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# Effect of Insulin and Dinoprostone on D-Glucose and 2-Deoxy-D-Glucose Uptake by Normal and Diabetic Rat Hepatocytes

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**Abstract** □ Suspensions of deaggregated hepatocytes were prepared by a collagenase perfusion technique from livers of both normal rats and rats rendered diabetic by streptozocin treatment. Uptake of D-glucose and 2-deoxy-D-glucose was estimated by adding $^{14}C$- or $^{3}H$-labeled hexose to a stirring suspension of cells in Krebs-Henseleit buffer at 37°, separating the cells by rapid centrifugation, and measuring radioactivity in the packed cell pellet. Uptake, calculated after correction for entrapped extracellular fluid, includes any hexose bound to, transported into, or otherwise immobilized in the cells. High concentrations of glucose (5–20 m*M*) establish an intracellular–extracellular distribution ratio near 1.0 within 1 min, indicating a facilitated diffusion transport system. In contrast, a low level of glucose (71 n*M*) almost immediately (<15 sec) establishes ratios of between 3 and 4, which suggests a significant amount of glucose binding to cell membrane. Such binding would not be detected at the high glucose levels because of its small magnitude. Hepatocytes from diabetic rats exhibit a decrease in this apparent binding to ~60% of normal; preincubation with 0.1 IU/ml insulin increases this toward normal values, although it does not affect the binding by normal hepatocytes themselves. Preincubation with 0.1 $\mu M$ dinoprostone depresses glucose binding in cells from both normal and diabetic rats. A low concentration (1.2 n*M*) of 2-deoxy-D-glucose establishes even higher intracellular–extracellular distribution ratios of between 4 and 6, but the apparent binding of this sugar is identical in normal and diabetic rat hepatocytes and is not affected by preincubation with either insulin or dinoprostone alone. However, combined treatment with both agents causes a significant increase in 2-deoxyglucose binding. The results suggest that insulin promotes formation of hexose-binding sites and conversion of these to specific glucose-binding sites while dinoprostone may act by blocking the latter conversion.

**Keyphrases** □ Insulin—effect on D-glucose and 2-deoxy-D-glucose uptake, normal and diabetic rat hepatocytes, dinoprostone □ Dinoprostone—effect on D-glucose and 2-deoxy-D-glucose uptake, normal and diabetic rat hepatocytes, insulin □ Hepatocytes—rat, normal and diabetic, effect of insulin, dinoprostone on D-glucose, 2-deoxy-D-glucose uptake

The roles of insulin and prostaglandins on glucose retention by adipocytes, muscle cells, and hepatocytes have been the subject of contention in the literature. Although some have denied the existence of any stimulatory action of insulin on sugar retention by normal hepatocytes (1, 2), others have demonstrated an increase in liver glycogen deposition in response to this hormone (3, 4). Treatment of diabetic animals with insulin is reported to augment glucose uptake by liver (5, 6), and insulin may also enhance sugar uptake by adipocytes (7) and muscle cells (8, 9). Dinoprostone stimulates glucose uptake and oxidation by adipocytes (10) and intravenous infusion of dinoprostone in humans increases blood glucose levels (11). The present study demonstrates that insulin specifically enhances binding of low levels of glucose to hepatocytes and that dinoprostone inhibits this binding

## EXPERIMENTAL

**Preparation of Rat Hepatocytes**—Liver parenchymal cells were isolated and prepared by the procedure of Bikhazi *et al.* (12), which modified procedures previously described by Berry and Friend (13) and Ingebretsen and Wagle (14).

**Tests for Cell Viability**—Trypan blue exclusion test for cellular viability was routinely performed before and after the experiments and preparations showing 90% viable cells or more were used.

The metabolic state of the hepatocytes during the incubation period was examined by following the respiration rate of cell suspensions in several separate experiments. Oxygen tension was recorded continuously with the aid of a biological oxygen monitor[1]. Rates were determined from the slopes of the oxygen tension curves and were calculated in terms of $\mu l$ of oxygen/mg of cell protein. Protein was determined by the biuret reaction, using bovine serum albumin as a standard on centrifuged cell pellets dissolved in 1 *N* NaOH to minimize possible interference from medium components.

**Preparation of Diabetic Rats**—Rats were rendered diabetic by streptozocin treatment following the procedure of Chandramouli and Carter (15) as modified by Bikhazi *et al.* (12).

