Mechanism of Insulin Action

Douglas R. Drury, M.D., Los Angeles, and*
Arne N. Wick, Ph.D.,† La Jolla, California

The primary action of insulin is familiar to everyone. It causes glucose to disappear from the blood. The blood sugar is in equilibrium with that in the interstitial fluid, so that when glucose is injected intravenously, it quickly distributes itself evenly in the extracellular compartment. Its passage from here into the cells is limited to a comparatively low rate. The glucose that insulin causes to leave the blood has but one place to go—into the cells.

Investigators in the past have proposed the theory that insulin primarily increases the oxidation of glucose, and that the insulin-induced disappearance of glucose from the blood was the result of increased oxidation of the glucose in the cells. Experimental work does not support this view. We can follow the behavior of the glucose molecule in the body by labeling it with radioactive carbon atoms (C^{14}). In order to carry out an accurate quantitative study of glucose metabolism, it is usually best to eliminate certain variable factors that are difficult to control, by removing the liver, pancreas, intestines, and kidney. Thus we have usually worked on the eviscerated rabbit. When an eviscerated rabbit is given enough insulin to produce a maximal effect, the disappearance rate of glucose immediately reaches a high level, with little or no further rise if administration of insulin is continued. The rate of oxidation of the glucose, as determined from the amount of tagged carbon in the expired carbon dioxide—increases slowly, and even after six hours of maximal insulin activity, only half of the disappearing glucose is being oxidized (table 1). The increased disappearance of glucose from the blood does not follow increased oxidation; the reverse is the case. Oxidation follows increased disappearance.

TABLE 1
Comparison of oxidation and disappearance of plasma glucose in an eviscerated rabbit maintained on maximum insulin

Hour	Glucose oxidized (per kg. per hr.) mg.	Glucose disappearing (per kg. per hr.) mg.
1	98	1,020
2	256	1,310
3	294	930
4	370	1,120
5	350	700
6	382	700

Another observation supports this concept. Insulin and glucose were administered for several hours to the eviscerated animal and the rate of disappearance and oxidation of glucose were determined. After five hours it was observed that 40 per cent of the respired carbon dioxide was coming from injected glucose. At this point a competitive fuel, beta-hydroxybutyric acid,[1] was injected. The rate of oxidation of glucose fell instead of continuing to rise, as it would otherwise have done (figure 1). The disappearance rate, however, continued the same as before instead of falling as it would have been expected to do if disappearance followed oxidation. These results indicate that insulin is not needed for the oxidation of glucose. This theory is well illustrated by the findings in the diabetic rat. When tagged glucose is fed to this animal its oxidation begins immediately, and within an hour, despite a large dilution of the fed glucose by body glucose, 50 per cent or more of the respired carbon dioxide is being derived from oxidation of the fed glucose. An eviscerated rat consumes much less glucose and even after four hours is utilizing glucose for only about one-quarter of its energy requirements (table 2). This difference is due to the presence of the liver in the diabetic animal. It appears that the liver changes some of the glucose to other compounds which can be more readily oxidized by the other tissues.

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From the Department of Physiology, University of Southern California, Los Angeles, and the Scripps Metabolic Clinic, La Jolla, California.

*Professor and Chairman, Dept. of Physiology, University of Southern California School of Medicine, Los Angeles.

†Scripps Metabolic Clinic, La Jolla, California.

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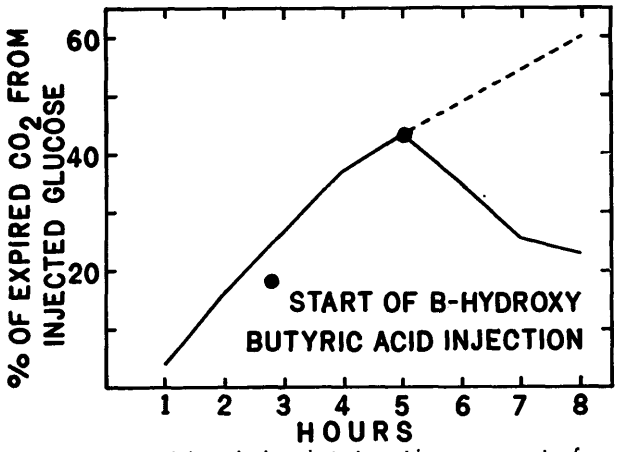

FIG. 1. Effect of beta-hydroxybutyric acid on per cent of expired carbon dioxide from injected glucose in insulinized animals. The glucose disappearance rate and carbon dioxide production remained the same throughout the experiment.

