Potential intercellular futile cycling of carbohydrates in diabetes

Nancy W. KLECKNER, Zenro KIZAKI and Ronald G. THURMAN*
Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, U.S.A.

INTRODUCTION

Data have accumulated over the past decade demonstrating that cells in different regions of the hepatic lobule do not contain similar amounts and activities of many key enzymes (Rapaport, 1976; Jungermann & Katz, 1982). Using micro-dissection techniques, Guder and co-workers (Guder & Schmidt, 1976) demonstrated quantitative differences in many enzyme activities in tissues from periportal and pericentral zones of the liver lobule. Major enzymes involved in gluconeogenesis are localized preferentially in periportal regions of the lobule (Babcock & Candell, 1974; Sasse et al., 1975), whereas many glycolytic enzymes predominate in pericentral areas (Sasse et al., 1975; Hildebrand, 1980). On the basis of these differences, Jungermann and his colleagues suggested that gluconeogenesis is localized in periportal regions and that glycolysis predominates in pericentral areas of the liver lobule (Jungermann et al., 1977; Jungermann & Katz, 1982). In spite of this information, flow of carbohydrate in the gluconeogenic pathway in intact cells located in periportal and pericentral regions cannot be deduced solely from differences in maximal activity of a few enzymes. Substrate concentrations and rates of cofactor supply, as well as local concentrations of intracellular modulators, could also be important. Therefore methods were developed to measure glucose synthesis in specific regions of the liver lobule mentally (Matsumura et al., 1984; Thurman & Kauffman, 1985; Lemasters et al., 1986). To determine metabolic flux rates in different regions of the liver lobule, miniature O₂ electrodes have been used to measure changes in rates of O₂ uptake in periportal and pericentral regions when substrate (e.g. lactate) was infused into livers from starved rats, perfused in the anterograde direction (Matsumura & Thurman, 1983, 1984; Matsumura et al., 1984). Glucose production was calculated from the extra O₂ uptake measured with miniature O₂ electrodes and the known stoichiometry between O₂ uptake and glucose production from lactate. In livers from normal rats, glucose was synthesized predominantly in periportal regions of the liver lobule; however, glucose was produced exclusively in periportal regions in livers from diabetic rats. In pericentral regions, O₂ uptake increased slightly in livers from normal rats, but declined significantly by 10 µmol/h per g in livers from diabetic rats. These data are consistent with the hypothesis that gluconeogenesis from lactate occurs exclusively in periportal regions of the liver lobule in livers from diabetic rats. A portion of this glucose is metabolized back to lactate in pericentral areas, leading to increased rates of glycolytic ATP production, thereby decreasing the demands for O₂. This production of glucose from lactate in periportal regions, followed by conversion of glucose back into lactate in pericentral areas, raises the possibility of intercellular futile cycling, stimulated by diabetes.

* To whom reprint requests should be addressed.

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Table 1. Estimation of fluid volume of perfused livers from normal and diabetic rats

Values are means ± S.D. for the numbers of rats shown in parentheses, treated as described in the legend to Fig. 2. Livers from rats that were treated with vehicle or were made diabetic were perfused with Krebs–Henseleit bicarbonate buffer. The flow was stopped, followed by the ligation of portal vein. The liver was then detached and weighed (weight of liver with fluid). The ligatures on the portal vein were removed and the liver was blotted dry and reweighed (wet weight). The dry weight of the liver was obtained after 48 h of drying (70 °C). The fluid content (intra- and extraacellular) was calculated by the formula (wt. of liver with fluid − liver dry wt.)/liver wet wt.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat wt. (g)</th>
<th>With fluid</th>
<th>Wet</th>
<th>Dry</th>
<th>Wet/dry</th>
<th>Fluid−dry wet</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (5)</td>
<td>181 ± 12</td>
<td>9.1 ± 0.8</td>
<td>8.1 ± 0.8</td>
<td>2.1 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>0.87 ± 0.03</td>
</tr>
<tr>
<td>Diabetes (6)</td>
<td>148 ± 14</td>
<td>8.8 ± 1.2</td>
<td>7.8 ± 1.1</td>
<td>2.0 ± 0.3</td>
<td>3.9 ± 0.1</td>
<td>0.87 ± 0.02</td>
</tr>
</tbody>
</table>

the anterograde direction (Kinugasa & Thurman, 1986). Interestingly, the lobular site of action of both hormones was switched rapidly to cells in the opposite end of the lobule when the direction of perfusion was reversed (Matsumura et al., 1984; Kinugasa & Thurman, 1986).

