dish. The organ was trimmed free of excess of fat and blood vessels, and cut into approx. 50 mg. slices; the slices were preincubated in 20 ml. of medium of glucose content 0·5 mg./ml. in a 250 ml. conical flask for 30 min. The flask was gassed with O_2+CO_2 (95:5) immediately before the start of each incubation.

Slices were then washed briefly in medium of composition similar to that in which they were subsequently to be incubated, and transferred individually to 25 ml. conical flasks each holding 1 ml. of medium containing glucose (0·6 or 3·0 mg./ml.), albumin (2 mg./ml.) and K^+ concentrations of 1, 3, 5, 8 or 10 mM. In a typical experiment four flasks contained medium of low glucose and normal K^+ content, six flasks medium of high glucose and normal K^+ content, and six flasks medium of high glucose and high or low concentrations of K^+.

The flasks were gassed once more with O_2+CO_2 (95:5), stoppered and incubated with gentle shaking for 30 min. at 37°. The pieces of tissue were then immediately removed from the medium and weighed to an accuracy of $\pm$2 mg. on a torsion balance. The medium was frozen and stored for assay of its insulin content at a later date. Rates of insulin release were calculated as mμg./min./g. wet wt. of pancreas. In experiments in which tolbutamide was used as a stimulus to insulin release, the substance was dissolved to give a concentration of 0·2 mg./ml. in medium of glucose content 0·6 mg./ml.

Isolation of islets of Langerhans. Small fragments of whole rabbit pancreas were incubated with solutions of collagenase; islets of Langerhans could then be separated in large numbers from the tissue fragments that remained.

Rabbits were killed by the method described above and the splenic portion of the pancreas was removed. This portion of the organ was placed in medium in a Petri dish and gross excess of fat was removed by trimming with scissors. Bicarbonate-buffered medium was then injected into the body of the tissue with a syringe fitted with a 20-gauge needle. Injections were made at several sites until the whole pancreas was grossly distended, a total volume of 7–10 ml. being used for this purpose. Excess of fat and blood vessels, which were now clearly visible, were then dissected away and the pancreas was chopped as finely as possible with scissors.

The pieces were transferred to a 15 ml. centrifuge tube, any that floated being removed since these consisted predominantly of fragments of adipose tissue. The pancreatic tissue that remained was spun down gently and the supernatant was pipetted off completely. The pieces were then poured into a 25 ml. conical flask and 2 ml. of a solution of collagenase (4·5 mg./ml.) in bicarbonate buffer of low glucose content (0·6 mg./ml.) was added. The flask was stoppered securely and shaken at a rate of about 200 cyc./min. in a metabolic shaker (Mickle Ltd., Gomshall, Surrey) at 37° for 20–30 min. The correct duration of incubation was determined in preliminary experiments by sampling the contents of the flask at 2 min. intervals and continuing the incubation until the islet tissue appeared to be predominantly free of acinar fragments. Subsequently the incubation time was judged sufficiently accurately by the size and appearance of the fragments in the flask.

The solution remaining at the end of the incubation was poured immediately into 20 ml. of medium of low glucose content (0·6 mg./ml.) and spun down gently. Islets and fragments of acinar tissue separated out at the bottom of the tube and the supernatant was rejected. The fragments were resuspended in a further 20 ml. of the same medium and again centrifuged, the washings being rejected. This washing was repeated a third time before suspension of the tissue in 2 ml. of medium and transference of the suspension to a Petri dish with a black base for further examination and separation of the islet tissue.

The tissue fragments in the Petri dish were diluted with a further 10 ml. of medium and examined with a dissecting microscope (Bausch and Lomb Ltd., Rochester, N.Y., U.S.A.) under a 10 × magnification. Under these conditions the islets are visible as yellowish-white spheres (Hellerstrom, 1964); fragments of acinar tissue were also present in large numbers. Islets were separated in a preliminary way, together with some fragments of acinar tissue also inadvertently picked up, by using finely drawn Pasteur pipettes; the islets were then transferred to a small test tube (6 cm. × 0·7 cm.). After completion of this initial separation, the contents of the test tube were pipetted into a second Petri dish with a blackened base and further diluted with medium. A second separation of the islet tissue to remove occasional fragments of acinar tissue was carried out by using finely drawn pipettes, the islets again being placed in a small test tube. On examination of the islets under 15 × magnification a third time after further dilution with medium, islets completely free of acinar fragments were visible in large numbers, the medium being almost completely free of acinar tissue. The islets were then pipetted into preincubation or other flasks before the appropriate experimental procedures. The total time taken from the death of the animal to the isolation of islets, ready for incubation, was 45–60 min. Yields of 120–450 islets were obtained from a single rabbit pancreas.

