Summary of Discussion

Dr. Donald F. Steiner expressed interest in Dr. Randle's idea of two types of glucose receptor sites for stimulation of insulin release. He recalled work of other investigators using rabbit islets, who showed that glucose and mannose stimulated insulin biosynthesis but fructose had no such effect. He asked Dr. Randle if the first site of narrow specificity might possibly be involved in both secretion and biosynthesis, whereas the second nonspecific site may not be involved in stimulating biosynthesis. *Dr. Philip J. Randle* suggested that one would like evidence that biosynthesis is mannoheptulose-sensitive, but there is no way to tell if the "potentiator site" is mannoheptulose-sensitive because glucose is needed to demonstrate the effect. He stated that glucosamine should stimulate biosynthesis on its own and ought to inhibit glucose stimulation. Glucosamine may be more tightly bound to some site than is glucose and the V_{max} for its effect is much lower. Therefore, in the presence of high glucose concentration, we would be seeing an effect of glucosamine and not glucose stimulation. The glucosamine response is much less readily suppressed by mannoheptulose than is that of glucose.

Dr. E. F. Pfeiffer cited experiments in which mannoheptulose decreased insulin secretion and biosynthesis to the same extent in the presence of 300 mg. glucose per 100 ml.

Dr. Patrick T. Grant commented on Dr. Randle's suggestion that ion flux may play some part in the secretory mechanism. Biosynthesis and the integrity of the polysomes in cell-free fish islet polysomes are highly dependent on metal ions including sodium, potassium, calcium, magnesium and especially zinc. It may be that ion flux is peculiar to polysome formation in the islet cells, since protein synthesis in other cell-free systems is not so dependent on metal ions.

Dr. Gerold M. Grodsky put three questions to Dr. Randle: (1) Could the potentiation of one sugar by another be an additive effect in a nonlinear system? Some sugars could have a very small effect at a lower threshold but, when added to the system above the threshold for glucose, could be magnified tremendously because the system would be operating at a very effective rate of change of insulin secretion. Dr. Grodsky cited his own observations that when a sugar or an agent augments glucose secretion, it does not do so beyond the V_{max}. For example, 50 mg. glucose per 100 ml. causes no stimulation of insulin secretion, 100 mg. glucose per 100 ml. causes slight glucose stimulation, but 150 mg. causes a tremendous stimulation of insulin release. However, going to 300-500 mg. per 100 ml. causes little extra effect on insulin output. (2) How effective was galactose as a stimulator of insulin release and is there any evidence that it is metabolized by the islets? (3) The studies of insulin secretion were performed in the presence of caffeine. Were the metabolite studies also done with caffeine in the medium so as to give identical conditions for comparison?

Dr. Randle replied: (1) It cannot be decided with certainty whether the potentiation of insulin secretion is a qualitative or quantitative effect. Caffeine was added to the system to make the responses as large as possible. Insulin secretory responses were done in the presence of 0.5 mg. of glucose per ml. He suggested that to answer the question of potentiation the potentiator should be tested throughout the full dose-response curve of glucose. (2) Galactose potentiation responses are very large in the rat but very small in the mouse. The rate of metabolism of fructose by the islets is only about 10 per cent that of glucose. This would be equivalent to a rate of glucose metabolism at a concentration below its stimulation threshold. Studies have been started on galactose metabolism by rat islets. Preliminary studies suggest that it is metabolized at about the same rate as fructose. (3) Caffeine did not affect the rate of glucose metabolism by the islets.

Dr. Franz M. Matschinsky cited other work showing similar lack of effect of caffeine on the rate of glucose oxidation by islets. Dr. Randle added that the islet metabolizes glucose at a high rate; that is, islets use about 25 per cent as much glucose per gm. as does the working heart. The effects of this high rate of glucose utilization during conditions when insulin is being neither synthesized nor secreted is not known.

