The Effect of Insulin on the Distribution of Glucose between the Blood Plasma and the Liver

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Abstract In normal fasted rats whole liver tissue contains as much glucose as the blood plasma, i.e., the ratio of the concentrations is about unity. The concentration of glucose in hepatic intracellular water is about 1.2 times higher than in plasma water. In rats injected with insulin the concentration of glucose in the liver falls to a lesser extent than in the plasma: resulting in a ratio of concentrations higher than unity. If insulin hypoglycemia is prevented by the ingestion of glucose the concentration ratio is less than in hypoglycemic rats but still significantly above unity. In normal rats the specific activities of plasma and hepatic glucose do not differ significantly at 7.5, 15, and 30 minutes after the intravenous injection of C14-labeled glucose. In rats injected with insulin the specific activity of glucose is higher in the plasma than in the liver at 7.5 and 15 minutes, but not at 30 minutes following the injection of tracer. In insulin-treated hypoglycemic rats considerably higher concentrations of labeled glucose are found in hepatic intracellular than in plasma water. The penetration of C14-glucose from plasma into hepatic intracellular water is found to be fast. Excess insulin causes an accumulation of glucose within the liver cells by retaining newly formed glucose and by the taking up of glucose from the plasma against an existing concentration gradient.

When determined by the dilution of injected C14-glucose, the apparent volume through which glucose seems to be distributed in dogs was found to increase following the injection of infusion of insulin (1, 2). This volume, V, was defined as the ratio N/C of the amount (N) of rapidly intermixing C14-glucose in the body to the concentration (C) of glucose in the plasma.

An increase of V can be the result of either or both of two processes: (a) the volume available for glucose to penetrate into increases whether the added volume (ΔV) contains glucose or not, that is whether N is changed or not. Or (b) gradients in the concentration may arise between different sites of the volume of fluid in which rapidly mixing free glucose is dissolved. As a result of
this the concentration of glucose in the plasma may be less than in other parts of this fluid.

Although there is some evidence mainly from in vitro experiments (3, 4) that insulin may increase the penetration of glucose into muscle tissue and even may cause its accumulation therein, the second possibility also merits investigation (5), and we are reporting the results of a study of it.

As the free glucose in liver tissue has been shown to mix relatively rapidly with plasma glucose (6), hepatic glucose is presumably part of the "glucose pool." In rats it has been demonstrated (7) that the concentration of glucose in whole liver tissue is equal to its concentration in the blood plasma; i.e., their ratio is near unity. If insulin decreases the concentration of glucose in plasma more than in the liver without cutting hepatic glucose off from the rest of the glucose pool, the increase of V might be explained. Such a mechanism, of course, supposes that the liver possesses an active transport mechanism for glucose which is activated by insulin, as it is in muscle tissue (3, 4), and also that glucose can be transported into the hepatic cells at a faster rate than it can be phosphorylated therein. To test this hypothesis the changes in the concentration of glucose were followed in the plasma, in whole liver tissue, and in hepatic intracellular water after the administration of insulin and of two other hormones. The rapidity of the intermixing of hepatic and plasma glucose was tested in experiments utilizing glucose uniformly labeled with C\textsubscript{14}.

**METHODS AND EXPERIMENTAL DESIGN**

The experiments were performed on inbred male rats weighing 155 to 247 gm. The rats were anesthetized by the intraperitoneal injection of nembutal (about 30 mg/kg) and 30 minutes later, through a midline abdominal incision, samples of liver were taken for the determination of free glucose, dry weight, and the sodium content. Blood was withdrawn from the heart, collected in a heparinized tube, and kept in ice cold water till it was centrifuged.

The water content of the individual liver samples was determined by weighing immediately after taking and after 20 hours' drying at 120°C. The water content of plasma was taken as 93 per cent (8). Extracellular and intracellular water were determined according to Manery and Hastings (9) using sodium rather than chloride as an extracellular constituent of liver tissue. This was justified as in the liver the size of the sodium space equals that of the chloride space (9). In our experiments the calculated average amount of intracellular water in the liver of eighty-five rats was 0.628 kg/kg cells compared with Manery's 0.646 kg/kg cells based on the chloride concentrations in liver and plasma of eight rats.

