REVIEW ARTICLE

Adipocytes, Available Energy and Endocrine Pancreas

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Over the past ten years, first in Boston, then in Geneva, I have been interested in two fascinating areas of diabetes-related research, that of adipose tissue metabolism and its hormonal control and, more recently, that of insulin secretion from the pancreas.

Actually, my involvement in the problem of insulin secretion was the consequence of studies, performed with R.J. Ho, J. Letarte, M. Touabi and A.E. Renold using isolated fat cells, during which we became increasingly aware of the important relationships exist-

“adipocyte” man and a “pancreas” man proved particularly rewarding since it led to establishing together the likely participation of adenylyl cyclase in the complex insulin-releasing mechanism (Lambert, Jeanrenaud and Renold, 1967).

This Minkowski Lecture, entitled “Adipocytes, available energy and endocrine pancreas”, will attempt to summarize some aspects of these two studies. I shall first discuss fat cell regulation, and then proceed to the problem of insulin secretion.

Fig. 1. Time course of potassium uptake by isolated mouse fat cells. Incubations carried out in 1.5 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin and 10 mM pyruvate). Cells first preincubated in a potassium-free buffer for 40 min, then further incubated for 0–40 min following addition of $^{42}\text{K}^+$ (6 mM final concentration). Results expressed as μmoles per gram dried fat cells. Data of Touabi and Jeanrenaud, 1970

ing among factors such as electrolytes, energy availability, and adenylyl cyclase activity in the regulation of adipocyte function (Jeanrenaud, 1968). As a consequence of this realization, and as will be described below, I later undertook a collaborative study with A. Lambert, working with a cultured foetal pancreas preparation, to examine the influence of these same factors, that of adenylyl cyclase in particular, on insulin secretion *in vitro*. This close collaboration between an

Fat cells carry out many energy-requiring processes (Goodman, 1966; Crofford, Minemura and Kono, 1969; Clausen and Rodbell, 1969; Clausen, Rodbell and Duanand, 1969; Touabi and Jeanrenaud, 1969; Vassalli and Jeanrenaud, 1970; Touabi and Jeanrenaud, 1970). Among those we have examined extensively are potassium uptake and accumulation (Touabi and Jeanrenaud, 1970), α -aminoisobutyric acid (AIB) uptake and accumulation (Touabi and Jeanrenaud, 1969,

1970; Vassalli and Jeanrenaud, 1970) and leucine uptake and incorporation into proteins (Jeanrenaud and Touabi, 1967; Touabi and Jeanrenaud, 1971). For the sake of clarity I shall present chiefly those results dealing with *potassium*, dividing this portion of the Lecture into two parts. In the first, the hormonal regulation of the transport of potassium, as we see it, will be reviewed. In the second, some effects of potassium on lipolysis and glucose metabolism will be examined. It should be borne in mind, however, that the regulation of amino acid uptake and/or incorporation into proteins appears to parallel that of potassium ion uptake rather closely, all these processes exhibiting similar energy requirements. On a few occasions I shall, therefore, also present results of studies carried out on AIB uptake or leucine incorporation into proteins to illustrate possible mechanisms of energy modulation.

Fig. 1 illustrates the actual uptake of ^{42}K by isolated fat cells. As can be seen, ^{42}K uptake increases rapidly with time, reaching a steady-state level within 20–40 min. The energy requirement for ^{42}K uptake is demonstrated in Fig. 2; it is evident that potassium

Fig. 2. Energy requirement of potassium uptake by isolated mouse fat cells. Incubations carried out in 1.5 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin). Cells first preincubated in a potassium-free buffer for 40 min with oligomycin $\pm$ unlabelled substrate(s), then further incubated for 40 min under the same conditions following addition of $^{42}\text{K}^+$ (6 mM final concentration). Each bar is the mean of 6 values $\pm$ S.E. Data of Touabi and Jeanrenaud, 1970

uptake is drastically curtailed when fat cells are incubated in the presence of oligomycin, and that the further addition of glucose to oligomycin-treated cells restores ^{42}K uptake towards normal. The latter finding is consistent with an effect of glucose on ATP pro-

duction via the glycolytic pathway. Such a concept is strengthened by the additional observation that the uptake of potassium is also markedly reduced upon addition of 2-deoxyglucose to fat cells incubated with

