Effect of Insulin on Utilization and Production of Circulating Glucose\textsuperscript{1,2,3}

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ABSTRACT

WALL, J. S., R. STEELE, R. C. DE BODO AND N. ALTSZULER. (New York U. Coll. Med., New York, and Brookhaven Natl. Lab., Upton, N. Y.) Effect of insulin on utilization and production of circulating glucose. Am. J. Physiol. 189(1): 43-50. 1957.—\textsuperscript{14}C\textsubscript{4} glucose was administered continuously to unanesthetized normal dogs by intravenous infusion following a priming dose at a rate which maintained the specific activity of the circulating glucose at a nearly constant level. Glucagon-free insulin was then administered intravenously in varying doses. Samples of blood were collected at intervals throughout the experiment and more frequently just subsequent to insulin injection. The glucose concentration of the circulating blood and the specific activity of this glucose, when considered together, allow calculations to be made of a) the outflow of glucose toward the tissue cells, and b) the inflow of glucose from the liver during the period after insulin injection. By this means it was shown that increased glucose uptake by the tissues is much more important than decreased delivery of glucose by the liver in bringing about insulin-induced hypoglycemia. It was also shown that recovery from hypoglycemia is brought about by increased glucose delivery by the liver and not by a decrease in tissue uptake of glucose below the preinsulin level. Alternative methods of calculation of absolute values for glucose inflow and outflow during periods of changing blood glucose concentrations are discussed and evaluated.

The mechanism whereby insulin lowers the blood glucose concentration is still not clearly understood. The blood glucose concentration in the postabsorptive state is a resultant of two processes: glucose utilization by the tissues and glucose replacement by the liver. Thus a decrease in the blood glucose concentration following insulin administration may be due to either an increase in the rate of glucose uptake by the tissues or a decrease in the rate of glucose inflow from the liver, or both.

The application of an isotope dilution technique, using \textsuperscript{14}C\textsubscript{4} glucose, has made it feasible to determine in unanesthetized dogs in the postabsorptive state, the size of the glucose pool and the rate of glucose inflow from the liver into the plasma \((1, 2)\). Under these conditions of constant blood glucose concentration, and in the absence of glycosuria, the rate of uptake of plasma glucose by the tissues equals the rate of glucose inflow into the plasma. This technique has now been further extended to permit the simultaneous determination of the rates of glucose inflow and outflow while the blood glu-

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\textsuperscript{2} Part of this research was carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

Glucose concentration is changing. It thus has become possible to determine the effect of administered insulin independently on each of the two rates of glucose flow.

The present study reports: a) the changes in the rates of plasma glucose inflow and outflow following the intravenous administration of a single dose of insulin to unanesthetized dogs in the postabsorptive state, and b) the changes in the two flow rates of glucose which result in the restoration of the plasma glucose concentration to the normal level following the insulin-induced hypoglycemia.

**Materials and Methods**

Six adult male and female dogs maintained on a standard diet (3) were used. The experiments were performed without anesthesia after a 17-18-hour fast. Several of the dogs had received C\textsuperscript{14} glucose previously, but a period of at least 6 weeks was allowed to elapse before they were used in the present studies.

A detailed account of the apparatus, preparation of the C\textsuperscript{14} glucose, most of the chemical analyses and the experimental procedure has been given (1, 2).

The C\textsuperscript{14} glucose was administered intravenously by an initial priming injection, followed immediately by a constant infusion which was continued throughout the entire experimental period.

After the radioactive glucose had been infused for 2 hours, insulin was injected intravenously. The glucagon-free insulin (Lilly) was dissolved in saline, adjusted to pH 3 with hydrochloric acid, just prior to each experiment. The doses of insulin varied from 0.025-0.25 u/kg body weight.

At intervals during the course of the experiment approximately 8-ml samples of blood were drawn from the exposed femoral vein (occasionally from the femoral artery), collected in heparinized tubes and the plasma rapidly separated by centrifugation, for the determinations of a) the plasma glucose concentration, and b) the C\textsuperscript{14} content of the plasma glucose. Samples of blood were taken more frequently just prior to and subsequent to the administration of insulin.