Glucose in the liver samples was determined by Appelboom's method "C" (10), which has been shown to give true glucose values. Three or four pieces of liver tissue (altogether about 80 to 150 mg) were dropped within 2 seconds from the time they had been excised into a 40 ml glass centrifuge tube containing 10 ml of hot distilled water. No pieces of liver were taken from already cut surfaces. The tubes were kept in boiling water for at least 45 minutes. Determination of hepatic glucose concentra-
tion in each rat was carried out in duplicate tubes, each of which yielded two further parallels in the procedure for the determination of glucose by the method of Nelson (12). For the homogenization of the boiled samples an all-glass homogenizer was used, and the homogenates were weighed in tared beakers with an accuracy of 1 mg. Deproteinization was carried out as described by Appelboom and at least 2 hours were allowed to elapse before the tubes were centrifuged. All glucose determinations were made on the day of the experiment, the balance of the supernatants being kept deep-frozen till the specific activities of glucose had been determined. It is realized that the rapidity with which the liver pieces are transferred into the boiling water is highly critical. Experiments pertinent to this problem are described in an Appendix to this paper.

Glucose in plasma was determined according to Somogyi (11) as modified by Nelson (12). The amount of intracellular glucose was calculated as the difference between the amount of glucose in whole liver tissue and that in the extracellular phase. The assumption that the concentration of glucose in plasma water is equal to its concentration in extracellular tissue was made.

Sodium in liver samples was determined by adding weighed amounts of liver tissue to 10 ml N/10 HNO₃ (13). One day later the samples were crushed and left in the acid for another day. After this the acid was neutralized by a few drops of concentrated NH₄OH thus precipitating a considerable amount of proteins. The samples were centrifuged and after the addition of a measured amount of lithium, their sodium content was determined by internal standard flame photometry. Plasma sodium was determined similarly using standard procedures.

In some experiments labeled glucose was injected intravenously at stated intervals (T) before the samples were taken. One μc corresponding to 1 mg of glucose was injected in all rats.

The specific activities of plasma glucose and of the glucose in whole liver tissue were determined from deproteinized plasma and liver homogenate by preparing glucosazones and plating them after transcrytallization (2). Counting was performed in an automatic windowless flow counter.

Specific activities were expressed as counts per minute per milligram glucose-carbon, and were corrected for body weight. The specific activity of hepatic intracellular glucose was calculated by the following formula:

\[ N_t \cdot (SA)_t = N_e \cdot (SA)_e + N_i \cdot (SA)_i \]

where \( N_t \) is the amount of glucose in total liver tissue, \( N_e \) in the extracellular phase, and \( N_i \) in the intracellular phase of liver tissue. \( (SA)_t \) is the specific activity of glucose in total liver, extracellular and intracellular respectively. Since \( (SA)_i \) is the only unknown it can be calculated. The product \( N \cdot (SA) \) measures the amount of labeled glucose in any phase to which \( N \) and \( (SA) \) are referred. If \( N' \) refers to the water content of the phase, \( N' \cdot (SA) \) gives the amount of labeled glucose in the respective water compartment. Thus \( N'_t \cdot (SA)_t \) gives the amount of labeled glucose in hepatic intracellular water, if \( N'_t \) is the amount of glucose in unit volume of intracellular water.