Fig. 3. Hormonal effects on potassium uptake by isolated mouse fat cells. Experimental procedure similar to that described in Fig. 1. Cells first preincubated in a potassium-free buffer for 40 min with agent(s) as indicated, then further incubated with agent(s) following addition of ^{42}K (6 mM final concentration). Data of Touabi and Jeanrenaud, 1970

Fig. 4. Adrenaline-induced lipolysis and potassium uptake by isolated mouse fat cells. Incubations carried out in 1.5 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin, 1 mM pyruvate). Cells first preincubated for 0–60 min in the presence of adrenaline (1 μg/ml), then further incubated under the same conditions for 5 min following addition of trace amounts of ^{42}K . ^{42}K uptake measured during the 5 min incubation. Cumulative glycerol and FFA release during preincubation *plus* incubation. Each point is the mean of 12 values $\pm$ S.E. Data of Touabi and Jeanrenaud, 1970

glucose and oligomycin (Fig. 2). Furthermore, it can also be observed that insulin, when added to oligomycin-treated cells together with glucose (but not with pyruvate), completely restores potassium uptake to normal.

Adrenaline markedly modifies potassium accumulation by fat cells as shown in Fig. 3. It is apparent that the presence of this hormone results in a large decrease in the uptake of this cation. In addition, although insulin by itself does not bring about any changes in basal uptake, it clearly prevents the drop in potassium uptake produced by the lipolytic hormone. This latter observation suggests that the drop in potassium uptake produced by adrenaline might be a consequence of some interference with the availability of energy within the adipocyte by the lipolytic process.

As a first step in checking this possibility, lipolysis and the uptake of potassium by fat cells were measured simultaneously. It is apparent from such experiments (Fig. 4) that during a 60 min incubation in the absence

line on another energy-requiring process, the uptake of AIB, is qualitatively similar to that observed on potassium uptake; net AIB uptake is decreased when lipolysis is stimulated.

In order to substantiate further the apparent inverse relationship existing between lipolysis and potassium uptake, the accumulation of potassium was then measured during both adrenaline-stimulated and insulin-inhibited lipolysis. Some of the results so obtained are shown in Fig. 6, where the particular case of adrenaline-induced lipolysis is illustrated; similar results have been obtained with ACTH, caffeine or theophylline. In the experiments illustrated in Fig. 6, the uptake of potassium has been measured together with the release of FFA and glycerol into the incubation medium. It is evident that when potassium uptake is high, lipolytic activity is low; when lipolysis is stimulated by adrenaline, either in the presence of unlabelled glucose or of pyruvate in the incubation

Fig. 5. Adrenaline-induced lipolysis and α -aminoisobutyric acid (AIB) uptake by isolated mouse fat cells. Experimental procedure similar to that described in Fig. 4, except that labelled AIB (1 mM final concentration) was used instead of labelled potassium. Each point is the mean of 6 values $\pm$ S.E. Data of Vassalli and Jeanrenaud, 1970

of hormone, potassium uptake remains at its optimum and lipolysis changes little. In contrast, when adrenaline is added, lipolysis, as measured by FFA and glycerol release into the medium, is stimulated and, as lipolysis increases, potassium uptake decreases. Similar results can be obtained when ACTH, caffeine or theophylline are used to stimulate lipolysis. It should be stressed, as shown in Fig. 5, that the effect of adrena-

Fig. 6. Relationship between lipolytic activity and potassium uptake by isolated mouse fat cells. Incubations carried out in 1.5 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin + 1 mM unlabelled substrate). Cells preincubated for 50 min with or without hormone(s) as indicated, then further incubated under the same conditions for 30 min following addition of trace amounts of ^{42}K . Each bar is the mean of 12 values $\pm$ S.E. Data of Touabi and Jeanrenaud, 1970

medium, potassium uptake is reduced to a large extent. In addition, when insulin is added to adrenaline-treated cells, lipolytic activity is significantly reduced and the drop in potassium uptake is almost completely prevented. These experiments suggest that during

lipolysis, free fatty acids may accumulate within the adipocyte and may directly, or indirectly through some of their derivatives, interfere with the availability of energy.