Incubation of islets of Langerhans. (*a*) Experiments studying influx of K^+. Islets were preincubated for 30 min.

in 5ml. of medium of glucose content 0·5mg./ml. and were then distributed, after rejection of the preincubation medium and washing, into 1ml. of medium containing glucose (0·5 or 2·5mg./ml.), albumin (2mg./ml.) and ^{42}KCl to a total concentration of 5mM in a 2in. × ¼in. tube. Three such tubes containing low concentrations of glucose and three containing high concentrations of glucose were used in most experiments. The tubes were placed inside 25ml. conical flasks, gassed thoroughly and incubated with gentle shaking in a water bath at 37° for 60min. At the end of this period the medium was pipetted off and a small portion was counted to determine its specific activity. The remainder of the medium was stored at −20° for 6 weeks so that its insulin content could be determined after the isotope had decayed. Islets were transferred by pipetting into a large centrifuge tube containing 40ml. of 0·25M-sucrose and centrifuged down, the supernatant being rejected. They were then resuspended in a similar volume of 0·25M-sucrose and again spun down. This washing procedure was repeated twice more, the final sucrose washings being retained for determination of their radioactivity. The islets were transferred to pointed 15ml. centrifuge tubes and washed in with sucrose, to a total volume of about 2ml. The tissue was then disrupted by the use of an ultrasonic disintegrator fitted with a ¼in. tip (Soniprobe; Dawe Instruments Ltd., Acton, London, W. 8). Each tube was treated for 2min., the temperature of the contents being maintained below 10° by using an ice bath.

The homogenates were transferred to standard flasks and made up to 5ml. with deionized water before immediate counting and determination of K^+ and insulin content at a later date.

The islets were washed free from the K^+ present in the incubation media by the centrifugation in the large volumes of sucrose mentioned above. Count rates for $^{42}K^+$ in the final sucrose supernatant never exceeded 1–2counts/min. above background. Extracts of islet tissue gave count rates in the range 10–20counts/min. above background.

The duration of ultrasonic disintegration applied gave maximal release of K^+ and insulin contents of the islet cells, and resulted in complete disintegration of the tissue as evidenced by phase-contrast microscopy.

Less than 10% of the insulin content of individual islets may be secreted during a 1hr. incubation of this type, and recovery of insulin from the extracts indicated that the extraction procedure gave consistent recoveries from the tissue present. There was probably some loss of islets during the centrifugation procedure because the recovery of insulin was 30–40% of that calculated for a number of islets used. Estimates of the K^+ fluxes from islets as a proportion of the total K^+ present are therefore likely to be overestimates of the true values.

(*b*) Experiments studying efflux of K^+. Rabbit tissues were labelled *in vivo* with $^{42}K^+$ by the intravenous injection of solutions of iso-osmotic ^{42}KCl in distilled water through a marginal ear vein; in most experiments a dosage of 100μc/kg. body wt. was used. Venous blood samples were taken from the other ear for determination of their radioactivity at 5min. after injection and immediately before the animals were killed 3hr. later. At the end of this time the rabbits were killed by the injection of Nembutal and the islets were separated from the pancreas in the way already described. The isolated islets were preincubated for 20min. in medium of low glucose content and then transferred in batches of 100 to flasks containing 1ml. of medium of low or high glucose content and albumin (2mg./ml.) for incubation in the same way as described.

At the end of the experiment the islets were removed from the medium, and the radioactivity of the medium and of the islets after homogenization by ultrasonic disruption was determined as described. The K^+ and insulin contents of the media were also determined at a later date.