**Experimental Design**

In the first series of experiments (Table I) the rats were divided into seven groups according to the treatments received. Group 1 contained control rats fasted for 20
### TABLE I

**CONCENTRATIONS OF GLUCOSE IN THE BLOOD PLASMA AND LIVER IN CONTROL AND TREATED RATS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>m mole glucose/kg plasma</th>
<th>m mole glucose/kg plasma water</th>
<th>m mole glucose/kg whole liver tissue</th>
<th>m mole glucose/kg cell water</th>
<th>Whole liver Plasma</th>
<th>Glucose in cell H₂O</th>
<th>Glucose in plasma H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control fasted</td>
<td>21</td>
<td>4.65±0.26</td>
<td>4.98±0.27</td>
<td>4.66±0.27</td>
<td>5.30±0.35</td>
<td>1.03±0.04</td>
<td>1.21±0.06</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control not fasted</td>
<td>9</td>
<td>8.14±0.43</td>
<td>8.75±0.46</td>
<td>7.07±0.45</td>
<td>9.70±0.40</td>
<td>0.87±0.03*</td>
<td>1.10±0.03</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Insulin &lt;1 u/kg</td>
<td>12</td>
<td>1.64±0.29</td>
<td>1.76±0.31</td>
<td>2.59±0.27</td>
<td>3.65±0.35</td>
<td>2.00±0.33‡</td>
<td>2.75±0.54‡</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Insulin &gt;2 u/kg</td>
<td>10</td>
<td>1.02±0.20</td>
<td>1.10±0.22</td>
<td>2.70±0.42</td>
<td>4.02±0.72</td>
<td>2.83±0.21†</td>
<td>4.04±0.35†</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Chronic insulin Tr.</td>
<td>5</td>
<td>1.34±0.20</td>
<td>1.44±0.22</td>
<td>3.17±0.28</td>
<td>4.76±0.47</td>
<td>2.53±0.33‡</td>
<td>3.41±0.49‡</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Adrenaline 0.5 to 1.0 mg/kg</td>
<td>4</td>
<td>20.94±0.85</td>
<td>22.52±0.92</td>
<td>18.03±0.68</td>
<td>28.08±1.10</td>
<td>0.86±0.01</td>
<td>1.25±0.02</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Glucagon 2.5 mg/kg</td>
<td>6</td>
<td>10.56±0.74</td>
<td>11.34±0.79</td>
<td>12.62±0.20</td>
<td>17.50±1.15</td>
<td>1.21±0.08*</td>
<td>1.57±0.15†</td>
<td></td>
</tr>
</tbody>
</table>

All insulin injections were given intraperitoneally, except four animals in group 4. Adrenaline and glucagon were injected subcutaneously. The last three groups were not fasted prior to the experiment. Figures following the ± sign are standard errors of the mean.

* Significantly different from group 1 at the 5 per cent level. † Significantly different from group 1 at the 1 per cent level.
TABLE II
CONCENTRATION OF GLUCOSE IN THE BLOOD PLASMA
AND THE LIVER IN CONTROL RATS AND RATS INJECTED
WITH INSULIN INTRAPERITONEALLY

All the rats received 4.5 gm/kg glucose per os 40 minutes prior to the collection of samples.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>m mole glucose kg plasma</th>
<th>m mole glucose kg plasma water</th>
<th>m mole glucose kg whole liver tissue</th>
<th>m mole glucose kg cell water</th>
<th>Whole liver glucose</th>
<th>Glucose in cell H₂O</th>
<th>Glucose in plasma H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>9</td>
<td>11.58±1.75</td>
<td>12.44±1.88</td>
<td>9.90±0.91</td>
<td>12.17±1.02</td>
<td>0.92±0.06</td>
<td>1.08±0.11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Insulin 3 to 6 u/kg</td>
<td>9</td>
<td>7.22±1.00</td>
<td>7.78±1.07</td>
<td>8.69±0.50</td>
<td>11.16±0.84</td>
<td>1.32±0.12</td>
<td>1.61±0.18†</td>
<td></td>
</tr>
</tbody>
</table>

Insulin was injected intraperitoneally. Figures following the ± sign are standard errors of the mean.
* Significantly different from group 1 at the 5 per cent level. † Significantly different from group 1 at the 1 per cent level.
hours prior to the experiment, receiving no treatment whatsoever. Group 2 was also made up of untreated rats, but these were not fasted prior to the experiment. Groups 3 and 4 were composed of fasted rats injected with a smaller (less than 1 u/kg) and a larger dose (more than 2 u/kg) of insulin (Insulin Toronto) respectively 30 to 32 minutes before they were sacrificed. All injections in group 3 were intraperitoneal, in group 4 four out of ten rats received a high dose of 8 to 12 u/kg insulin intravenously, while the remaining six animals received 2.5 to 3 u/kg intraperitoneally.