It was then necessary to determine whether or not available energy actually changed under these con-

ditions. ATP content of the adrenaline-treated cells when compared with that of the control cells. Since a high adrenaline concentration was used in these particular experiments, it should be pointed out that a significant fall in the level of ATP can be obtained with concentrations of adrenaline as low as 0.006 $\mu\text{g/ml}$, provided

Fig. 7. Effect of adrenaline on ATP content of isolated mouse fat cells. Incubations carried out in 1 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml, no added substrate) with or without adrenaline. Results expressed as nmoles ATP per gram dried fat cells. Data of Bihler and Jeanrenaud, 1970

Fig. 8. Prevention by insulin of the effect of adrenaline on ATP content of isolated mouse fat cells. Experimental procedure similar to that described in Fig. 7. Data of Bihler and Jeanrenaud, 1970

ditions. Fig. 7 shows a time course, carried out with fat cells incubated in the absence or presence of adrenaline, during which the level of cellular ATP has been measured (Bihler and Jeanrenaud, 1970). It is clearly evident that there is a progressive, marked drop in the

the cells are incubated long enough in the presence of the hormone. Other experiments have indicated that a significant drop in ATP can be observed in fat cells incubated with adrenaline for as little as 20 min. In addition, as shown in Fig. 8, the drop in ATP observed in the presence of adrenaline (as well as in that, although not presented here, of caffeine) is largely prevented by the superimposed addition of insulin to the cells, even in the absence of glucose in the medium. This observation fits with the previous finding that the decrease in potassium uptake produced by adrenaline can be overcome by insulin. A last observation of interest is the finding that the decrease in cellular ATP produced by adrenaline can also be prevented by the addition of another lipolytic agent, ouabain (Bihler and Jeanrenaud, 1970).

As yet, there is no direct evidence as to the mechanism by which adrenaline modulates the level of energy within adipocytes. The recent work of S.W. Cushman and J.J. Heindel in this laboratory does, however, supports the concept that intracellular FFA (referred to as cell-associated fatty acid or CAFA) may play at least a role in such modulation (Cushman, Heindel and Jeanrenaud, 1970, 1971; Heindel, Cushman and Jeanrenaud, 1971). *First*, the fall in ATP observed during adrenaline stimulation has been shown to follow an observed rise in CAFA. *Second*, as depicted in Fig. 9,

the addition of a long-chain fatty acid such as palmitate or a short-chain one such as octanoate to fat cells results in a significant decrease in energy-requiring processes such as AIB uptake, paralleled by a drop in cellular ATP (Bihler and Jeanrenaud, 1970). *Third*, Heindel and Cushman have shown that in the presence of a fixed concentration of adrenaline, CAFA levels can be increased by lowering the extracellular concentration of albumin from 3.5 g/100 ml to 0.5 g/100 ml, presumably as a result of the decrease in extracellular binding sites available for released fatty acids. The possible result of such an increase in CAFA levels is shown, for the particular case of leucine incorporation into proteins, in Fig. 10. It is apparent that as the albumin concentration in the presence of a fixed dose of adrenaline is lowered, the adrenaline-produced drop in cellular ATP content becomes greater. Moreover, the small adrenaline-induced drop in leucine incorporation into proteins observed in cells incubated in medium with a high albumin concentration contrasts with the considerably greater drop measured in those incubated in medium with a low albumin concentration. Further studies with the same experimental design do show that the rise in CAFA so obtained also correlates with a decrease in potassium or AIB uptake by fat cells. *Fourth*, Cushman and Heindel have also shown that the rise in CAFA levels produced by adrenaline or caffeine can be reduced by insulin. This appears to correspond, as depicted in Fig. 11, to the observed prevention by insulin of the drop in cellular

Fig. 9. Effect of fatty acids on AIB uptake by isolated mouse fat cells. Incubations carried out in 1.5 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin). Cells preincubated for 60 min in the presence of various concentrations of fatty acids, then further incubated under the same conditions for 30 min following addition of labelled AIB (1 mM final concentration). Each bar is the mean of 6–12 values $\pm$ S.E. Data of Vassalli and Jeanrenaud, 1970

ATP as well as of the decrease in leucine incorporation into proteins produced by caffeine, effects which coincide with some decrease in lipolytic activity.

When studied in more detail, it appears that the following sequence in time is observed when fat cells

are exposed to adrenaline: within 1 min, glycerol is released and CAFA levels rise; FFA release is not detected until three minutes; CAFA levels continue to rise slowly with time until a critical extracellular ratio of FFA released to albumin present of 3–4/1 is reached; at that time, the rate of CAFA increase becomes larger while glycerol and FFA output slows; following this “second” phase of CAFA increase, an ATP drop is observed which slightly precedes or coincides with the first appearance of decreases in potassium or AIB uptake. This latter observation supports the possible existence of a cause-effect relationship between alterations in CAFA levels and the drop in ATP.