The apparent specific radioactivity of K^+ in the islets at the start of the experiment was determined from the radioactivity present in the medium and that remaining in the islet extracts at the end of the experiment. Net K^+ efflux was then calculated from the radioactivity present in the incubation media. It was assumed that the labelling of K^+ within the islets was uniform; no correction was made for further K^+ loss from the islets during the washing procedure.

In a typical experiment the specific radioactivity of K^+ in the blood of rabbits was 1302counts/min./μmole 5min. after injection, and 217counts/min./μmole 3hr. after injection.

RESULTS

Effects of K^+ *on rates of insulin secretion.* Table 1 shows the results of studies of the effects of alterations of the K^+ content of the incubation medium on insulin release by rabbit pancreas slices in response to glucose (2·5mg./ml.). The K^+ concentration of the incubation medium was altered from 5mM to 1, 3, 8 or 10mM in different experiments; the mean serum K^+ concentrations of 11 rabbits was determined as 4·41mM.

No significant changes in rates of insulin secretion were seen on decreasing the K^+ concentration of the incubation medium to 3mM, though significant inhibition of secretion was seen on decreasing the K^+ concentration to 1mM. Conversely, an increase of K^+ concentration of the medium from 5 to 8mM was without significant effect, whereas in the presence of 10mM-K^+ rates of insulin release were significantly increased (Table 1).

In a similar series of experiments it was shown (Table 2) that insulin release induced by tolbutamide (0·2mg./ml.) was partly but significantly inhibited in the presence of 1mM-K^+ and markedly increased if 10mM-K^+ was present in the incubation medium.

Influx of $^{42}K^+$ *into isolated islets of Langerhans.* (*a*) Glucose-induced secretion of insulin. Table 3 shows that the rate of uptake of $^{42}K^+$ by isolated islets of Langerhans may be significantly increased when the glucose concentration of the incubation medium is increased from 0·50 to 2·50mg./ml. In these conditions there was also a marked increase in the rate of release of insulin from the islets. In separate experiments, the rate of uptake of $^{42}K^+$ was found to be linear over 1hr.; in this time the specific radioactivity of the K^+ within the islets may approach 10% of that in the incubation

Table 1. *Effect of* K^+ *concentration of incubation medium on rates of insulin release in response to glucose by rabbit pancreas slices*

Pancreas slices were preincubated for 30min. in medium of K^+ content 5mM and glucose content 0·5mg./ml. They were then transferred individually to medium containing albumin (2mg./ml.) and the concentrations of glucose and K^+ shown, and incubated for 30min. The slices were then removed and weighed, and the insulin content of the media was determined by immunoassay. Results are given as means ± S.E.M. with the numbers of observations in parentheses.

Concn. of glucose in incubation medium (mg./ml.)	Concn. of K^+ in incubation medium (mM)	Rate of insulin release (mμg./min./g. of pancreas)	*P* value
0·6	5·0	2·30 ± 0·21 (20)	
3·0	5·0	9·45 ± 0·74 (30)	< 0·01
3·0	1·0	6·70 ± 0·63 (30)	
0·6	5·0	2·34 ± 0·19 (12)	
3·0	5·0	9·05 ± 0·73 (18)	> 0·50
3·0	3·0	8·45 ± 0·61 (18)	
0·6	5·0	2·35 ± 0·21 (12)	
3·0	5·0	12·2 ± 0·61 (18)	> 0·50
3·0	8·0	12·6 ± 0·65 (18)	
0·6	5·0	2·40 ± 0·19 (20)	
3·0	5·0	9·55 ± 0·73 (30)	< 0·005
3·0	10·0	12·85 ± 0·83 (30)	

Table 2. *Effect of* K^+ *concentration of incubation medium on rates of insulin release in response to tolbutamide by rabbit pancreas slices*

The experimental procedure was similar to that used for the experiments shown in Table 1. Tolbutamide (0·2mg./ml.) was used in place of glucose (3·0mg./ml.) as a stimulus to insulin secretion. Glucose (0·5mg./ml.) was present in all media. Results are given as means ± S.E.M. with the numbers of observations in parentheses.

