EDITORIALS

THE "PERMEABILITY" HYPOTHESIS OF THE ACTION OF INSULIN

The first experimental evidence that the permeability of certain cells to the entrance of glucose or other sugars was influenced by insulin was published some five years ago.[1, 2, 3] Since this time the total experimental evidence has been multiplied by the publication of many papers and has established the validity of this hypothesis that changes in permeability of certain tissues play a major role in the mechanism of the action of insulin. A recent comprehensive review by Ross[4] has been published. The history of the subject, however, goes back much further than 1949, since in 1914 Hoeber[5] suggested the possibility that in diabetes mellitus the permeability of certain cells to glucose was so impaired as to prevent its penetration to intracellular sites of enzymatic action. On the basis of these speculations Hoeber discussed, as a working hypothesis, what he called a "membrane theory" of diabetes. However, he published no experimental evidence in support.

Lundsgaard in 1939[6] reported that effects of insulin on cell-free systems could not be demonstrated. He postulated, therefore, that its action was associated with cell structure. In general, the concept that insulin action cannot be demonstrated in cell-free systems has been supported by the work of others.[7] Lundsgaard's experiments on perfused hind limb preparations demonstrated the increased disappearance of glucose from the perfusing medium and also showed that no free glucose could be demonstrated in the cell. On the basis of this evidence he conceived the possibility that insulin accelerated the migration of glucose into striated muscle cells through some unknown mechanism acting upon the surface of the cell. However, the possibility that insulin acted upon hexokinase was not ruled out by this type of experiment.

The term, "permeability," has been used with different meanings in the literature. For our purposes, its simplest interpretation may be regarded as indicating that the cell membrane permits the passage of certain solutes into the interior of the cell while barring or slowing the entrance of others. Theories as to the mechanism of this permeability have been freely presented in the literature, but in its application to the problem of the action of insulin authors have tended to avoid any implications as to mechanisms involved. For this reason, some have preferred to use the term, "transport" across cell boundaries, again without implication as to mechanism. But at the moment a rational selection of any descriptive term is impossible until more definite experimental evidence is available upon which a choice may be based.

Ross[4] has outlined an interesting teleological discussion for the necessity of a mechanism by which insulin accelerates the entrance of glucose, particularly into muscle cells. The need for a rapid increase of metabolic activity in muscle demands at certain times a more rapid entry into the cell of glucose, which is the chief easily available, energy-producing nutrient for muscle metabolism. Evolution then has developed this special hormonal accelerating mechanism in order that muscle may be capable of large ranges of energy-producing metabolism. The hydrophilic glucose must pass the hydrophobic barrier of the lipoid phase of the cell membrane, and insulin, by some unknown mechanism, increases the permeability of the cell membrane by diminishing the differences between these two antagonistic properties of solute and cell membrane. The alternative would have been to depend upon the rapid formation of energy-producing metabolites of lipophilic compounds, for example, acetate, pyruvate and lactate. Indeed, in diabetes mellitus the organism does precisely this, since by accelerating ketone production from fat in the liver it furnishes peripheral muscle with large amounts of energy-producing metabolites. Ketone bodies readily enter and are easily metabolized by muscle. Unfortunately, no regulatory mechanism for this type of metabolism has been established, and in consequence, the uncontrolled ketone production in excess frequently results in fatal acidosis.

The experimental evidence for the permeability hypothesis of insulin action has been obtained in two ways: (1) By measuring the volume of distribution of selected sugars in the eviscerated animal, or (2) by demonstrating an increase of free glucose or other sugars intracellularly by appropriate methods. In a series of papers, Levine and his colleagues[1, 2, 3] studied the effect of insulin upon the volume of distribution of d-galactose. This sugar was chosen because it is not utilized by skeletal muscle, although it is transformed into glucose in the liver, kidneys and intestinal tract. In the eviscer-

ated animal these transformations are eliminated. Levine and others[1, 2, 3] showed that without insulin the initial volume of distribution of d-galactose was approximately 45 per cent of the body weight, but the simultaneous injection of exogenous insulin increased this to 70 per cent, approximately the volume of total body water. Since all sugars are not insulin-responsive when studied by this method, it was natural to conclude that molecular configuration conferred some degree of specificity of the action of insulin upon permeability. The experiments of Levine, and Haft, Mirsky and Perisutti,[8] and also those of Drury and Wick,[9] clearly established this possibility.