![Figure 1](https://jgp.rupress.org/)

**Figure 1.** Specific activity of glucose in the blood plasma, whole liver tissue, and hepatic intracellular water in control fasted rats, after different lengths of time for the intermixing of tracer glucose have been allowed. Ordinate, specific activity of glucose (cpm/mg glucose-carbon 10^{-3}). Abscissa, intermixing time (T) in minutes. n, number of experiments at any given T. Mean values are shown.

A separate group of five animals (group 5) was treated with insulin for 4 months, receiving daily 3 u NPH insulin in the morning and 5 u PZI in the late afternoon. A diet rich in carbohydrates was fed to this group ad libitum. The last injection of insulin was given 1 to 2 hours prior to the experiment and food was withdrawn at this time. Group 6 had been injected subcutaneously with 0.5 to 1.0 mg/kg adrenaline (Parke-Davis) suspended in oil, 30 minutes before the liver samples were taken. Group 7 comprised six rats injected subcutaneously with 2.5 mg/kg glucagon (Connaught) suspended in 0.9 per cent NaCl solution, 30 minutes before being sacrificed. The rats of groups 5, 6, and 7 were not fasted.

In the second series (Table II) two groups of rats have been compared: both re-
ceived 1 ml 45 per cent glucose solution/100 gm of body weight by gastric tube 10 minutes before the injection of nembutal. The first group served as controls, the second received 3 to 6 u/kg insulin intraperitoneally 30 minutes prior to the time of taking the samples.

Statistical evaluation of differences was made by the t-test (14).

![Figure 2](https://jgp.rupress.org/)

**Figure 2.** Specific activity of glucose in the blood plasma, whole liver tissue, and hepatic intracellular water in fasted rats injected with insulin (1 to 2.5 u/kg) after different lengths of time for the intermixing of tracer glucose have been allowed. Ordinate, specific activity of glucose (cpm/mg glucose-carbon10^-3). Abscissa, intermixing time (T) in minutes. n, number of experiments at any given T. Mean values are shown.

**RESULTS**

Table I shows the concentrations of glucose in millimoles per kilo of the blood plasma, plasma water, whole liver tissue, and in hepatic intracellular water. The second last column gives the average ratios of glucose concentrations in whole liver tissue over plasma. The last one gives the ratio of the concentration of glucose in hepatic intracellular water over plasma water.

It is evident that in normal fasting rats whole liver tissue contains glucose in about the same concentration as the plasma. Calculated, however, on the basis of water content the concentration of glucose in the water of liver cells is
about 20 per cent higher than in plasma water. In non-fasting rats the ratios of whole liver to plasma glucose concentrations seem to be slightly smaller. The difference is, however, of no statistical significance when the concentrations are calculated in the respective amounts of cell and plasma water.

Insulin seems to decrease the concentration of glucose in the plasma to a greater extent than in the liver. This was found in rats given a single dose of insulin as well as in animals receiving prolonged treatment with the hormone.

**Figure 3.** The ratio of the specific activities of hepatic intracellular glucose over plasma glucose in control and insulin-treated fasted rats, after different lengths of time for the intermixing of tracer glucose have been allowed. Ordinate, ratio of specific activities. Abscissa, intermixing time (T) in minutes. Each point is the average of three to five experiments. Mean values and standard errors are shown. Dose of insulin, 1 to 2.5 u/kg.

Adrenaline did not significantly alter the ratio of the concentrations of glucose in hepatic cellular and plasma water. Glucagon caused a statistically significant ($P < 0.01$) increase in the ratio of glucose in cell water/plasma water. The increase in the ratio of whole liver tissue to plasma glucose was less significant ($P < 0.05$).