**Isotopically Labeled Hexose**—In all experiments with labeled glucose, 10 $\mu$Ci of [1-$^{14}$C]D-glucose (58 mCi/mmole) was added to 226 ml of buffer to give a final concentration of 71 n*M*. In experiments with labeled 2-deoxyglucose, 5 $\mu$Ci of [1-$^{3}$H]-2-deoxy-D-glucose (18.8 Ci/mmole) was added to the same volume to give a final concentration of 1.2 n*M*.

**Determination of Hepatocyte Count and Mean Diameter**—Hepatocyte count ($1.2 \times 10^8$ cells per liver) and mean diameter (20 $\mu$m) were estimated by the procedure of Bikhazi *et al.* (12) using an automated counting machine[2].

**Uptake of D-Glucose and 2-Deoxy-D-glucose by Hepatocytes**—In general, $15 \times 10^6$ cells obtained from one rat liver were added to 225 ml of stirred Krebs–Henseleit solution in a water-jacketed beaker at 37° (the data in Tables I and II represent results from six different animals). After 45 min a small volume of isotopically labeled hexose was added, and 1.5 ml aliquots of suspension were periodically withdrawn, transferred to

[1] Yellow Springs Instruments, Yellow Springs, Ohio.
[2] Coulter Counter model A, Coulter Electronics, Hialeah, Fla.



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**Table I—Uptake of Very Low Concentrations of Glucose by Normal and Diabetic Hepatocytes** [a]

| Additions | Total Uptake, p moles/$10^6$ cells | Calculated Intracellular Concentration, n*M* | Excess Uptake, n*M* | Binding, % of Normal | Exp |
|---|---|---|---|---|---|
| Normal | | | | | |
| | 1.128 ± 0.044 | 268 | 205 | (100) | a |
| +I | 1.150 ± 0.039 | 274 | 211 | 103 | b |
| +P | 0.972 ± 0.022 | 232 | 169 | 82 | c |
| +I+P | 0.983 ± 0.006 | 234 | 171 | 83 | d |
| Diabetic | | | | | |
| | 0.761 ± 0.011 | 181 | 118 | 58 | e |
| +I | 0.894 ± 0.017 | 213 | 150 | 73 | f |
| +P | 0.733 ± 0.033 | 174 | 111 | 54 | g |
| +I+P | 0.800 ± 0.017 | 190 | 127 | 62 | h |

[a] Significant differences ($N = 6$). $0.01 < p < 0.02$ (b,c), (b,d), (f,g); $p < 0.01$ (e,f), (f,h), (a,e), (b,f), (c,g), (d,h). No other combinations show significant differences. Extracellular concentration of glucose: 71 n*M*. Expected intracellular concentration of glucose based on uptake at high concentration (Table III): 63 n*M*. Intracellular concentration was calculated as described in Table III and 63 n*M* was subtracted to obtain excess uptake. Excess uptake for the normal control was set equal to 100% to obtain percent binding.
Conditions: (+I), preincubated in the presence of 0.10 IU/ml of insulin for 45 min; (+P), preincubated in the presence of 0.10 μ*M* of dinoprostone for 45 min; (+I+P) preincubated in the presence of both insulin and dinoprostone. The error given is the *SEM*.

a 1.5-ml plastic centrifuge tube, and centrifuged at 10,000×*g* for 30 sec in a microcentrifuge[3]. The supernatant solution was removed, the sedimented cell pellet was rinsed 2–3 times with phosphate buffer, and the tip of the plastic tube containing the pellet was cut with a knife and placed in a scintillation vial. The pellet was solubilized with 1 ml of 1% papain solution, 5 ml of scintillation fluid was added, and radioactivity measured using a three-channel liquid scintillation spectrometer[4]. The amount of hexose retained in the pellet was estimated from the radioactivity measured in standard samples of the hexose.

The effects of insulin and dinoprostone and a combination of these two substances were evaluated with a series of four incubations carried out in parallel. A concentrated cell suspension was divided into 4 aliquots, each containing ~15 × $10^6$ cells. The first aliquot was added to 225 ml of buffer solution; the second was added to the same volume of buffer solution containing 0.10 IU of insulin/ml; the third to the buffer solution containing 0.10 μ*M* dinoprostone; and the fourth to the buffer solution containing both insulin and dinoprostone. After 45 min of preincubation, labeled hexose was added and the procedure described above was followed.

Uptake of high concentrations (5, 10, and 20 m*M*) of glucose was measured after addition of normal hepatocytes to 225 ml of buffer containing the indicated concentrations of glucose plus a tracer dose of [1-$^{14}$C]D-glucose.