**Experimental Design**

The advantages of measuring the size and turnover rate of the glucose pool by a technique whereby the C\textsuperscript{14} glucose is administered intravenously as a priming dose following by a constant infusion, have been discussed (1). An analysis of the process of the mixing of the C\textsuperscript{14} glucose with the endogenous glucose and the calculations of the pool size and turnover rate in the postabsorptive state have been given in detail (1). The quantities of glucose contained in both the radioactive priming injection and the constant infusion were minute and introduced insignificant quantities of glucose into the body glucose pool.

A 2-hour interval of constant infusion after the injection of the priming dose and prior to the administration of insulin was chosen to allow adequate time for the plasma glucose specific activity to reach a relatively constant level and to permit sufficient serial sampling of the plasma glucose for the calculation of the glucose pool and turnover rate prior to the insulin injection. The preinsulin period was kept as brief as possible to minimize errors due to recycling (i.e., the release into the blood of labeled glucose resynthesized from C\textsuperscript{14} fragments derived from the injected C\textsuperscript{14} glucose), which become evident in prolonged experiments.

Two alternative explanations may be offered as causes for the fall in the blood glucose concentration following insulin administration. If insulin promotes the uptake of glucose by the tissue cells without affecting the inflow of glucose from the liver, then the specific activity of the plasma glucose would remain unchanged during the fall of the plasma glucose concentration. In this case the rate of C\textsuperscript{12} glucose inflow from the liver would remain in balance with the constant C\textsuperscript{14} glucose infusion and the prevailing plasma glucose specific activity would thereby be maintained unchanged. The process of glucose uptake by the tissues cannot alter the plasma glucose specific activity since C\textsuperscript{12} and C\textsuperscript{14} molecules are taken up in proportion to their concentrations in the plasma.

If, however, insulin diminishes the release of glucose from the liver, then the fall in the plasma glucose concentration would be accompanied by a rise in the plasma glucose specific activity. In this case the C\textsuperscript{14} glucose delivered by the constant infusion would be diluted by lesser amounts of unlabeled inflowing endogenous glucose, and the net effect would be as if there were an influx into the plasma of glucose of higher specific activity than the tagged glucose already present in the plasma.

Both of these effects, the increased glucose uptake by the tissues and the diminished glucose inflow from the liver, which contribute to the lowering of the blood glucose concentration, may be occurring simultaneously. A method for the quantitative evaluation of each of these effects in contributing to the hypoglycemia is given below.
The restoration to normal of the decreased blood glucose concentration which follows the insulin injection may also be ascribed to either of two alternative mechanisms or to both occurring simultaneously. One possibility is that the release of glucose from the liver might be increased. This would result in a fall of the plasma glucose specific activity, the C^14 glucose infusion being constant. If, on the other hand, the restoration of the blood glucose level were due only to a great diminution of the glucose outflow from the plasma, then this effect would not cause a change in the plasma glucose specific activity.

**METHOD OF CALCULATION**

The magnitude of the changes in the rates of glucose inflow from the liver into the plasma and the glucose outflow from the plasma following an injection of insulin, can be determined from the changes in the plasma glucose concentration and the plasma glucose specific activity. The following equation relates these factors in an ideal instantaneously mixing system:

\[
\frac{dX}{dt} = F - X \left( \frac{g - a}{C_0 + at} \right)
\]

\(X\) = total \(\mu\text{c} \text{ C}^{14}\) present in the body pool of glucose; \(t\) = time in minutes elapsed since the beginning of the observation period; \(F\) = infusion rate of \(\text{C}^{14}\) glucose in \(\mu\text{c/min. ('weightless') amount of C}^{14}\) glucose; \(g\) = inflow of \(\text{C}^{14}\) glucose from the liver in grams carbon per minute; \(a\) = increase in total quantity of glucose carbon in the body pool expressed in grams carbon per minute as determined by the observed change in glucose concentration during \(t\) minutes of observation; \(C_0\) = total amount of glucose carbon in the body pool at the beginning of the observation period.