To explore the rate of hypoglycemia per se in the response to insulin, rats received 1 ml/100 gm of 45 per cent glucose solution by gastric tube 10 minutes prior to the injection of nembutal. The data in Table II indicate that most but
not all the effect of insulin is abolished if hypoglycemia is prevented. The ratio of the glucose concentrations in hepatic cell water and in plasma water differs significantly from unity. The same ratios in group 1 of Table I and group 1 of Table II do not differ significantly. There is a significant difference, however, between these ratios in group 1 (Table I) and group 2 of Table II ($P < 0.01$).

![Graph showing ratio of concentrations of labeled glucose in hepatic cell water and plasma-water](image)

**Figure 4.** The ratio in the concentration of labeled glucose in plasma-water and hepatic intracellular water in control and insulin-treated fasted rats after different lengths of time for the intermixing of tracer glucose have been allowed. Ordinate, ratio of the concentrations of labeled glucose in hepatic cell water and liver water. Abscissa, intermixing time ($T$) in minutes. Each point is the average of three to five experiments. Mean values and standard errors are shown. Dose of insulin, 1 to 2.5 u/kg.

Mean specific activities of plasma, whole liver, and intracellular liver glucose are presented in Fig. 1 for control and in Fig. 2 for insulin-treated animals, after $T = 7.5, 15,$ and $30$ minutes. The specific activities of glucose in plasma and in liver do not differ significantly at any of the times in control animals. The specific activities of glucose in plasma are significantly higher than in the liver at $T = 7.5$ minutes ($P < 0.05$), 15 minutes ($P < 0.05$), and 30 minutes ($P < 0.02$) in the animals treated with insulin.

The specific activity of glucose in liver is significantly less in insulin-treated than it is in control rats at $T = 7.5$ and 15 minutes ($P < 0.05$) but not at $T = 30$ minutes.
Fig. 3 shows the average ratios of the specific activities of hepatic intracellular glucose to plasma glucose in control and insulin-treated animals. In control rats this ratio is 0.81, 0.96, and 0.86 at $T = 7.5$, 15, and 30 minutes respectively; in the treated rats the ratios after the same intermixing times are 0.48, 0.66, and 0.63. The difference between the two groups is significant at $T = 7.5$ minutes ($P < 0.05$), at the borderline of significance at $T = 15$ minutes ($P \simeq 0.05$), and not significant at $T = 30$ minutes. The averages of the ratios of plasma over liver glucose specific activities differ significantly from unity in insulin-treated but not in control rats.

The ratio of the concentrations of labeled glucose in hepatic intracellular water over plasma water of control and insulin-treated rats is given in Fig. 4. It is evident that in normal rats this ratio is close to one even after 7.5 minutes of intermixing time. In insulin-treated rats, however, liver cell water contains more labeled glucose than does plasma water. The differences are significant at $T = 15$ ($P < 0.02$) and 30 minutes ($P < 0.05$) but not at $T = 7.5$ minutes.

**DISCUSSION**

**A. Concentration of Free Glucose in Liver Tissue**

In the fasting rat the concentration of glucose in whole liver tissue was found to be equal to the concentration in plasma by Gey (7). Essentially similar results were obtained by Cahill et al. (6) at least in the normal range of blood sugar levels. Our results correspond with theirs, although three different techniques for the determination of hepatic glucose have been employed. It seems that a ratio of one for whole liver glucose/plasma glucose concentration is characteristic of the fasting rat. Consistently higher ratios have been described for other species, such as dogs (10, 15), rabbits (16, 17), and guinea pigs (16, 17). However, the ratio of glucose in cell water/plasma water is slightly, but significantly higher than one. The validity of this statement rests on the assumptions that all the intracellular water is available for the distribution of glucose and all glucose within the cells is freely soluble in water.