**Measurement of Extracellular Space in the Packed Pellet**—The general procedure for uptake of hexoses was applied, as described above, except that labeled hexose was replaced by 5 μCi of [$^3$H]inulin. The radioactivity retained in a packed cell pellet of known volume was used to calculate the volume of medium contained within the pellet, assuming that inulin is excluded from the intracellular space. The inulin space (extracellular space) in the packed cell pellet ranged from 35 to 40%, and averaged 38%, a value consistent with the extracellular space around closely packed spheres. All estimates of uptake were corrected for inclusion of 38% of extracellular fluid in the packed cell pellet.

**Statistical Treatment**—Data were statistically treated and the tests of significance were checked by the standard error of the mean (*SEM*) using the Student's *t* test distribution table.

## RESULTS

**Viability of Hepatocytes During the Course of the Experiments**—The trypan blue exclusion test indicates that almost all cells retain their ability to exclude the dye over the 45-min period of incubation. Moreover, the mean cell diameter remains constant during the incubation in either insulin or dinoprostone. A $QO_2$ of ~1.0 μl/mg dry weight/hr is estimated which is lower than that reported for liver slices, but is within the range of normal mammalian cells (16). The protein

[3] Eppendorf Microcentrifuge, model 5412, Brinkmann Instruments, Westbury, N.Y.
[4] Tricarb, model 3320, Packard Instrument Co., Downers Grove, Ill.

**Table II—Uptake of Very Low Concentrations of 2-Deoxyglucose by Normal and Diabetic Hepatocytes** [a]

| Additions | Total Uptake, p moles/$10^6$ cells | Calculated Intracellular Concentration, n*M* | Excess Uptake, n*M* | Binding, % of Normal | Exp |
|---|---|---|---|---|---|
| Normal | | | | | |
| | 0.0168 ± 0.0013 | 4.00 | 3.00 | (100) | a |
| +I | 0.0188 ± 0.0005 | 4.48 | 3.48 | 116 | b |
| +P | 0.0187 ± 0.0008 | 4.45 | 3.45 | 115 | c |
| +I+P | 0.0298 ± 0.0015 | 7.10 | 6.10 | 205 | d |
| Diabetic | | | | | |
| | 0.0181 ± 0.0004 | 4.30 | 3.30 | 110 | e |
| +I | 0.0188 ± 0.0004 | 4.48 | 3.48 | 116 | f |
| +P | 0.0178 ± 0.0002 | 4.24 | 3.24 | 108 | g |
| +I+P | 0.0257 ± 0.0008 | 6.12 | 5.12 | 172 | h |

[a] Significant differences ($N = 6$). $p < 0.01$ (a,d), (b,d), (c,d), (e,h), (f,h), (g,h). No other combinations show significant differences. Extracellular concentration of 2-deoxyglucose: 1.18 n*M*. Expected intracellular concentration based on Table III: 1.00 n*M*. Other calculations and conditions are as described in Table I.

content of hepatocytes is ~16% of the wet weight, near the value reported for whole liver (16). The usual hexose uptake experiments were conducted after the steady-state rate of respiration had been established.

**Uptake of High Concentrations of Glucose by Hepatocytes of Normal Rats**—The data in Fig. 1 and Table III indicate a carrier-mediated facilitated mode of transport for glucose as expected with most mammalian cells. The intracellular concentration of glucose reaches an equilibrium value near the extracellular concentration within 1.0 min. The initial velocity of transport is too great to be estimated by the procedure used; hence, calculation of a $K_M$ for glucose transport is impossible, although it is known to be in the millimolar range for other cell types (3).

**Uptake of a Low Concentration of Glucose**—Table I summarizes the uptake of glucose by hepatocytes from both normal and diabetic rats in the presence and absence of insulin and dinoprostone. Several features were evident:

1. The uptake exceeded that expected of a facilitated diffusion transport. Instead of a ratio of near 1.0 (0.885 in Table III), ratios between 2.5 and 3.5 were obtained for the intracellular–extracellular distribution.
2. Uptake was greatly reduced in hepatocytes from diabetic animals under all conditions. In terms of percent binding, untreated cells from