When there is a rise or fall of blood sugar \((a \neq 0)\), and glucose influx from the liver continues \((g \neq 0)\), and when utilization by the tissues is maintained \((g - a \neq 0)\), the integrated equation yields:

\[
\frac{SA_t}{t} = \frac{F}{g} \left( \frac{C_0}{C_0 + at} \right)^{g/a}
\]

\[
\frac{SA_0}{t} = \frac{F}{g}
\]

wherein: \(SA_0\) = specific activity of pool glucose in \(\mu\text{c/g}\) carbon at the beginning of the observation period; \(SA_t\) = specific activity of pool glucose at time \(t\).

After substituting the experimentally known values for a given time interval in equation 2, the inflow of glucose into the pool \((g)\) for that period may be determined by trial and error. The outflow of glucose of the body pool is equal to \(g - a\).

It is impossible to collect a sample which is characteristic of the entire glucose pool during the transient state following insulin injection. The available part of the glucose pool is the blood; the plasma glucose concentration and plasma glucose specific activity can be determined. The employment of the changes of plasma glucose specific activity and plasma glucose concentrations as a means of approximating glucose inflow to and outflow from the body pool are subject to the qualifications discussed below:

As shown previously (1) the entire body glucose pool can be represented as a central plasma compartment and two peripheral compartments thought to be located in the interstitial fluids. One of these interstitial fluid compartments exchanges glucose very rapidly with the plasma so that an intravenous dose of \(\text{C}^{14}\) glucose mixes completely in this 'fast' compartment in less than 5 minutes (1). The glucose contained in the plasma plus that contained in the 'fast' compartment is about half of the entire amount of glucose in the body pool. The other half of the body glucose pool is located in a 'slow' compartment which exchanges glucose with the plasma so slowly that more than an hour is required for equilibration of an intravenous dose of \(\text{C}^{14}\) glucose with the 'slow' compartment. Therefore, when rapid changes in plasma glucose concentration occur after insulin these are indicative of what is happening in the 'fast' compartment but are nearly independent of what may be happening in the 'slow' compartment. An approximate calculation can be made for what can be observed experimentally by treating the plasma glucose plus the 'fast' compartment glucose as an ideal homogeneously mixed pool as described above. The size of this pool is taken to be 50% of the whole pool during the preinsulin period; after insulin its size is thought of as varying in direct proportion with the plasma glucose concentration.

In order to evaluate the magnitude of the
Fig. 1. Curves for inflow and outflow of glucose subsequent to insulin injection derived from the operation of an electronic analogue of the glucose pool. Curves (x—x) represent the operation of the full three-compartment (three condenser) system; curves (O--O) represent the simplified system in which the slow compartment is eliminated and the other two compartments are separated by zero resistance; curves (Δ—Δ) represent another simplified system in which all three compartments are retained but are separated from each other by zero resistance so as to act as a single condenser (single well-mixed pool).

Errors which may be introduced into the calculations by the above described procedure, an electronic analogue of the dog body glucose pool was constructed.4 In such an analogue the flow of glucose is represented by the flow of electrons, each compartment of the body glucose pool is represented by a fixed capacitor of appropriate size, and the restrictions in flow of glucose into and out of the various compartments are represented by electrical resistors.

In the particular model constructed two identical analogues were used in parallel, one representing C12 glucose concentrations (as the electrical charges on the condensers) and flows, the other representing C14 glucose concentrations and flows. It was assumed that the effect of insulin is to reduce the resistance to the flow of glucose out of the ‘fast’ compartment of the body glucose pool, so provision was made for a two-gang variable resistance to bring about a simultaneous and equal change in electrical resistance in both the C12 and the C14 analogues at this location. One servo-mechanism was set to follow the observed changes in C14 glucose concentration in the plasma compartment and to bring about these changes automatically and accurately by increasing or decreasing the setting of the variable resistor. The inflow of C14 glucose (the infusion) remained constant and was represented by a constant current inflow device feeding into the plasma compartment of the C14 analogue. The inflow of C12 glucose, on the other hand (inflow from the liver), was not kept constant. It was increased or decreased by the operation of a second servo-mechanism which was set to track the observed changes in C12 glucose concentration in the plasma compartment. It will be remembered that the changes in the variable resistor leading out of the ‘fast’ compartment of the C14 analogue were reproduced in the C12 analogue; hence the C12 servo-mechanism was forced to compensate for changing outflow of C12 glucose while keeping the C12 glucose concentration in the plasma compartment on the track by increasing or decreasing the inflow of electrons into the plasma compartment of the C12 analogue.