In animals treated with large doses of adrenaline the ratio of the glucose concentrations in liver cell water and plasma water does not differ significantly from that in control rats. Thus the breakdown of glycogen, manifesting itself in considerably elevated plasma glucose levels, is in itself insufficient to raise this ratio. This would mean, that the amount of glucose originating from the breakdown of glycogen does not accumulate in the liver cells but diffuses out rapidly. The slight but significantly higher ratio found in glucagon-treated rats may be the result of the presence of insulin in the sample of glucagon injected. The small differences found in the ratios between fed and fasted rats are not significant, indicating that the penetration of parenterally administered sugar into the liver cell water is rapid.
It is of some importance to refer glucose concentration in tissues to intracellular water because the ratio of concentration in whole liver/plasma may in some instances be misleading. The relatively low value of this ratio in hyperglycemic animals may be due to a “masking” effect of a relatively large amount of extracellular glucose in the whole tissue relative to the glucose within the cells.

B. The Specific Activity vs. Time Curve of Hepatic and Plasma Glucose
Since the \( SA \) vs. time curves in Fig. 1 do not intersect at any point although in these animals the amount of rapidly intermixing glucose was in or close to the dynamic steady state, it may be concluded that neither a catenated nor a mammillary two compartment system would correctly depict the exchanges of plasma glucose and hepatic glucose. It seems that the exchange of glucose through the hepatic cell membrane is so rapid that when an intermixing time of about 7.5 minutes has been allowed, hepatic and plasma glucose behave as a single “pool.” This is reflected also in the apparently linear relationship between the logarithm of the \( SA \) of plasma glucose and intermixing time in these experiments, and further in the finding that hepatic intracellular water and plasma water contain labeled glucose in equal concentrations even after 7.5 minutes’ intermixing (Fig. 4). A similar length of time (6 to 9 minutes) to reach a linear relationship of log \( SA \) of plasma glucose to intermixing time of tracer was reported in dogs (18).

In insulin-treated rats (Fig. 2) the amount of rapidly mixing glucose was definitely not in the dynamic steady state and valid conclusions regarding compartmentalization cannot be drawn (19). Comparing, however, Fig. 2 with Fig. 1 certain conclusions are permissible. It seems that in rats with insulin-induced hypoglycemia the complete mixing of hepatic and plasma glucose is slower. The reason for this is a rapid new formation of hepatic glucose. This is indicated by the low specific activity of hepatic glucose as compared to the \( SA \) of plasma glucose in these animals at \( T = 7.5 \) and even to some extent when \( T = 15 \) minutes (see also Fig. 3). A breakdown of glycogen (20) and an increase of glucose output from the liver (2) were described in insulin hypoglycemia. It seems that in insulin-treated rats \( N \) does not behave as if in a single compartment unless a definitely longer (15 minutes or more) time is allowed for mixing of tracer with the glucose to be traced.

Fig. 3 indicates that there is a small difference in the specific activity of plasma and hepatic intracellular glucose. In control rats, assumed to be in the dynamic steady state our data seem to indicate that about 80 to 90 per cent of hepatic glucose exchanges completely with plasma glucose.

C. The Effect of Insulin
In insulin-treated rats hepatic glucose, in spite of its higher concentrations, is not compartmentalized but intermixes freely with plasma glucose. The data
presented in Fig. 4 demonstrate, that in control animals even after \( T = 7.5 \) minutes, the concentration of labeled glucose is the same in plasma and in intracellular hepatic water. After insulin significantly more labeled glucose is maintained in unit volume of hepatic cell water than in plasma water if 15 minutes or more are allowed after the injection of tracer. This increased penetration of labeled glucose is against a concentration gradient, as in insulin-treated rats more glucose was found to be in hepatic intracellular water than in plasma water. It seems therefore, that the liver has the ability, at least under the effect of an excess of insulin, to transport glucose actively through the cell membrane. The design of the reported experiments does not permit the calculation of the rate of this transport of glucose. Neither do the experiments supply information about the biochemical mechanism of this active (i.e. energy-requiring) process. They demonstrate, however, that in insulin-treated rats more labeled glucose was taken up by the cells from the plasma than was released or metabolized by them. The existence of such an active transport supports the hypothesis of Levine et al. according to which insulin increases the rate of transport of glucose into the cells (21). The accumulation of glucose in the liver, as compared with some other organs, may be explained by the unusually low hexokinase activity in liver tissue (22). Our findings, however, indicate one more factor: the relatively low specific activity of hepatic glucose in insulin-treated animals shows that the glucose entering from the plasma is diluted by a larger amount of endogenous glucose in the liver, and thus the glucose which is in excess in the hepatic cells of insulin-treated animals, originates partly from intracellular sources. This leads to the conclusion that glucose whether taken up from the plasma or formed within the liver cells is, at least temporarily, retained intracellularly under the action of insulin. A part of these effects appears to be a result of hypoglycemia rather than a specific effect of insulin, since Table II shows that in the absence of hypoglycemia the effect of insulin is smaller. To assess the relative importance of low plasma glucose level in the response described further experiments are needed.