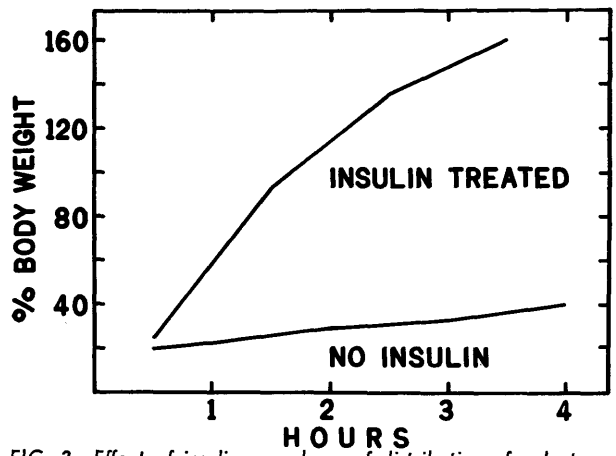

FIG. 2. Effect of insulin on volume of distribution of galactose-1-C^{14} in the eviscerated nephrectomized rabbit. No carrier galactose added. The volumes of distribution greater than total body water indicate a higher concentration of galactose in the cells than in the extracellular fluid.

TABLE 2
Comparison of glucose oxidation rates in the diabetic and the eviscerated rat

Hour	Per cent of respired carbon dioxide coming from glucose: Diabetic rat	Eviscerated rat
1	48	11
2	53	20
3	62	21
4	79	27

Our results support this view. If the blood of an intact rat is examined an hour or two after feeding with tagged glucose, a considerable amount of radioactivity is detected in nonglucose compounds. These compounds are ether-soluble. The molecules are probably smaller than glucose and hence quickly gain entry into the cell, so that they serve as quick fuel for the tissues. Acetic[2] and lactic acids[3] are examples of such compounds.

Such observations lead to the conclusion that insulin has little to do with the direct oxidation of glucose. Fed glucose can be quickly metabolized by diabetic animals by virtue of its being transformed into easily available fuels by the liver. Diabetic rats can derive their metabolic needs from carbohydrate, provided that they have constant access to it in their food, but they cannot store it for future needs. Glucose that has passed into the cell could also serve as a fuel in competition with other oxidizable compounds.

This view is strengthened by a report from Levine and his co-workers[4] to the effect that the intracellular transfer of galactose is accelerated by insulin. Galactose is oxidized little, if any, by the extrahepatic tissues. Insulin merely causes this hexose to be transferred into the cells, where it accumulates as a seemingly inert substance. We have obtained similar results,[5] as illustrated in figure 2. Galactose is closely related chemically to glucose, and it would appear that insulin acts on it because of this similarity. There are other sugars closely related to glucose that are also acted on by insulin. The Michael Reese workers[6] have reported that xylose is one of these. We have found that mannose also is such a sugar,[7] and furthermore that it is oxidized by the peripheral tissues, indicating that the intracellular enzymes can act on it after it is transferred into the cell. Reactivity of sugars to insulin, however, is highly specific. We have previously reported that fructose[8] and sorbitol[9] do not enter the cells in the living animal even when maximal insulin activity is induced. Data in table 3 show that gluconic acid, glucuronic acid, and 3-methyl glucose behave similarly. Figure 3 shows the structural configuration of these compounds with respect to glucose. For comparative purposes the structural configuration of the compounds that respond to insulin injection is illustrated in figure 4.

TABLE 3
Volume of distribution of glucose derivatives before and after insulin administration in eviscerated rabbits. Volume expressed as per cent of body weight. Figures in parentheses refer to time of plasma sampling after initial injection.