Addition to incubation media	Concn. of K^+ in incubation medium (mM)	Rate of insulin release from pancreas slices (mμg./min./g. of pancreas)	*P* value
None	5	3·35 ± 0·43 (16)	
Tolbutamide (0·2mg./ml.)	5	7·70 ± 0·68 (24)	< 0·05
Tolbutamide (0·2mg./ml.)	1	5·70 ± 0·62 (24)	
None	5	3·40 ± 0·45 (20)	
Tolbutamide (0·2mg./ml.)	5	7·60 ± 0·74 (30)	< 0·05
Tolbutamide (0·2mg./ml.)	10	9·70 ± 0·69 (30)	

Table 3. *Rate of influx of* K^+ *into isolated islets of Langerhans during incubation in media of glucose content 0·5 or 2·5mg./ml., or containing tolbutamide (0·2mg./ml.)*

Groups of 40 islets of Langerhans isolated from rabbit pancreas were preincubated for 30min. in medium of glucose content 0·5mg./ml. They were then transferred to media containing 5mM-^{42}KCl together with glucose or tolbutamide and incubated for a further 1hr. period. Results are expressed as means ± S.E.M.

Addition to basal medium	Rate of insulin release (μμg./min./islet)	Sp. radioactivity of K^+ extracted from islets (counts/min./μmole)	K^+ content of islet extracts (mμmoles)	Insulin content of islet extracts (μg.)	No. of observations
Glucose (0·5mg./ml.)	47 ± 6	204 ± 45	48 ± 6	0·72 ± 0·07	14
Glucose (2·5mg./ml.)	89 ± 11	466 ± 95	39 ± 4	0·80 ± 0·07	14
P value	< 0·02	< 0·05	< 0·3	> 0·5	
Glucose (0·5mg./ml.)	45 ± 8	194 ± 39	49 ± 7	0·69 ± 0·05	13
Glucose (0·5mg./ml.) + tolbutamide (0·2mg./ml.)	79 ± 9·5	237 ± 62	38 ± 6	0·57 ± 0·06	13
P value	< 0·02	> 0·5	< 0·4	> 0·2	

medium. This rate of equilibration appears to be comparable with that reported for smooth muscle by Goodford & Hermansen (1961).

The total K^+ content of the islets was decreased after 1 hr. of incubation in medium of high glucose content relative to the K^+ content of the controls in medium of low glucose content, though this decrease was not significant (Table 3). The recoveries of tissue after incubation appeared to be comparable in both sets of flasks, the weight of insulin that was recoverable being very similar in both cases (Table 3). During an incubation of this duration less than 10% of the insulin content of the islets is released into the incubation medium of high glucose concentration.

(*b*) Tolbutamide-induced secretion of insulin. In experiments in which tolbutamide (0·2 mg./ml.) was used as a stimulus to insulin release in place of glucose, there was no significant change between the rate of uptake of K^+ by the flasks containing tolbutamide, relative to controls containing only low concentrations of glucose (Table 3). A significant increase in rates of insulin secretion into the media containing tolbutamide was seen in these experiments. The extractable K^+ and insulin contents both fell slightly in flasks containing tolbutamide, but these differences were not significant (Table 3).

(*c*) Effect of ouabain. Islets of Langerhans were incubated in medium containing 1·5 mg. of glucose/ml. in the presence or absence of ouabain (0·1 mM) for 1 hr. Table 4 shows that the rate of influx of K^+ into the islets as indicated by the specific radioactivity of the K^+ extracted after incubation is markedly decreased in the presence of this compound. No significant increase in the rate of release of insulin was observed in the presence of ouabain in these experiments.

Efflux of $^{42}K^+$ from islets. Table 5 shows the results of experiments in which the efflux of $^{42}K^+$ from islets previously labelled *in vivo* was studied in media of low and high glucose content. The animals were killed after 3 hr., this time being chosen as providing optimum radioactivity in the islets after separation and extraction. The efflux was expressed as the quantity of $^{42}K^+$ present in the islets at the

Table 4. *Effect of ouabain* (0·1 mM) *on influx of* K^+ *into isolated islets of Langerhans*

Islets isolated from rabbit pancreas were preincubated for 30 min. in medium of low glucose content. They were then transferred to flasks containing medium made up with 5 mM-^{42}KCl and glucose (1·5 mg./ml.) and incubated for 1 hr. Islets were washed free of excess of medium, disrupted ultrasonically, and the specific radioactivity of K^+, as well as the K^+ and insulin contents of islets and media, was determined. Results are given as means ± S.E.M.