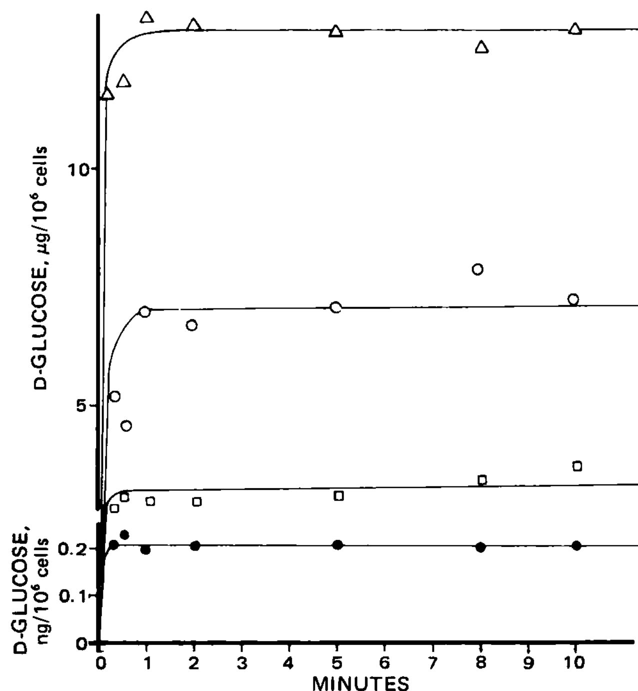


**Figure 1**—*Uptake of D-glucose by normal rat hepatocytes. Glucose concentrations key: (●) 71 nM, (□) 5 mM, (○) 10 mM, and (△) 20 mM. Note that the scale used for the low concentration differs from the scale for the three high concentrations.*

**Table III—Uptake of High Concentrations of Glucose by Normal Hepatocytes** [a]

| Extracellular Concentration, mM | Uptake, nmoles/$10^6$ cells | Intracellular Concentration, mM | Concentration Ratio, Intracell/Extracell |
|---|---|---|---|
| 5.0 | 18 | 4.3 | 0.87 |
| 10.0 | 39 | 9.3 | 0.93 |
| 19.9 | 72 | 17.1 | 0.86 |
| | | Mean | 0.885 |

[a] Uptake was estimated from the levels shown in Fig. 1. Intracellular concentration was based on cell volume estimated from a mean cell diameter of 20 μm, which gave a volume of 4.2 μl/$10^6$ cells. All values for uptake have been corrected for an estimated 38% inclusion of extracellular fluid in the packed cell pellet (see text).

diabetic rats were found to bind only 58% as much glucose as their normal counterparts.

3. Insulin significantly increased (but not to the normal value) the uptake by the cells from diabetic animals, but had no effect on hepatocytes from normal rats.

4. Dinoprostone tended to depress glucose uptake in both normal hepatocytes and in insulin-treated cells from diabetic rats.

**Uptake of a Low Concentration of 2-Deoxyglucose**—As in the case of glucose, the uptake of 2-deoxyglucose considerably exceeded the uptake expected from facilitated diffusion and gave even higher intracellular–extracellular distribution ratios (Table II). However, there are several striking differences between the glucose and 2-deoxyglucose uptake patterns.

1. There was no difference in the uptake of 2-deoxyglucose with or without insulin when cells from normal and diabetic animals were compared.

2. Dinoprostone had no depressing effect on 2-deoxyglucose uptake.

3. The only significant effect of any treatment was the marked increase in uptake and apparent binding when either cell type was exposed to a combination of insulin and dinoprostone.

## DISCUSSION

A previous report (2) showed that insulin had no effect on the uptake of sugars by enzymatically dispersed normal hepatocytes. The effect of insulin in the present study was rendered evident by two factors: first, the use of hepatocytes from diabetic rats, which had a depressed glucose uptake responsive to insulin, in contrast to hepatocytes from normal rats which may already have been maximally stimulated; and second, the use of an extremely low level of glucose, much less than the physiological concentrations (~5 m$M$) usually employed in such experiments.

The excess uptake detected by the use of a very low glucose concentration probably reflects some change in the glucose binding sites in the cell. The possibility that this excess uptake is attributable to an active transport system cannot be ruled out absolutely, although it appears unlikely because of the speed of equilibration which appears within 15 sec (Fig. 1). Assuming that the excess does indeed represent glucose binding, the question remains as to what relationship this binding has to the transport mechanism and whether it indicates some change in a transport carrier protein.

The most striking difference between the excess uptake of a low level of glucose and an even lower level of 2-deoxyglucose was the lack of effect of diabetes and insulin on the 2-deoxyglucose uptake. This may indicate that different sites and/or some differential change in relative affinities of the same sites are involved in the binding of the two sugars. In either case, it appeared that the insulin effect is specific for glucose.

In conclusion insulin increases the uptake of glucose by hepatocytes from diabetic rats and not by those from normal rats, possibly because the normal cells are already maximally stimulated as is evident at low concentrations (<100 n$M$) of glucose. The insulin effect is specific for glucose since the hormone does not enhance binding of 2-deoxy-D-glucose. The results suggest that insulin promotes formation of hexose binding sites and conversion of these to specific glucose binding sites, while dinoprostone may act by blocking the latter conversion.

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