The compartment sizes and the steady-state rates of glucose flow were adjusted to match the dog body glucose pool described in a previous publication (1); the curves for servo-mechanism tracking were the C14 glucose concentration (μg/ml plasma) and the C12 glucose concentration (mg glucose/ml plasma) observed in a typical dog after administration of 0.1 U insulin/kg body weight intravenously.

Figure 1 represents results from the electronic analogue when operated in three different ways. The solid lines represent glucose flows (metered electron inflow into the C12 analogue and the sum of the metered electron outflows from the ‘fast’ and ‘slow’ compartments of the C12 analogue) when the complete three-compartment system is operating. The broken lines represent

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4 The authors wish to thank Dr. Robert M. Sugarman and Mr. George Hoppen, Dept. of Instrumentation, Brookhaven National Laboratory, for developing the electronic analogue of the dog body glucose pool which was used in this study.

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glucose flows when the analogue is rewired a) to isolate the 'slow' compartment capacitor from the circuit, and b) to bring to zero the resistance between the plasma compartment capacitor and the 'fast' compartment capacitor. These changes are the equivalent of the simplifying assumptions made in the calculations described in the present report. The third type of line (dot-dash line) represents glucose flows when the resistances between all three capacitors are brought to zero. This corresponds to the situation when the whole glucose pool is assumed to be a single well mixed pool and represents an alternative simple method of computation which was at first considered.

It is seen from figure 1 that either method of simplified computation gives useful and unconfusing information about the time sequence of glucose inflow and outflow subsequent to insulin. As a quantitative measure of glucose flows the particular simple computation adopted in the present paper is better if it be assumed that insulin increases glucose outflow only from the fast compartment of the glucose pool. The alternative simple method of computation would give better quantitative results if insulin would have an equal influence on glucose outflow from both the 'fast' and 'slow' compartments. However, it is felt that inasmuch as glucose is so slow in diffusing in and out of the 'slow' compartment, the large protein molecule of insulin must be even slower in penetrating.

An additional advantage of the simplified computation which was adopted for this paper is that it deals only with that part of the pool wherein changes in glucose concentration and specific activity are reflected promptly in the circulating blood and hence in the experimental data.

RESULTS

In figure 2 are illustrated the changes in plasma glucose concentration and plasma glucose radioactivity in a normal dog following the intravenous injection of insulin, 0.063 U/kg. The calculated rates of glucose inflow and outflow are also shown in figure 2. Immediately following the administration of insulin a decrease in plasma glucose concentration and a slight elevation of the plasma glucose specific activity were observed. The calculated glucose uptake by the tissues underwent a large increase during this period, and the calculated inflow of glucose from the liver underwent a transient decrease. It is evident that the hypoglycemia resulting from the administration of insulin was due mainly to the increased uptake of glucose by the tissues. Although the diminished inflow contributed to the lowering of the blood sugar concentration, it was of much lesser importance in this respect than the increased uptake of glucose by the tissues.

As illustrated in figure 2, the return of the plasma glucose concentration to its control value was accompanied by a marked decline in the plasma glucose specific activity. The calculated glucose outflow did not fall below the preinsulin level during this period, whereas the calculated glucose inflow increased considerably. Thus, the recovery process was due entirely to a rapidly increased inflow of glucose from the liver into the glucose pool. It can be noted that after the plasma glucose concentration had returned to the preinsulin value, the plasma glucose specific activity was slowly rising toward its preinsulin value. Here the specific activity was tending toward an asymptotic final value determined by the C^14 glucose infused and the C^13 glucose delivered by the liver in the resting state, just as in the final hour of the preinsulin infusion.