Addition to basal medium	Rate of insulin release (μμg./min./islet)	Sp. radioactivity of K^+ extracted from islets (counts/min./μmole)	K^+ content of islet extracts (mμmoles)	Insulin content of islet extracts (μg.)	No. of observations
Glucose (1·5 mg./ml.)	75 ± 5	212 ± 32	45 ± 5	0·70 ± 0·07	12
Glucose (1·5 mg./ml.) + ouabain (0·1 mM)	86 ± 9	135 ± 18	38 ± 6	0·67 ± 0·08	12
P value	< 0·30	< 0·05	< 0·40	> 0·50	

Table 5. *Efflux of* K^+ *and insulin release from isolated islets of Langerhans in the presence of* 0·5 *and* 2·5 *mg. of glucose/ml.*

Rabbits were injected with solution of ^{42}KCl, the pancreas being removed for separation of the islets after 3 hr. Islets were preincubated for 15 min. in medium of low glucose content, and were then incubated in batches of 100 in medium of glucose content 0·5 or 2·5 mg./ml. for 1 hr. The incubation medium was separated off and its $^{42}K^+$ content and insulin content were determined. Islets were disrupted ultrasonically before determination of the specific radioactivity of K^+ and the insulin content of the extracts. The specific radioactivity of the K^+ in the islets at the start of the incubation was determined and the efflux of K^+ during the incubation was thus calculated. Results are given as means ± S.E.M. of 11 experiments.

Glucose content of medium (mg./ml.)	Rate of insulin release (μμg./min./islet)	K^+ extractable from islets (μmole)	Insulin extractable from islets (μg.)	Sp. radioactivity of K^+ in islets (counts/min./μmole)	K^+ efflux from islets (μmole/hr.)
0·5	51 ± 8	0·121 ± 0·009	1·88 ± 0·16	150 ± 40	0·038 ± 0·007
2·5	115 ± 15	0·113 ± 0·010	1·79 ± 0·18	165 ± 33·2	0·045 ± 0·008
P value	< 0·005	> 0·5	> 0·5	> 0·5	> 0·5

start of the experiment that had emerged during 1 hr. of incubation. Low count rates in these experiments made accurate determinations difficult, and wide variation was experienced from flask to flask; it was necessary to use batches of 100 islets in each flask to obtain satisfactory results. Table 5 shows that the rate of efflux of K^+ was increased by a mean of 19% in these experiments, but that this increase was not statistically significant.

DISCUSSION

Use of isolated islets of Langerhans for metabolic studies. Methods have recently become available for the isolation of intact islets of Langerhans from mammalian pancreas in large numbers by the use of collagenase (Moskalewski, 1965; Kostianovsky & Lacy, 1966). Islets separated by such methods appear to respond to factors that regulate insulin synthesis and secretion in a way very similar to slices of whole pancreas (Howell & Taylor, 1966; Lacy & Kostianovsky, 1967; Montague, Howell & Taylor, 1967). Moreover, the appearance of islets in the electron microscope is normal (Lacy & Kostianovsky, 1967). In the method described by Kostianovsky & Lacy (1966), the pancreas was distended by injection of saline into the pancreas via the common bile duct before removal of the organ and treatment with collagenase. In the present studies it was found that distension of the organ by the subperitoneal injection of saline after removal from the animal was equally satisfactory. It seemed essential to disrupt the tissue in this way to obtain effective action of the collagenase. This method is readily applicable to the separation of islets from the pancreas of other small mammals such as the rat (Montague *et al.* 1967), in which the pancreatic duct may not be easily available for cannulation.

In the rat, 80% of the cells within the islets of Langerhans appear to consist of insulin-producing β-cells (Carpenter, 1966), and a similar preponderance of β-cells exists in rabbit islets (Gomori, 1939). Islets prepared in this way appear to be devoid of acinar tissue, and it therefore seems likely that results obtained from such a preparation may represent data for the most part derived from a study of the β-cells.