	Before insulin		After insulin	
Glucuronic acid	17(30′)	18(90′)	19(150′)	17(210′)
Gluconic acid	26(30′)	27(60′)	28(90′)	29(120′)
3-Methyl glucose	16(60′)	16(120′)	19(180′)	19(240′)

Recent studies of glucosamine indicate that insulin acts on it.[10] It passes into the cells much more rapidly when insulin is present (figure 5). At the same time the transfer rate for glucose is depressed. These two compounds compete in entering into the reaction accele-

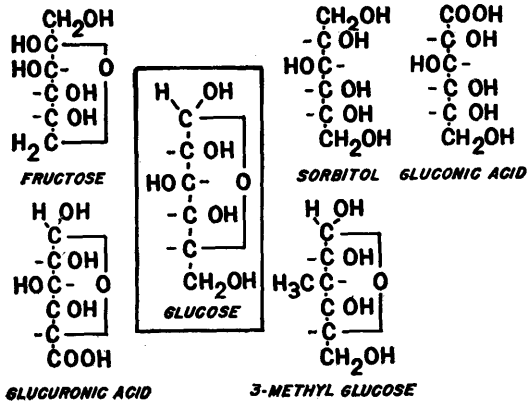

FIG. 3. Structural configuration of compounds related to glucose that do not enter the cells of the extrahepatic tissues in the living animal.

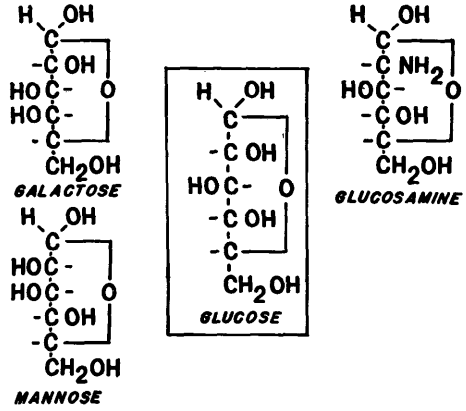

FIG. 4. Structural configuration of compounds that respond to the insulin action.

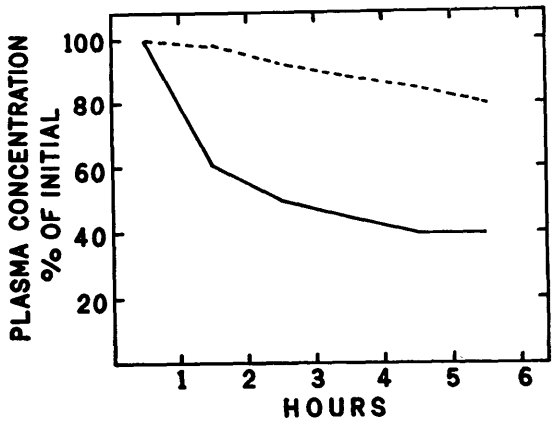

FIG. 5. Data showing the effect of insulin on the plasma disappearance of injected D-glucosamine-HCl in the eviscerated rabbit. Solid line represents an insulin treated animal and the broken line—no insulin.

rated by insulin which brings about entry of the sugars into the cells. The mechanism does not seem to be one of simple substitution. Glucosamine appears to block the process, since the entry of a given amount into the cells—for example, 100 mg.—will inhibit the entry of two to three times as much glucose. With present evidence we cannot state whether glucosamine behaves like galactose—having its intracellular transfer rate but not its oxidation rate accelerated by insulin—or whether it acts like glucose and mannose, whose transfer rate and oxidation rate are both accelerated by insulin. To answer this question will require the use of tagged glucosamine.

SUMMARY

Insulin acts on a group of compounds closely related to glucose. The effect common to these substances is an acceleration of the intracellular transfer of the hexose. With only two of these sugars, glucose and mannose, is combustion accelerated by insulin. All the data support the view that this increase is secondary to the increased amount of the sugar entering the cells.

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DISCUSSION

HELEN E. MARTIN, M.D., (*Los Angeles*): The studies made by Drs. Drury and Wick quite clearly show that the major site of insulin action is near the cell surface where in some undetermined fashion insulin effects the transfer of glucose into the cell. Their recent work on the competition between glucosamine and glucose when insulin is used is of great interest.