Fig. 10. Effect of varying the medium albumin concentration in the presence of a fixed dose of adrenaline upon labelled leucine incorporation into proteins by isolated mouse fat cells. Experimental conditions as indicated. Data of Touabi and Jeanrenaud, 1971

It should be mentioned at this point that Hepp, Challoner and Williams (1968) have observed that in all situations where CAFA levels are, according to our experiments, elevated by lipolytic agents, ^{32}P incorporation into ATP is markedly diminished and that in-

sulin, even in the absence of glucose, reverses these effects. Finally, the still hypothetical action of intracellular free fatty acids in interfering with phosphorylation and energy availability is certainly strengthened by the observation of Clausen and collaborators (Clausen and Rodbell, 1969; Clausen, Rodbell and Dunand, 1969) that although adipose cell ghosts (a preparation devoid of lipids) do accumulate potassium or AIB by an energy-

Fig. 11. Effect of lipolysis on labelled leucine incorporation into proteins and on cellular ATP in isolated mouse fat cells. Experimental conditions as indicated. Data of Touabi and Jeanrenaud, 1971

requiring process, such an accumulation is totally unaffected by the presence of lipolytic hormones.

Having examined some aspects of potassium uptake and the relationship between potassium uptake and the modulation of ATP metabolism, I shall summarize briefly, as an example of the influence upon intermediary metabolism of a cation requiring energy for its transport, some of the effects which potassium appears to exert upon lipolysis and glucose metabolism.

As illustrated in Fig. 12, the uptake of potassium by the adipocyte is considerably lowered by treatment with ouabain and so is the intracellular potassium content (Touabi and Jeanrenaud, 1969; Letarte, Jeanrenaud and Renold, 1969). This is an interesting observation in view of the fact, shown in Fig. 13, that ouabain markedly decreases adrenaline-produced lipolysis, an inhibitory effect that is proportional to the

Fig. 12. Effect of ouabain on potassium uptake by isolated mouse fat cells. Experimental conditions similar to those described in Fig. 1. Cells preincubated for 30 min with or without ouabain in a potassium-free buffer, then further incubated under the same conditions for 0–60 min following addition of $^{42}K^+$ (6 mM final concentration). Each point is the mean of 6 values $\pm$ S.E. Data of Touabi and Jeanrenaud, 1970

Fig. 13. Effect of ouabain on adrenaline-induced lipolysis in isolated rat fat cells. Incubations carried out in 2 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin, no substrate). Data of Ho, Jeanrenaud, and Renold, 1967

concentration of this cardiac glycoside in the incubation medium. Further experiments have indicated that lipolysis produced by cyclic AMP or caffeine is *unaffected* by ouabain, whereas that produced by all fast-acting lipolytic hormones (ACTH, glucagon, adrenaline) is (Ho, Jeanrenaud, Posternak and Renold, 1967). Thus, ouabain appears to have no influence on the action of cyclic AMP once formed; its effect must,

therefore, be related to some action on the formation of cyclic AMP. Indeed, as illustrated in Fig. 14, pretreatment of adipose tissue with ouabain markedly decreases adenyl cyclase activity and this effect can be mimicked by removing potassium from the incubation medium.

The inhibitory effect of ouabain on the formation of cyclic AMP is corroborated by the finding that the stimulation of glycogen breakdown in isolated fat cells by lipolytic hormones is also inhibited by this agent. It should be pointed out, however, that when the concentration of lipolytic hormone in the incubation medium is increased, the degree of inhibition of hormone-induced lipolysis by ouabain as well as by potassium

Fig. 14. Effect of ouabain or potassium-lack on adenyl cyclase activity of rat adipose tissue homogenate. Data of Ho, Jeanrenaud, Posternak, and Renold, 1967