Calculation of K^+ fluxes. It has been assumed throughout the present experiments that the movements of K^+ measured have predominantly represented exchange between incubation media and the intracellular compartment of the tissue. It seems likely that interference from much more rapid exchange of K^+ that may occur with the extracellular part of the islet tissue would be minimized as a result of rapid dilution of this pool occurring during the washing procedures. Further evidence that the uptake of K^+ measured in the experiments does occur into the intracellular compartment is provided by the linear uptake of $^{42}K^+$ over a 1 hr. period, and by the fact that influx is inhibited by ouabain (Table 4). Neither of these conditions would obtain if exchange with the extracellular fluid were the phenomenon that was predominantly being measured.

Influence of K^+ concentration on insulin secretion. The above results indicate that an increase in the K^+ concentration of the incubation medium in which pancreas slices are incubated results in a significant increase in the rate of release of insulin from the slices, both in response to tolbutamide as well as in response to glucose. Alteration of the K^+ concentration of incubation media between 3 and 8 mM appears to be without effect on rates of insulin secretion *in vitro*. Changes in serum K^+ concentrations *in vivo* outside this range are unlikely to occur physiologically, but may be found in certain pathological states. Other investigators have obtained similar effects of K^+ concentration on the secretion of insulin, either in perfusion experiments (Grodsky & Bennett, 1966) or in experiments with isolated rabbit pancreas slices (Milner & Hales, 1967*a*).

Influx of K^+ into the islets during secretion. It is clear from Table 3 that there is a marked increase in the quantity of $^{42}K^+$ that enters the islets during a 1 hr. period when the glucose concentration of the incubation medium is raised from 0·5 to 2·5 mg./ml. However, in the presence of tolbutamide, a pharmacological agent that induces secretion of insulin, no increase in the rate of uptake of K^+ relative to control islets was seen. It therefore appears that secretion of insulin is not obligatorily linked with the increased uptake of K^+ by the islet cells. Tolbutamide, as well as high concentrations of glucose, induces insulin secretion by the movement of β-granules to the plasma membrane, with subsequent release of their insulin contents into the extracellular space (Lacy, 1961; Williamson, Lacy & Grisham, 1961). It seems possible that the increased uptake of K^+ that occurs in the presence of high concentrations of glucose may be related to aspects of the metabolism of glucose within the β-cell that are not directly concerned with insulin release.

The presence of ouabain at a concentration of 0·1 mM partly inhibited the uptake of K^+ by islet cells. At this concentration, ouabain inhibits an Na^+-plus-K^+-dependent adenosine triphosphatase present in the membrane of many mammalian cells (Post, Merritt, Kinsolving & Albright, 1960). This enzyme may therefore also play a role in the transport of K^+ into islets. By contrast ouabain did not affect rates of insulin release in our experiments. However, ouabain may increase the rates of

insulin secretion when present at lower concentrations (Milner & Hales, 1967*a*). The effect of ouabain on secretion at these concentrations may conceivably not be related to its action in inhibiting adenosine triphosphatase activity; thus ouabain has been shown to produce a maximal inhibition of Ca^{2+} uptake by cardiac muscle fragments at a concentration of 1μM with little apparent effect at 0·1mM (Lee & Choi, 1966).

Efflux of K^+. During investigations of the influx of K^+ into islets of Langerhans, it was noted that islets that had been stimulated to secrete insulin generally contained less K^+ on extraction at the end of the experiment than did islets incubated in the control flasks, though these decreases were not statistically significant. In an attempt to determine whether or not there was an increased efflux of $^{42}K^+$ from the islets when secretion was occurring, it was not found possible to label the islets with $^{42}K^+$ by incubation *in vitro* because of the relatively short survival time of the tissue in these conditions (about 2hr. after the completion of separation), and of the relatively long period required for partial equilibration of the extracellular and intracellular K^+ pools. Rabbits were therefore injected *in vivo* with an iso-osmotic solution of ^{42}KCl.

Alteration of the glucose concentration of the medium appears to have no significant effect on K^+ efflux (Table 5). The small loss of total K^+, and slightly increased efflux of $^{42}K^+$, that occurs during incubation in medium of high glucose concentration may be related to the passive loss of K^+ during the processes of granule extrusion.