Diabetes is characterized by high glucagon/insulin ratio and high glucose concentrations in blood. Glucose production from lactate and other gluconeogenic precursors has been reported to be higher in diabetics than in hepatocytes (Wagle & Ingebritson, 1975), and activities of some gluconeogenic enzymes are higher in livers from diabetic rats (Wagle, 1964; Prinz & Seubert, 1964; Lardy et al., 1965). These previous studies, however, did not provide direct information on the lobular distribution of gluconeogenesis in diabetes. The purpose of the present study was therefore to determine the effect of experimental diabetes on sublobular compartmentation of gluconeogenesis in the isolated perfused rat liver. Local rates of oxygen uptake were measured before, during and after lactate infusion by using a miniature $O_2$ electrode and the stopped-flow $O_2$-uptake technique (Matsumura & Thurman, 1983). The data indicate that gluconeogenesis from lactate occurs exclusively in periportal regions of the liver lobule in livers from diabetic rats. Moreover, the data are consistent with the hypothesis that newly synthesized glucose was recovered into lactate in pericentral regions only in livers from diabetic rats. Preliminary accounts of this work have appeared elsewhere (Kleckner et al., 1986).

MATERIALS AND METHODS

Animals and liver perfusion

Female Sprague–Dawley rats were maintained on lab chow ad libitum. They received sodium phenobarbital in drinking water (1 mg/ml) for 1–4 weeks before perfusion experiments, to enhance pigmentation differences between periportal and pericentral regions of the liver lobule (Ji et al., 1980; Lemasters et al., 1986).

Diabetes was produced by intravenous injection of alloxan monohydrate (120 mg/kg) in saline after starvation for 24 h. Blood samples were taken 48 h after injection and were analysed for glucose by standard enzymic procedures (Bergmeyer, 1974). Rats with blood glucose concentrations greater than 250 mg/dl were considered diabetic. At 2 days after injection of alloxan or vehicle, normal and diabetic rats were starved for 24 h before liver perfusion. All rats used in stopped-flow $O_2$-uptake experiments were injected intraperitoneally with galactosamine (1.5 g/kg) 3 h before perfusion to inhibit glycogen synthesis (Decker & Keppler, 1972). All livers from rats treated in this manner took up $O_2$ at similar rates to controls, and did not release lactate dehydrogenase or take up Trypan Blue, indicating that galactosamine treatment was not hepatotoxic under these conditions. Livers were perfused with Krebs–Henseleit (1932) bicarbonate buffer (pH 7.4, 37 °C) saturated with $O_2/CO_2$ (19:1) in a non-recirculating system. Perfusate was pumped into the liver through the portal vein and was collected via a cannula inserted into the inferior vena cava. Effluent perfusate was channeled past a Teflon-shielded Clark-type platinum $O_2$ electrode and through a sampling well before being discarded. Rates of $O_2$ uptake by the whole organ were calculated from the difference between influent and effluent $O_2$ concentration, liver wet weight and perfusate flow rate.

Sodium lactate in perfusate (pH 7.4) was infused into the liver at a final concentration of 2 mm. Samples of effluent perfusate were collected and analysed for glucose, lactate and ketone bodies enzymically (Bergmeyer, 1974). Rates of metabolite production were calculated from influent minus effluent perfusate metabolite concentrations, the flow rate and the liver wet weight.