Fig. 15. Effect of potassium-lack on adrenaline-induced lipolysis in isolated rat fat cells. Incubations carried out in 2 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin, no substrate). Data of Ho, Jeanrenaud, Posternak, and Renold, 1967

lack (Fig. 15) is reduced, indicating that these effects are reversible and depend upon the concentration of the hormone. Although the reversibility of these inhibitory actions cannot yet be explained in mechanistic terms, it is conceivable that it represents a modulation by changes in cation concentration of the affinity between lipolytic hormones and adenyl cyclase, or between lipolytic hormones and the cell membrane. If the affinity were so decreased, but not abolished, by the absence of potassium, it should be understandable, as it is observed, that the effect of potassium depletion might be counteracted by increasing the concentration

of lipolytic hormones, whatever the mechanism(s) involved. It thus appears that potassium ion probably modulates hormone-stimulated lipolysis via changes in the activity of the adenyl cyclase. Furthermore, as potassium uptake has been shown to be markedly reduced by lipolytic agents (Touabi and Jeanrenaud, 1970), it is conceivable that stimulated lipolysis might result in changes in ionic flux, and eventually in a decrease in intracellular potassium content that might limit the lipolytic process.

While studying the effects of ouabain and of potassium depletion on fat cells, we also observed, as illustrated in Fig. 16, that potassium loss, produced by either of the two treatments, has a stimulatory effect on glucose metabolism (Ho and Jeanrenaud, 1967; Letarte, Jeanrenaud and Renold, 1969). The pattern of glucose metabolism so obtained is actually, when studied in detail, very similar to that produced by insulin (Ho and Jeanrenaud, 1967). Moreover, kinetic evidence indicates that the absence of potassium results in an increase in the maximal velocity of overall glucose metabolism without an alteration in the apparent K_m of the reactions involved. It is clear

Fig. 16. Effect of potassium-lack on glucose metabolism by isolated mouse fat cells. Incubations carried out in 2 ml Krebs-Ringer bicarbonate buffer (3.5 g/100 ml albumin) with or without 6 mM potassium. Each point is the mean of 6 values $\pm$ S.E. Data of Letarte, Jeanrenaud, and Renold, 1969

that such experiments, based on the analysis of end products of glucose metabolism, must be interpreted with great caution. Nevertheless, they are consistent with the concept that lowering the intracellular potassium may render the sugar transport system more mobile and effective (Letarte, Jeanrenaud and Renold, 1969). Consonant with such a concept are the experiments of Clausen (1969), carried out with 3-O-methyl glucose, which clearly indicate that the glucose transport system *per se* is stimulated in adipose tissue in all situations where, according to his and our own studies, the intracellular potassium content is reduced (i.e. incuba-

tion of adipose tissue in a potassium-free buffer, or in the presence of adrenaline, ACTH, dinitrophenol or cyanide).

As summarized in Fig. 17, these studies and those carried out by other investigators (Jeanrenaud and Hepp, 1970; Ho, England, Johnson and Meng, 1970; Hollenberg, Vost and Patten, 1970; Hollenberg and Patten, 1970; Angel, Desai, and Halperin, 1971) suggest that insulin, lipolytic agents and electrolytes, potassium in particular, all appear to influence the level of one or more of three critical intracellular compounds: cyclic AMP, FFA (i.e. CAFA) and ATP. The three overlapping circles of the figure further indicate that these intracellular compounds probably influence each other as well. For example, when intracellular FFA accumulates, ATP decreases; when adenyl cy-

Fig. 17. Possible interrelationships between hormones and intermediary metabolism in isolated fat cells

clase activity decreases, cyclic AMP and FFA levels decrease and ATP levels return to normal; when a rise in CAFA is produced by lowering the medium albumin concentration, the level of ATP decreases without, apparently, changing adenyl cyclase activity (Cushman, Heindel and Jeanrenaud, 1971). Moreover, the respective levels of these three compounds appear, as previously discussed, to eventually govern many aspects of intermediary metabolism such as glucose transport and metabolism, lipolysis, potassium uptake, and amino acid uptake or incorporation into proteins, all activities which in turn, as depicted by the five overlapping circles of the figure, influence each other.

The integrated picture is, therefore, exceedingly difficult to understand in terms of "primary" and "secondary" events. Finally, since very little is known about blood flow through the adipose mass and, therefore, about the availability of albumin, it is not known whether changes in CAFA and ATP do actually occur under physiological conditions or only, if at all, under extreme conditions such as starvation, stress or diabetes.