Role of K^+ *in insulin secretion.* There has recently been some interest in the possibility that electrical activity at the plasma membrane, resulting in alterations in the degree of polarization of the membrane, might influence rates of secretion by gland cells. For example, intracellular membrane potentials in thyroid tissue may be lower in the presence of thyroid-stimulating hormone (Woodbury & Woodbury, 1963). However, in adrenal medullary cells, which possess granules that may be extruded by processes analogous to those seen in β-cells, there is little evidence for a direct relationship between the polarization of the cell membrane and hormone release (Douglas, Kanno & Sampson, 1967*a*,*b*). In the absence of direct measurements of membrane potentials in β-cells, it is not at present possible to decide with certainty whether depolarization accompanies insulin secretion.

Earlier experiments in which effects of high concentrations of K^+ were investigated are consistent with depolarization of β-cell membranes accompanying secretion. Thus the effects both of glucose and of tolbutamide (in which secretion is believed to be a consequence of granule extrusion) are increased by high concentrations of K^+ and are inhibited in low K^+. However, later experiments did not suggest that if depolarization takes place this is accompanied by K^+ fluxes. Thus whereas glucose-induced release of insulin is accompanied by an increased $^{42}K^+$ uptake by islets, this is not so when release is due to tolbutamide. In addition, K^+ efflux does not seem to accompany secretory activity. It is always possible that depolarization can take place unaccompanied by measurable fluxes of K^+, but until experiments have been carried out on β-cell membrane potentials this cannot be certain.

The mechanism by which alterations of K^+ concentration of incubation media affect insulin secretion is unknown at present. The effects of K^+ do not appear merely to sensitize the cells to effects of secretory agents such as glucose or tolbutamide, since high concentrations of K^+ may induce secretion in the complete absence of glucose (Grodsky & Bennett, 1966).

In the absence of conclusive evidence of the importance of electrical activity in the initiation of insulin secretion, it seems possible that K^+ exerts its effects by altering the activities of enzymes or enzyme systems within the β-cells. Though alteration of K^+ concentrations of incubation media appears to cause alterations in glycolysis in some tissues (McIlwain, 1952; Rolleston & Newsholme, 1967), it seems unlikely that the effects of K^+ on insulin secretion are mediated in this way. Thus alteration of secretion in response to tolbutamide is also seen (Table 2); tolbutamide induces insulin secretion by a mechanism that appears to be independent of glucose metabolism (Coore & Randle, 1964). In addition, the metabolism of glucose by glycolysis is not believed to be the signal for insulin release (Coore & Randle, 1964).

Alteration of the K^+ content of incubation media may, however, affect metabolism in other ways. Thus an effect of K^+ on the regulation of mitochondrial respiration has been described (Blond & Whittam, 1965). There is also the possibility that high concentrations of K^+ may increase concentrations of 3′,5′-(cyclic)-AMP in islet tissue, as is the case in salivary gland (Bdolah & Schramm, 1965), brain (Sutherland & Rall, 1967) and diaphragm (Lundholm, Rall & Vamos, 1967). Some evidence has recently been presented that 3′,5′-(cyclic)-AMP might be important in the mediation of insulin secretion (Turtle, Littleton & Kipnis, 1967; Lambert, Jeanrenaud & Renold, 1967; Sussman & Vaughan, 1967).

Though serum K^+ concentrations do not seem to exercise an important controlling influence except at unphysiological concentrations, this may not be the case for other cations. Thus it has been shown that physiological concentrations of Ca^{2+} are necessary for the maintenance of insulin secretion

(Grodsky & Bennett, 1966; Milner & Hales, 1967*b*). Fluxes of Na^+ and of Ca^{2+} in islet tissue might be important in regulating insulin secretion.