In figure 3 are compared the effects of two doses of insulin (0.025 and 0.10 U/kg) on the plasma glucose concentration, the rate of glucose outflow, and the rate of glucose inflow of normal
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FIG. 3. Comparison of the effects of intravenous injections of different doses of insulin in normal dogs on the plasma glucose concentrations (A), the glucose outflow rates from the body glucose pools (B), and the glucose inflow rates into the pools (C).

dogs. It can be seen that the degree of the hypoglycemia and the time necessary for the plasma sugar concentration to return to the preinsulin level depended upon the amount of insulin injected (fig. 3A).

It is evident that the magnitude and duration of the effect of injected insulin on the rate of glucose uptake by the tissues depended upon the quantity of insulin administered (fig. 3B). With the larger doses of insulin the rate of uptake was increased more, and the duration of this effect was prolonged. The total amounts of glucose taken up by the tissues following insulin may be calculated from the areas under the curves (fig. 3B).

The rate and amount of glucose inflow from the liver following insulin injection were related to the degree of hypoglycemia produced (fig. 3A and 3C). However, there was no apparent critical level of hypoglycemia that had to be reached in order for the liver to start to deliver increased amounts of glucose into the pool. Thus with the larger doses of insulin the blood glucose concentration fell to lower levels than with the small doses, before an increased inflow of glucose from the liver was evoked.

DISCUSSION

It is evident from the present studies that the administration of insulin to normal dogs in the postabsorptive state resulted in a greatly increased uptake of plasma glucose by the tissues. This effect and the, quantitatively less significant, transient diminution of glucose release from the liver, were responsible for the decrease in the plasma glucose concentration observed following the injection of insulin. The resulting hypoglycemia evoked a rapid release of glucose from the liver, sufficient to restore the plasma glucose concentration to normal.

The action of insulin in bringing about an increased uptake of glucose in the tissues in the normal animal is in harmony with the current concepts of insulin action which are based on experiments performed under a variety of conditions, e.g., in the eviscerated, eviscerated-nephrectomized preparations, isolated diaphragms, etc. (4, 5).

The finding that the injection of insulin is initially followed by a transient diminution of the release of glucose from the liver is in accord with similar observations made by Bearn et al. (6) in human beings, employing hepatic catheterization technique. More recently, Henderson et al. (7) have demonstrated, using C\textsuperscript{14} glucose, that the intravenous injection of insulin in anesthetized depancreatized dogs reduced the release of hepatic glucose.

It should be noted that a transient decrease in C\textsuperscript{12} glucose output by the liver, which is what is discernible by the C\textsuperscript{14} glucose technique, would not necessarily mean that a decrease in net glucose output by the liver was taking place. A shift in the output by the liver to C\textsuperscript{14} glucose in place of C\textsuperscript{12} glucose (more of the output being derived from C\textsuperscript{14} tagged liver constituents, for example) would be construed as decreased liver glucose output by the C\textsuperscript{14} technique. As evidenced by the experimental results, insulin does promote the uptake of glucose from
the glucose pool by the tissues and possibly a portion of the C14-labeled glucose is incorporated into liver glycogen. Stetten and Stetten (8) have demonstrated that the newly incorporated glucose residues occupy terminal portions of the polymer. It would appear probable that these residues would be most readily released during the subsequent recovery period after insulin-induced hypoglycemia. This would result in an apparently lower value for glucose output by the liver (as calculated by the change in specific activity) than actually occurs since the calculation is based on the assumption that glucose derived from the liver is unlabeled. The fact that the hepatic vein catheterization technique (6) also revealed a decreased glucose output by the liver is very reassuring.

The restoration of the plasma glucose concentration to normal following the insulin-induced hypoglycemia was shown to be due to an increased release of glucose from the liver. From the observed effects with the various dosages of insulin (fig. 3) it is apparent that with increasing doses there was a greater glucose uptake by the tissues and a greater decrease in the plasma glucose concentration. Furthermore, the increased severity of the hypoglycemia resulted in a greater release of glucose from the liver.