Glucose was included among the insulin responsive sugars by Levine because of its molecular similarity in carbons 1, 2, and 3 with other responsive sugars. But Park and his co-workers [10, 11] first established that it was transported into the cell as free glucose, its phosphorylation to hexose-6-phosphate being secondary to entrance into the cell. They obtained this proof by (1) increasing the glucose concentration in the medium in which their muscles were equilibrated to very high levels, thus swamping the action of hexokinase, or (2) by chilling their systems to 12° C., thus practically eliminating the phosphorylating reaction. Park and his co-workers clearly showed that insulin had an accelerating effect on increasing the concentration of free glucose within the cell, thus establishing the fact that insulin acted upon permeability or transport of this sugar rather than upon its phosphorylation.

To date the evidence appears to show clearly that the permeability hypothesis of insulin action has only been established in the case of skeletal muscle, fat, adipose tissue, and heart. There is no clear-cut evidence that all or even part of the action of insulin upon hepatic metabolism can be explained upon the basis of the permeability hypothesis. In the case of the intestinal mucosa and the renal tubule it has been clearly shown that glucose transport in these sites is not accelerated by insulin. A unique situation exists with respect to the brain. Although blood-brain barriers to certain substances have been clearly established in this organ, no barrier appears to affect the entrance of glucose. It appears that insulin is not concerned in this transport, nor, indeed, has it ever been established that insulin has any effect upon metabolic reactions within the brain. There appears to be no clear-cut evidence to the effect that metabolic processes within the brain are disturbed even in severe diabetic states, unless there is some purely secondary effect due to such factors as acidosis due to severe ketosis.

Ross[4] has published an interesting speculative discussion proposing a definite mechanism by which insulin accelerates the transport of glucose across certain cell membranes. He proposes the existence of a "glucose transferase" which requires the presence of insulin for its complete functioning. This complex existing upon the lipoidal cell membrane has the ability to combine with glucose in a manner similar to the combination of an enzyme and its substrate. This glucose-insulin-transferase combination has the ability to transfer the hydrophilic glucose through the hydrophobic cell membrane into the cell interior. Within the cell glucose reacts with the hexokinase. The insulin-transferase complex is then free to repeat its transporting cycle.

Much remains to be done on this aspect of insulin action. But it appears to be established that the cell membrane is not an inert physical structure whose functions are limited solely by its osmotic properties. As Ross[4] has pointed out, "diseases of permeability" are possible. Apparently diabetes mellitus, in part at least, is the first disease to be assigned to this category.

REFERENCES

1 Levine, R., and Goldstein, M. S.: On the mechanism of action of insulin. Rec. Prog. Hormone Res. *11*:343, 1955.

2 Levine, R.; Goldstein, M.; Huddlestun, B.; and Klein, S.: Action of insulin on the permeability of cells to free hexoses, as studied by its effect on the distribution of galactose. Am. J. Physiol. *163*:70, 1950.

3 Levine, R.; Goldstein, M.; Klein, S.; and Huddlestun, B.: The action of insulin on the distribution of galactose in eviscerated nephrectomised dogs. J. Biol. Chem. *179*:985, 1949.

4 Ross, E. J.: The "permeability" hypothesis of the action of insulin. Medicine *35*:355, 1956.

5 Hoeber, R.: In discussion of paper by Kozawa, S.: Beiträge zum arteigenen Verhalten der roten Blutkörperchen. III. Artdifferenzen in der Durchlässigkeit der roten Blutkörperchen. Biochem. Zeitschr. *60*:231, 1914.

6 Lundsgaard, E.: On the mode of action of insulin. Upsala Lakareförenings Forhandlinger *45*:143, 1939.

7 Stadie, W. S.: Current concepts of the action of insulin. Physiol. Rev. *34*:52, 1954.

8 Haft, D.; Mirsky, I. A.; and Perisutti, G.: Influence of insulin on uptake of monosaccharides by isolated rat diaphragm. Proc. Soc. Exp. Biol. and Med. *82*:60, 1953.

9 Drury, D. R.; and Wick, A. N.: Insulin and the volume of distribution of glucose. Am. J. Physiol. *166*:159, 1951.

10 Park, C. R.; Bornstein, J.; and Post, R. L.: Effect of insulin on free glucose content on rat diaphragm in vitro. Am. J. Physiol. *182*:12, 1955.

11 Park, C. R.; and Johnson, L. H.: Effect of insulin on transport of glucose and galactose into cells of rat muscle and brain. Am. J. Physiol. *182*:17, 1955.

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