Measurement of rates of $O_2$ uptake in periportal and pericentral regions of the liver lobule

A miniature $O_2$ electrode was constructed as described by Matsumura & Thurman (1983) from 50 μm-thick platinum wire pulled under heat in a glass capillary. The electrode was coated with an acrylic ester polymer to form an $O_2$-permeable membrane and was used with standard polarization circuitry with an Ag/AgCl reference electrode. The electrode was used to measure $O_2$ partial pressure in various regions of the liver lobule by placing it on periportal (light spots) or pericentral (dark areas) regions of the liver lobule on the liver surface (Lemasters et al., 1986). $O_2$ uptake by hepatocytes under the electrode was measured by stopping influent and effluent perfusate flow simultaneously (stopped-flow $O_2$-uptake technique). $O_2$ uptake was determined from the rate of decrease in $O_2$ concentration per unit time measured by the electrode (d$O_2$/dt) and the fluid content
Fig. 1. Rates of glucose production and $O_2$ uptake by perfused livers from normal and alloxan-diabetic rats

All rats received phenobarbital in drinking water (1 g/l) from 1 to 4 weeks before injection with alloxan (120 mg/kg). Rats were treated 3 days later with galactosamine (1.5 g/kg) 3 h before liver perfusion, to inhibit glycogen synthesis (right panels), or with saline vehicle (left panels). Infusion of lactate is indicated by horizontal bars and arrows. Results of typical experiments are shown.

per g of liver, which was 0.87 ml/g under stopped-flow conditions in livers from both normal and diabetic rats (Table 1).

RESULTS

Effect of lactate on rates of glucose production and $O_2$ uptake by perfused livers from normal and diabetic rats

Fig. 1 shows rates of glucose production and $O_2$ uptake in perfused livers from normal (upper panels) and diabetic (lower panels) rats which had been pretreated with galactosamine (right panels) or vehicle (left panels). In the absence of galactosamine pretreatment, basal rates of $O_2$ uptake were similar in livers from control and diabetic rats. However, glucose output was very high (> 60 µmol/h per g) initially in livers from diabetic rats and declined steadily, before reaching a steady rate of around 25 µmol/h per g after 40 min of perfusion. This elevated rate of glucose output is presumably due to the high rate of glycolysis present in livers from alloxan-diabetic rats (Exton et al., 1972). In contrast, glucose output in livers from normal rats was less than 10 µmol/h per g and was constant for up to 60 min of perfusion. When lactate was infused, both glucose production and oxygen uptake increased rapidly in livers from untreated and diabetic rats. When glucose synthesis from lactate was inhibited with 3-mercaptopicolinic acid (250 µM), an inhibitor of phosphoenolpyruvate carboxykinase (DiTullio et al., 1974), $O_2$ uptake in all groups studied was negligible.

Galactosamine is a potent inhibitor of glycogen synthesis, which causes glycogen concentrations to decline precipitously (Decker & Keppler, 1972). After galactosamine pretreatment for 3 h, basal rates of glucose output in livers from both normal and diabetic rats were about 10 µmol/h per g (Fig. 1, right panels). Thus galactosamine treatment prevented glucose production from glycogen, but had no effect on basal rates of $O_2$ uptake under these conditions. Therefore all subsequent experiments in this study were performed with galactosamine-treated rats.

Glucose synthesis and $O_2$ uptake were measured in livers from normal and diabetic rats when lactate was increased in steps from 0.1 to 2.0 mM (Fig. 2). Stepwise increase in glucose production and $O_2$ uptake were observed in both groups, which returned to basal values when lactate infusion was terminated (Fig. 2). Similar titrations in livers from diabetic rats produced stepwise increase in glucose and $O_2$ uptake; however, maximal rates of glucose production were about 50% lower than in livers from normal rats.

Basal rates of glucose output by the whole organ were comparable (Fig. 2, Table 2) in livers from normal and diabetic rats in this study after treatment with galactosamine (< 10 µmol/h per g). When lactate was infused, however, rates of glucose output were significantly higher in livers from control (39 µmol/h per g) than from diabetic rats (25 µmol/h per g; Table 2). After lactate infusion was terminated, rates of glucose output returned to basal values in both groups.