Dr. Stefan S. Fajans asked if amino acids may directly stimulate insulin secretion or whether they must be metabolized in order to have this effect. Nonmetabolizable analogs of leucine cause insulin stimulation in dogs. He suggested that transport of amino acids across cell membranes may stimulate transport receptor sites in the process of stimulation of insulin release. Likewise, a nonmetabolizable analog of arginine will stimulate insulin as well as glucagon release. Mannoheptulose inhibits

glucose and arginine stimulation of insulin release. This effect may be due to (1) the possibility that in order for arginine to stimulate insulin release the islets may have to metabolize glucose simultaneously or (2) possibly mannoheptulose competes for transfer of arginine across cell membranes, a mechanism similar to that proposed for glucose by Dr. Matschinsky. He also pointed out that mannoheptulose potentiates rather than inhibits the stimulation of insulin release by leucine and its nonmetabolizable analog.

Dr. Randle wondered if glucose could be replaced by leucine and still get an effect of arginine on the stimulation of insulin secretion. Dr. Fajans pointed out that arginine-induced insulin release in vivo is greatly potentiated by glucose. In the presence of hypoglycemia very little arginine-induced insulin release can be observed. However, in vitro studies have shown that arginine will stimulate insulin release in the absence of glucose in the media. *Dr. Willy J. Malaisse* pointed out that leucine can replace glucose for arginine-induced insulin release and arginine can act without glucose in the media.

Dr. Joseph Larner commented on the large effect of caffeine on insulin release reported by several of the investigators. He wondered if anyone had measured cyclic AMP content in islets and correlated with these the magnitude of insulin release. *Dr. Albert E. Renold* pointed out that there had been very few such studies in islets and none have measured cyclic AMP levels in isolated beta cells. Dr. Grodsky cited work of other investigators in which caffeine added to the media caused a rise in cyclic AMP levels in the islets, but these did not correlate with the phases of insulin release. Dr. Renold emphasized that these measurements were made in intact islets.

Dr. Arthur A. Like pointed out that many diabetic beta cells seemed to be able to secrete large amounts of insulin even though by electron microscopy they appear to be degranulated. He raised the question as to whether there may be different mechanisms of insulin secretion in degranulated islets.

Dr. Steiner added that, in view of the fact that glucose does not cause a change in cyclic AMP levels in the islets, protein kinase activity was measured in islets in his laboratories. Protein kinase activity was found to be cyclic AMP-dependent, but it was not found to be dependent upon glucose, glucose-6-phosphate or other glucose metabolites. Dr. Renold emphasized again that we are dealing with intact islets which have both alpha and beta cells—which may respond in opposite directions to stimulation. We must be particularly cautious in interpreting changes in cyclic AMP levels, especially when they are negative.

Dr. John Logothetopoulous asked Dr. Matschinsky two questions: (1) How does he explain the increased insulin release when the infusion was stopped? (2) How does he explain the lack of change in glucose-6-phosphate and fructose-6-phosphate levels if it is accepted that there is an increased flux of the glucolytic pathway? Dr. Matschinsky (1) speculated that if the pancreas is perfused with a secretagog, insulin may accumulate at the periphery of the cell. If a stimulator, such as glucosamine, may also be a partial agonist, then removal of the agonistic component may lead to a concentration where the stimulatory effect is far more pronounced than the inhibitory action and thus possibly result in a markedly augmented response. (2) Experiments in the muscle of the fly have shown that flux changes which vary by factors of 100 are accompanied by such a well-regulated system that the alteration in levels of metabolites is very small—in the order of only 10-15 per cent. There probably are alterations in levels of islet cell metabolites that cannot be detected by present methods, but they are associated with the fluctuation in regulatory steps which are activated. *Dr. George F. Cahill, Jr.,* pointed out the paradox in the data of Dr. Hellerström and Dr. Randle between the increase in the rate of glucose metabolism and the small increase in oxygen consumption. Dr. Randle explained that he used bicarbonate buffer and Dr. Hellerström used phosphate buffer; therefore the results could not feasibly be compared. He also pointed out from his data that 30 per cent of the glucose utilized by the islets is oxidized. The fate of the remainder of the glucose is unknown; some goes to lactate.

—JAMES E. VANCE, M.D.