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REFERENCES

Bdolah, A. & Schramm, M. (1965). *Biochem. biophys. Res. Commun.* **18**, 452.
Blond, D. M. & Whittam, R. (1965). *Biochem. J.* **97**, 523.
Carpenter, A.-M. (1966). *J. Histochem. Cytochem.* **14**, 834.
Conn, J. W. (1965). *New Engl. J. Med.* **273**, 1135.
Coore, H. G. & Randle, P. J. (1964). *Biochem. J.* **63**, 66.
Dicker, S. E. (1966). *J. Physiol.* **185**, 429.
Douglas, W. W., Kanno, T. & Sampson, S. R. (1967*a*). *J. Physiol.* **188**, 107.
Douglas, W. W., Kanno, T. & Sampson, S. R. (1967*b*). *J. Physiol.* **191**, 107.
Douglas, W. W. & Poisner, A. M. (1964). *J. Physiol.* **172**, 1.
Fuhrman, F. A. (1951). *Amer. J. Physiol.* **167**, 314.
Gardner, L. I., Talbot, N. B., Cook, C. D., Berman, H. & Concepcion, U. R. (1950). *J. Lab. clin. Med.* **35**, 592.
Gey, G. O. & Gey, M. K. (1936). *Amer. J. Cancer*, **27**, 45.
Gomori, G. (1939). *Anat. Rec.* **74**, 439.
Goodford, P. J. & Hermansen, K. (1961). *J. Physiol.* **158**, 426.
Grodsky, G. M. & Bennett, L. L. (1966). *Diabetes*, **15**, 910.
Hales, C. N. & Randle, P. J. (1963). *Biochem. J.* **88**, 137.
Hellerstrom, C. (1964). *Acta endocr., Kbh.*, **45**, 122.
Howell, S. L. & Taylor, K. W. (1966). *Biochim. biophys. Acta*, **130**, 519.
Howell, S. L. & Taylor, K. W. (1967). *Biochem. J.* **102**, 922.
Kostianovsky, M. & Lacy, P. E. (1966). *Fed. Proc.* **25**, 377.
Lacy, P. E. (1961). *Amer. J. Med.* **31**, 851.
Lacy, P. E. & Kostianovsky, M. (1967). *Diabetes*, **16**, 35.
Lambert, A. E., Jeanrenaud, B. & Renold, A. E. (1967). *Lancet*, i, 819.
Lee, K. S. & Choi, S. J. (1966). *J. exp. Pharmacol. Therap.* **153**, 114.
Lundholm, L., Rall, T. & Vamos, N. (1967). *Acta physiol. scand.* **70**, 127.
McIlwain, H. (1952). *Biochem. J.* **52**, 289.
Milner, R. D. G. & Hales, C. N. (1967*a*). *Biochim. biophys. Acta*, **135**, 375.
Milner, R. D. G. & Hales, C. N. (1967*b*). *Diabetologia*, **3**, 47.
Mondon, C. E., Burton, S. D., Grodsky, G. M. & Ishida, T. (1967). *Diabetes*, **16**, 510.
Montague, W., Howell, S. L. & Taylor, K. W. (1967). *Nature, Lond.*, **215**, 1088.
Moskalewski, S. (1965). *Gen. comp. Endocrin.* **5**, 342.
Post, R. L., Merritt, C. R., Kinsolving, C. R. & Albright, C. D. (1960). *J. biol. Chem.* **235**, 1796.
Rolleston, F. S. & Newsholme, E. A. (1967). *Biochem. J.* **104**, 524.
Sagild, U., Andersen, V. & Andreasen, P. B. (1961). *Acta med. scand.* **169**, 243.
Spergel, G., Bleicher, S. J., Goldberg, M., Adesman, J. & Goldner, M. G. (1967). *Metabolism*, **16**, 581.
Spergel, G., Schmidt, P., Stern, A. & Bleicher, S. J. (1967). *Diabetes*, **16**, 312.
Sussman, K. E. & Vaughan, E. D. (1967). *Diabetes*, **16**, 449.
Sutherland, E. W. & Rall, T. (1967). *Fed. Proc.* **26**, 707.
Turtle, J. R., Littleton, G. K. & Kipnis, D. M. (1967). *Nature, Lond.*, **213**, 727.
Vogt, M. (1952). *Brit. J. Pharmacol.* **7**, 325.
Williamson, J. R., Lacy, P. E. & Grisham, J. W. (1961). *Diabetes*, **10**, 460.
Woodbury, D. M. & Woodbury, J. W. (1963). *J. Physiol.* **169**, 553.