The main features of adipose tissue regulation, revealed by the explosive phase of adipose tissue research beginning in 1956, are now relatively well known (Renold and Cahill, 1965). Recently, it has been the trend to use fat cells as a tool for the better understanding of fundamental mechanisms (Birnbaumer and Rodbell, 1969; Angel, 1969; Kono, 1969; Jeanrenaud and Hepp, 1970; Flatt, 1970; Björntorp and Karlsson, 1970). One of these, pinocytosis, first described by Barnett and Ball (1960) and later by Williamson and Lacy (1965) and by Cushman (1970), is still poorly understood in terms of its regulation and its precise role in cell function. Pinocytosis appears, however, to be stimulated in the fat cell by the level of intracellular FFA (Cushman, 1970) and might well, therefore, be related to some of the phenomena described in Fig. 17. Preliminary studies from our laboratory, carried out by Cushman and Orci, indicate, as shown in Fig. 18, that pinocytic activity measured by the horse-radish peroxidase technique is detectable in non-stimulated fat cells within a few minutes. As can be seen, pinocytic vesicles form along the cell membrane and appear to migrate towards the interior of the cell (Fig. 18a, b); they also, possibly at a later time, appear to coalesce as rosettes (Fig. 18c). Since pinocytosis could be responsible for, among other things, the in and out transport of fatty acids and/or lipoproteins, about which very little is known, these experiments represent an exciting inquiry into the hormonally-controlled transport devices of the adipocyte.

As mentioned initially, cell energy and electrolytes were at the origin of the collaborative study I carried out with André Lambert on the problem of insulin secretion from the cultured foetal pancreas (Lambert, Jeanrenaud and Renold, 1967; Lambert, Junod, Stauffacher, Jeanrenaud and Renold, 1969; Lambert, Jeanrenaud, Junod and Renold, 1969). The foetal pancreas preparation, as originally designed by Vecchio, Luyckx and Renold (1967), had two peculiar properties: a) insulin release was triggered only by high concentrations of glucose, and even then, only moderately so, and b) glucagon stimulated insulin output only when glucose was present in the incubation medium. These were indeed two puzzling findings in view of the known stimulatory effect of glucose and of glucagon on insulin secretion observed in several other preparations *in vitro* (for review see Malaisse, 1969; Lambert, 1970).

The experience gathered with the fat cell preparation, where adenyl cyclase had become a permanent

Fig. 18. Pinocytotic activity in rat, isolated fat cells. Cells incubated in Krebs-Ringer bicarbonate albumin buffer for 5 min (Fig. 18a and b) and 30 min (Fig. 18c), then fixed and stained by the peroxidase tracer technique (R.C. Graham and M.J. Karnovsky, *J. Histochem. Cytochem.*, **14**, 291, 1966). Fig. 18a: pinocytic vesicles (v) containing reaction product are seen along the surface of the cell. L = fat droplets. Enlargement $\times 75600$. Fig. 18b: arrows indicate a cluster of microvesicles containing the reaction product. Enlargement $\times 80640$. Fig. 18c: numerous microvesicular rosettes (arrows) containing the reaction product. Enlargement $\times 82320$. Micrograph of Orci, Cushman and Jeanrenaud (unpublished observations)

preoccupation, led us to approach the insulin secretion problem with Sutherland's hypothesis in mind (Sutherland, Oye and Butcher, 1965), and eventually to propose, as did a few investigators at about the same time (Sussman and Vaughan, 1967; Malaisse, Malaisse-Lagae and Mayhew, 1967; Turtle, Littleton and Kipnis, 1967), that cyclic AMP might play a role in the insulin-releasing mechanism (Lambert, Jeanrenaud and Renold, 1967). We hypothesized that glucose might trigger insulin release by furnishing ATP, viewed as a precursor of cyclic AMP, but that somehow, cyclic AMP could *not* accumulate in our preparation, perhaps because of a relatively high phosphodiesterase activity. The addition of the phosphodiesterase blocker, caffeine, to the medium in the *absence* of glucose (Fig. 19) indicated that our hypothesis might be correct since insulin release by the pancreatic explants was then definitely increased above control values. Furthermore, as also depicted in Fig. 19, when an energy-yielding substrate such as glucose or pyruvate was added *together* with caffeine, a considerably higher insulin release was observed. These observations were the

Fig. 19. Insulin release from cultured foetal rat pancreas: effect of substrate and caffeine, singly and combined. Data of Lambert, Jeanrenaud, and Renold, 1967; and of Lambert et al. 1969

starting point for many exciting experiments which I shall now summarize, and which clearly indicate that both cyclic AMP and some energy source are needed to elicit maximal insulin release from the foetal pancreas preparation.