D. Conclusions Affecting the Measurement of Accumulation and Rates of Transfer of Glucose by Methods Applying Labeled Glucose as Tracer

The demonstration that gradients in the concentration may occur in the fluid in which rapidly mixing glucose is dissolved, deprives the concept of the “glucose space” or the more cautious “apparent distribution volume of glucose” of any anatomical interpretation, because this would postulate a uniform concentration of glucose throughout this volume (5). Moreover, if such gradients should arise during an experiment as a result of some procedure, the constancy of the operationally defined (23) apparent glucose volume cannot be assumed. This in turn affects the validity or at least the accuracy of those methods of measuring transfer rates of glucose, which depend on the assumption of the
constancy of this apparent space. On the other hand the described experiments explain the mechanism, at least partially of our earlier finding (12) in normal dogs in which insulin increased the apparent distribution volume \( V \) of glucose. It can be calculated that an effect on the liver of the magnitude shown in group 3 of Table I could be in itself adequate to raise \( V \) to about 45 to 50 per cent of body weight.

It therefore seems advisable to use the concept of glucose pool more cautiously. The concept of a “metabolic pool” assumes uniform intermixing; i.e., the same specific activity of the constituent molecules (24). In the case of glucose this is not absolutely true as is evident from Fig. 3. More important, however, is the finding that the time necessary for the intermixing of tracer glucose in the body may be different under different experimental circumstances, at least in the species we have studied. This may lead to erroneous conclusions unless adequate precautions are taken.

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APPENDIX

To obtain quantitative information about the effect of the length of the time span between the withdrawal of the liver sample and its immersion into hot water on the concentration of glucose in the liver, four experiments were carried out on two fasted and two fed rats. Glucose uniformly labeled with C\(^{14}\) was injected intravenously 20 minutes before the rats were sacrificed. Samples were excised from the liver as described and transferred into hot water (a) as fast as possible, i.e. 1 to 2 seconds after excision, (b) after holding them in a pair of forceps for 5 seconds after excision, (c) holding them for 15 seconds. After all samples had been taken as much blood was withdrawn from the heart as was possible. The concentrations of glucose in whole liver tissue and plasma as well as their specific activities are shown in Fig. 5. It appears, that in fed rats the concentration of glucose in freshly excised liver increases approximately linearly with time at a rate of about 0.29 m mole/kg/sec. In fasted animals there was a somewhat larger increase between samples (a) and (b), but no further increase was found between the samples delayed for 5 and 15 seconds. These experiments, however, need further confirmation.

Assuming that sample (a) was taken 1 second prior to the immersion into hot water, a straight line function was fitted to the natural logarithms of the specific activities of hepatic glucose vs. the time which elapsed between the withdrawal and the immersion of samples. The rate constants were calculated \(-0.019\) and \(-0.059\) per second for the fasted, and \(-0.029\) and \(-0.020\) per second for the fed rats. These observations seem to indicate that in normal fed rats each second, from the 1st to the 5th, between the excision and the immersion of the sample increases the
concentration of hepatic glucose by about 3 to 4 per cent, in fasted rats a similar delay causes an error of about 6 per cent. The relative changes in the specific activity of hepatic glucose are given by the rate constants and are in three out of four experiments about minus 2 per cent per second.

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