It has always been puzzling to understand why one diabetic patient takes three thousand units of insulin and another ten units, and why the insulin dose may fluctuate rather widely, particularly during infections and other stress situations, in the same individual. There are relatively few substances that directly antagonize insulin action which can be measured. Glucosamine may be such a substance. It is present throughout the body, as an important constituent of polysaccharides. The polysaccharides occur in many substances, such as thrombin, prothrombin, heparin. Bacterial antigenic properties are largely due to polysaccharides, of which glucosamine is a very important constituent. Also, it is the prosthetic carbohydrate combined with protein, in the well-known mucoproteins that are assuming such importance. Mucoprotein is presumably the substance laid down in the glomerular capillaries in the Kimmelsteil-Wilson lesion.

There have been some studies that have shown that glucosamine may reach levels around 100 mg. per 100 cc. in the blood. It has been shown to be elevated in diabetic renal disease and nondiabetic renal disease. Thus, it is very tempting to the clinician to speculate about the role of this substance acting as a competitor to glucose. This idea of competitive situations in the body is one that is being studied increasingly in many areas—such as in the renal tubular transport mechanisms. It may well be that there are important competitive mechanisms at the cell surface.

I would like to ask Dr. Drury to elaborate further on what to me is a very puzzling difference between the untreated diabetic rat who apparently can use glucose with a good deal of ease and very rapidly, as compared to the untreated diabetic dog who can use very little glucose, and is unable to get it into the liver or use it as a quick fuel, or the severely diabetic man who certainly is unable to use glucose quickly without insulin.

JOSEPH M. LOONEY, M.D., (*Boston*): As is well-known, the action of insulin is on the phosphorylation of these compounds. I wonder if the authors have studied the phosphate metabolism of the blood along with these changes in their sugar.

ARNOLD LAZAROW, M.D., (*Minneapolis*): I should like to ask Drs. Drury and Wick if they have evidence for action on the liver itself.

DOUGLAS R. DRURY, M.D., (*San Francisco*): Regarding the action of insulin on the liver, we have not studied it directly, and I do not know of any work by other workers that indicates any direct action of insulin on liver slices. We have not studied phosphate metabolism in our animals.

As to the importance of glucosamine clinically, knowledge of the substance is too recent for one to form a definite opinion. I know of only a few clinical articles on it. One trouble is that the authors have not distinguished too well between free glucosamine and combined glucosamine. There seems to be plenty of combined and polymerized glucosamine in the body. I doubt that free glucosamine could exist in a very high concentration in the body, because the liver would change it over to other compounds.

As to metabolism in the diabetic dog: All the diabetic animals have their own way of doing things, and the diabetic dog is certainly different from the diabetic rat. That, of course, is not to say that the primary action of insulin is any different in the two. The different manifestations of diabetes in the two animals may be due to some primary defect to which the two animals respond in different ways. There are, I suppose, a hundred different abnormalities in the diabetic. Certainly all cannot be due to differences of a hundred different insulin mechanisms. I don't know, though, that you can say that the diabetic dog or diabetic man is not burning quite a bit of glucose. No one has studied the animals while they are being fed a high carbohydrate diet.

I think the group at Berkeley have studied the diabetic dog and have found that about 20 per cent of the carbon dioxide came from the oxidation of glucose. The liver of the diabetic dog, though, certainly in a fasting condition, is doing a similar thing to what the diabetic rat's liver is doing when glucose is fed—it is making a quick fuel for the peripheral tissues. In the fasted diabetic dog the liver makes the quick fuel, betahydroxybutyric acid, out of fat.

SUMMARIO IN INTERLINGUA

Mechanismo del Action de Insulina

Insulina age super un gruppo de compositos intimemente affin a glucosa. Omne iste substantias ha le effecto de accelerar le transferimento intracellular del hexosa. In le caso de solmente duo de iste sucros—glucosa e mannosa—le combustion es accelerate per insulina. Omne le datos disponibile supporta le conception que iste acceleration es secundari al augmento del quantitate de sucro que entra in le cellulas.