Fig. 20 shows that not only glucose, but a variety of substrates is able to increase the output of insulin. Thus, sugars such as glucose, mannose and, although not represented in the figure, fructose and galactose; amino acids such as lysine, arginine, threonine, leucine, histidine and phenylalanine; and a fatty acid such as

octanoate, all increase to varying degrees the release of insulin from the foetal pancreas. The finding that substrates from each of the three classical classes of fuel, carbohydrates, amino acids and fatty acids, increase the release of insulin in the presence of caffeine, together with the observation that pyruvate also has a marked stimulatory effect (Fig. 19), suggests that all these substrates might function through a common mediator, perhaps pyruvate itself,

Fig. 20. Insulin release from cultured foetal rat pancreas: effect of substrates. Data of Lambert, Jeanrenaud, Junod, and Renold, 1969

Fig. 21. Insulin release from cultured foetal rat pancreas: energy requirement. Data of Lambert, Junod, Stauffacher, Jeanrenaud, and Renold, 1969

acting either as a source of ATP or through some other mechanism(s). For the particular case of glucose, the studies by Hellman and Larsson (1961), by Humbel and Renold, (1963), by Matschinsky and Ellerman (1968), and by Ashcroft and Randle (1970) have shown that glucose is indeed metabolized by the islets of Langerhans. Moreover, other experiments (Hellerström, 1967; Ashcroft and Randle, 1968; Ashcroft, Hedeskov and Randle, 1970) have indicated that some parallelism appears to exist between glucose metabolism by the pancreas and the ability of glucose to pro-

mote insulin release. In our own studies, the need for energy in the stimulation of insulin output is further shown by the finding that agents interfering with glucose metabolism, such as mannoheptulose or 2-deoxyglucose, decrease the output of the hormone from our preparation and that, as shown in Fig. 21, glucose- or pyruvate-induced insulin release is markedly reduced by oligomycin added to the pancreas preparation at a concentration known to inhibit the production of ATP.

The role of cyclic AMP in triggering the release of insulin from our preparation is best illustrated in Fig. 22. As can be seen, the presence of glucagon results in a modest release of insulin. As mentioned earlier (see Fig. 19), caffeine alone, in the absence of glucose, is

Fig. 22. Insulin release from cultured foetal pancreas: effect of glucagon and caffeine, singly and combined. Data of Lambert, Jeanrenaud, and Renold, 1967; and of Lambert, Jeanrenaud, Junod, and Renold, 1969

Fig. 23. Insulin release from cultured foetal pancreas: effect of glucagon and glucose, singly and combined. Data of Lambert, Jeanrenaud, and Renold, 1967

also stimulatory. Of more importance, however, is the observation that, again in the absence of glucose, the simultaneous addition of glucagon *and* caffeine elicits a release of insulin that is twice that observed with caffeine alone. This is a rather clear indication that this

preparation contains a relatively high phosphodiesterase activity, and that the blockade of this activity, for example by caffeine, is one of the necessary factors in achieving the optimal appearance of insulin in the incubation medium.

The presumed need for both energy *and* cyclic AMP in producing maximal release of insulin is illustrated by the experiments summarized in Fig. 23. It is evident that whereas glucose alone or glucagon alone are only very mildly stimulatory in the absence of caffeine, they become powerful stimuli when combined. This is in keeping with other experiments in which we have

Fig. 24. Insulin release from cultured foetal pancreas: effect of potassium. Data of Lambert, Jeanrenaud, Junod, and Renold, 1969

Fig. 25. Insulin release from cultured foetal pancreas: effect of calcium. Data of Lambert, Jeanrenaud, Junod, and Renold, 1969

found that over a large range of concentrations, dibutyryl cyclic AMP, alone or in the presence of caffeine, is ineffective in stimulating the release of insulin. However, as soon as glucose is added to the medium, dibutyryl cyclic AMP becomes an extremely

powerful stimulator (Lambert, Orci, Jeanrenaud, Rouiller and Renold, 1969). This latter observation suggests that despite the morphological evidence for glycogen granules in our preparation (Orci *et al.*, 1970), these stores may not be able to provide energy sufficient to permit the insulin-releasing action of cyclic AMP.