In bringing about the release of glucose from the liver in response to hypoglycemia, adrenaline does not play an indispensable role inasmuch as the adrenalectomized animal is still capable of rapidly restoring its blood glucose concentration to normal following an insulin-induced hypoglycemia (9).

It is important to realize the implications involved if the action of insulin in reducing liver glucose output were to be ascribed to the operation of the same fundamental mechanism as its action in increasing the uptake of glucose by the extrahepatic tissues.

In the extrahepatic tissues the direction of glucose flow is from outside to inside the cells in both the postprandial and the postabsorptive states. In the liver the net glucose flow may be either toward the cell interior, as in the postprandial state, or toward the cell exterior as in the postabsorptive state.

If in the liver in the postabsorptive state glucose is to be thought of as flowing from a region of lower concentration outside the cell, then an action of insulin to facilitate the transport of glucose in both directions across the cell membrane (increased permeability) would result in a net increase in glucose output—the opposite of what is observed to happen. Thus, if it is to be claimed that the universal and sole primary action of insulin is to accelerate the operation of a transport mechanism by which glucose moves from the outside to the inside of cells, then the observed effect of insulin on the liver in the postabsorptive state demands that the transport mechanism which is postulated be one which transports glucose selectively in the inward direction and one which is capable of transporting it against a glucose concentration gradient. Also, there is required in the case of the liver a second transport process (diffusion for example), which is not accelerated by insulin and by which glucose moves from the inside to the outside of the hepatic cell. If the outward flow of glucose were to remain unchanged while insulin were to accelerate a process putting some glucose back into the cells, the net effect would be the observed diminution, after insulin, of glucose output by the liver.

An alternative view with regard to the liver might be that in the postabsorptive state insulin decreases the intracellular glucose concentration by increasing the rate at which glucose is converted to a phosphorylated metabolic intermediate. This would reduce the concentration gradient between the inside and the outside of the cell and so reduce the rate of glucose output. In the case of the extrahepatic tissues, however, the current concept is that the intracellular glucose concentration is near zero prior to the action of insulin; if this is so it would not be possible for an increased rate of phosphorylation to increase significantly the rate of glucose entry into the extrahepatic cell. On the other hand, the phosphorylative process might occur in the cell wall and be part of the glucose transport mechanism which moves glucose from outside to inside the cell. If the liver cell were like the extrahepatic cell with regard to such a phosphorylative transport mechanism, then the movement of glucose into the hepatic cell after insulin in the postabsorptive state would not in the strict sense occur against a concentration gradient for it would not be glucose...
which entered the cell but a metabolic interme-
diate of glucose.

The question arises to what extent do the results of these studies apply to the physiological role of insulin. In the present experiments a single dose of insulin was injected in the post-
absorptive state. Although the administration of a large dose of insulin is undoubtedly un-
physiological, since in the normal animal insulin is not secreted in such amounts as to produce frank hypoglycemia, there is good evidence that some insulin is constantly secreted in the postabsorptive state (6). Several investigators (10–13) have shown that a constant injection of insulin was necessary to maintain a constant normal blood sugar level in depancreatized dogs. The short duration of the effect of injected in-
sulin on the glucose uptake in the present studies is consistent with the above finding. On the other hand, Drury and Greeley (13) claimed a longer period of action for insulin; however, anesthesia and larger doses of insulin were used in their experiments, which would tend to extend the duration of the effects.

In view of the convincing evidence that in-
sulin is continuously secreted in small amounts in the postabsorptive state, it is reasonable to assume that under physiological conditions it exerts effects similar to those observed in the present study.

In the postprandial state the secretion of insulin is increased in response to hyperglyce-
mia. Searle and Chaikoff (14) have recently reported that in the normal dog the injection of a large dose of glucose resulted in a cessation of glucose release by the liver, until the hypergly-
cemia subsided. It may well be that this cessation of glucose production by the liver was due to the increased secretion of insulin during the postprandial period. If so, the present findings of a diminution of glucose release by the liver in the insulin-injected animal in the postabsorptive state may likewise reflect an aspect of the physiological action of insulin.

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REFERENCES