When increases in glucose production and $O_2$ uptake were compared, excellent correlations ($r = 0.94$) were observed in both normal and diabetic livers (Fig. 3). However, the stoichiometry between glucose production...
Fig. 2. Titration of gluconeogenesis and $O_2$ uptake with lactate in perfused livers from normal (a) and diabetic (b) rats

Phenobarbital-treated rats were starved for 24 h before experiments and were injected with galactosamine (1.5 g/kg) 3 h before perfusion. Alloxan-diabetes was induced in group b as described in the Materials and methods section. Livers were perfused in a non-recirculating system in the anterograde direction, and lactate was infused at the concentrations indicated in the horizontal bar (lower panel) at times denoted by arrows with a precision infusion pump. Upper panel: production of glucose. Middle panel: $O_2$ uptake. Rates were calculated from the influent-effluent concentration differences and the constant flow rate. Results of typical experiments are shown.

Table 2. Effect of lactate on gluconeogenesis and $O_2$ uptake in periportal and pericentral regions of the liver lobule in livers from normal and diabetic rats

Values are means $\pm$ S.D. for livers from six normal and five diabetic rats. Periportal and pericentral $O_2$ uptakes were calculated from the decrease in $O_2$ concentration when a miniature $O_2$ electrode was placed on the surface of the liver in the presence or absence of 2 mM-lactate and the flow was stopped. Whole-organ $O_2$ uptake was calculated from influent and effluent perfusate $O_2$ concentrations. Glucose output was determined enzymically in effluent perfusate samples and rates were calculated from the flow rate and liver wet wt. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for comparison with group directly above in the same column; †$P < 0.01$ for comparison with treated group in normal rats.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Addition</th>
<th>Periportal</th>
<th>Pericentral</th>
<th>Whole organ</th>
<th>Glucose output (umol/h per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>None</td>
<td>136 $\pm$ 22</td>
<td>56 $\pm$ 11</td>
<td>120 $\pm$ 9</td>
<td>5.7 $\pm$ 1.2</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>183 $\pm$ 34**</td>
<td>67 $\pm$ 7**</td>
<td>158 $\pm$ 17***</td>
<td>38.9 $\pm$ 6.2***</td>
</tr>
<tr>
<td></td>
<td>Lactate off</td>
<td>131 $\pm$ 16**</td>
<td>55 $\pm$ 14*</td>
<td>114 $\pm$ 10***</td>
<td>8.5 $\pm$ 1.0***</td>
</tr>
<tr>
<td>Diabetic</td>
<td>None</td>
<td>162 $\pm$ 21</td>
<td>73 $\pm$ 9</td>
<td>133 $\pm$ 16</td>
<td>6.9 $\pm$ 3.8</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>207 $\pm$ 23*</td>
<td>63 $\pm$ 11**</td>
<td>157 $\pm$ 14***</td>
<td>24.7 $\pm$ 6.1****†</td>
</tr>
<tr>
<td></td>
<td>Lactate off</td>
<td>157 $\pm$ 35**</td>
<td>72 $\pm$ 12*</td>
<td>122 $\pm$ 13***</td>
<td>5.2 $\pm$ 3.0***</td>
</tr>
</tbody>
</table>

and $O_2$ uptake was around 1.0 in livers from control rats, but was near 0.5 in livers from diabetic rats (Fig. 3). For an interpretation of this phenomenon, see below (the Discussion section).

Effect of lactate on $O_2$ uptake in periportal and pericentral regions of the liver lobule