As might be expected from previous studies in many different tissues, electrolytes do influence the secretion of insulin. As shown in Fig. 24, changes in potassium concentration between 6 and 12 mM do not alter insulin output; however, both complete removal of potassium from the medium and exposure of the preparation to high potassium concentrations have very marked stimulatory effects. Similarly, the same importance of calcium for the release of insulin from other pancreatic preparations (Curry, Bennett and Grodsky, 1968; Hales and Milner, 1968; Malaisse-Lagae and

mones such as glucagon may act via stimulation of adenylyl cyclase although no direct evidence for such a mechanism has yet been obtained. These hormones may influence the uptake of substrate, thereby changing the energy state of the B-cell. Cyclic AMP might also influence substrate utilization. However, the breakdown of glycogen, if it does occur, does not appear to be of importance in our preparation. On the other hand, cyclic AMP could, as previously shown for the adipocyte (Fig. 17), alter the transport of electrolytes such as potassium, calcium, etc. Perhaps of even more importance, this nucleotide might control the intracellular distribution of these ions, that of divalent calcium in particular, as suggested by evidence obtained from other secretory cells (Rasmussen, 1970). Such an alteration in the intracellular "milieu" of the B-cell could result in changes in cell membrane permeability, in B-granule membrane permeability, or in the function as well as morphology of intracellular organelles, as recently proposed (Lacy, Howell, Young and Fink, 1968; Lacy, 1970); any or all of these changes could eventually be responsible for the release of insulin. Thus it is apparent that, as for adipose tissue, the cascade of reactions following an increase in cyclic AMP concentration within the cell is certainly very complex. For that reason, while considering this last scheme of this Lecture, I cannot help but think of the scheme previously discussed for the regulation of adipose tissue function (Fig. 17) and suggest that, despite (possibly because of) the more recent hypotheses proposed for the mechanism of insulin release (Luft and Randle, 1970), our knowledge of the endocrine pancreas, and of the insulin-releasing system, might be approaching that stage, reached at an earlier time with the adipocyte, where it will become increasingly difficult to discern primary events from secondary ones, and physiological from *in vitro* regulation.

Fig. 26. Schema of possible relationships leading to insulin release

Malaisse, 1971) can also be observed in our foetal pancreas explants. Thus, as shown in Fig. 25, the omission of calcium from the medium results in a decrease in the release of insulin. Although the omission of magnesium *per se* is not inhibitory, the absence of both calcium and magnesium, achieved by adding EDTA to the incubation medium, completely abolishes insulin output from the explants whatever the stimulus tested.

A summary of the tentative relationships that might, on the basis of our experiments, be important for the regulation of insulin release is illustrated in Fig. 26. It would appear that both cyclic AMP and an energy source, possibly ATP derived from pyruvate, are needed to activate the insulin-releasing system. As in fat cells, it is possible that the level of ATP influences that of cyclic AMP. Insulin-releasing hor-

In ending this Minkowski Lecture, I should like to pay tribute to the many people I have had the privilege of meeting and working with during my scientific career. I owe a great debt to the dedicated group of Dr. G.W. Thorn at the Peter Bent Brigham Hospital in Boston, the city in which I was introduced to medical and biochemical research. I am, in addition, greatly indebted to Albert E. Renold, a long time advisor and friend, head of the Institut de Biochimie clinique in Geneva (Fig. 27). Surprisingly, the official Institut sign shown in Fig. 27 reads "Chemin privé", i.e. private way. However, nowhere have I seen a "chemin privé" so open to the world, thanks to the art of A.E. Renold of bringing people together, and to his deep conviction that science can only be international in nature. The work presented during this Lecture is the result of the collaborative work of many researchers of many nationalities. Among these, I should like to thank, particularly and heartedly, R.J. Ho, J. Letarte, I. Bihler, and M. Touabi. My very sincere thanks also go to more recent collaborators: S.W. Cushman, J.J. Heindel, F. Assimacopoulos and L. Orci; the name of W. Stauffacher should also be cited. Special mention is due for A.E. Lambert as we have been engaged in collaborative project, the fascinating and many facets of which I shall certainly never forget. I

must at this point pay an additional tribute to a compound which has frequently been added to fat cells and which represents the origin of my collaboration with Lambert: caffeine.

Finally, and most especially, I should like to thank my wife Inès who has accepted with good humour the many drawbacks of having a scientist as a husband. More important, our mutual interest for our own respective work has made our life together an exceedingly stimulating and interesting one; to Inès this Lecture is dedicated.

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