Experiments to determine local rates of $O_2$ uptake were performed before, during and after infusion of lactate into perfused livers, as depicted by the hatched areas in Fig. 4. $O_2$ concentration was higher in periportal regions than in pericentral regions in livers from both normal and diabetic rats as a result of the natural $O_2$ gradient in the liver (Fig. 5). Experimental diabetes did not alter local $O_2$ concentrations. When perfusate flow through the liver was stopped, $O_2$ concentration decreased rapidly in both regions of the liver lobule; however, the rate of decrease was 2–3 times greater in periportal than in pericentral regions in livers from both groups of rats (Table 2). Subsequently, rates of $O_2$ uptake were calculated from the rates of change in $O_2$ concentration and the fluid content of the liver. Basal rates of $O_2$ uptake in periportal regions were not significantly different in livers from diabetic and normal rats. Basal rates of $O_2$ uptake were 2–3-fold higher in periportal than in pericentral regions in livers from both normal and diabetic rats, in agreement with previous studies (Matsumura et al., 1984; Kinugasa & Thurman, 1986). Rates of $O_2$ uptake increased after lactate infusion in periportal regions in livers from both normal and diabetic rats (Table 2). In pericentral regions, basal rates of $O_2$ uptake were also similar in livers from normal and diabetic rats. Lactate increased the rate of $O_2$ uptake significantly in pericentral regions in livers from normal rats by about 10 umol/h per g; however, it decreased $O_2$ uptake in livers from diabetic rats significantly by
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Fig. 3. Relationship between O₂ uptake and glucose production in livers from normal (○) and diabetic (▲) rats

Conditions were as in Fig. 2. The best-fit linear-regression line from data from five livers perfused at various concentrations (0.1–2.0 mM) of lactate is shown. The slope for data from normal liver was 1.03 ± 0.09, and that for data from diabetic liver was 0.48 ± 0.05 (mean ± S.E.M.): r = 0.94 for both.

10 μmol/h per g (Table 2). After lactate infusion was terminated, rates of O₂ uptake and glucose production returned to near-basal values in both regions of the lobule in livers from both normal and diabetic rats (Table 2).

Lactate uptake and ketone-body (β-hydroxybutyrate and acetoacetate) production were monitored also in livers from normal and diabetic rats. Lactate uptake increased as [lactate] was increased in steps in these experiments (Table 3). Maximal rates of lactate uptake were about 2-fold higher after lactate infusion (2 mM) in livers from normal than from diabetic rats. Under these conditions, rates of ketone-body production were similar in livers from both groups studied (Table 3).

DISCUSSION

Glucose synthesis in livers from galactosamine-treated rats

The lactate-induced increase in O₂ uptake observed in periportal plus pericentral regions of the normal galactosamine-treated liver can account for the glucose produced by the whole organ. In livers where endogenous long-chain fatty acids are a primary fuel, 5.4 mol of ATP is produced per mol of O₂ consumed, instead of 6 if all substrates were linked to NADH (5.4/6.0 = 0.9; Thurman & Scholz, 1977). Therefore, the theoretical ratio of 0.9 mol of glucose produced by gluconeogenesis per mol of O₂ consumed, and the fact that about 50% of the liver lobule is portal and about 50% is central (Lemasters et al., 1986), can be used to compare local rates of glucose synthesis with measurements of glucose production by the whole organ. For example, rates of glucose synthesis by periportal regions of 47 μmol/h per g, plus 11 μmol/h per g by pericentral areas, is nearly equivalent to whole-organ rates of 33 μmol/h per g (47/2 + 11/2 = 29 μmol/h per g). The extra O₂ consumed owing to lactate infusion in the diabetic liver was also not far from predicted values.

In livers from galactosamine-treated control rats, the increase in O₂ uptake owing to addition of lactate occurs predominantly in periportal regions of the liver lobule (Table 2), confirming previous work with untreated rats from this laboratory (Matsumura et al., 1984). A small increase in O₂ uptake also occurred in pericentral regions in livers from normal rats, consistent with previous work.
Table 3. Ketone-body production and lactate uptake in livers from normal and diabetic rats

<table>
<thead>
<tr>
<th>Lactate (mM)</th>
<th>Production of β-hydroxybutyrate + acetocetate (μmol/h per g)</th>
<th>Lactate uptake (μmol/h per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Diabetic</td>
</tr>
<tr>
<td>0</td>
<td>16 ± 6</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>0.1</td>
<td>16 ± 6</td>
<td>13 ± 6</td>
</tr>
<tr>
<td>0.5</td>
<td>19 ± 6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>1.0</td>
<td>24 ± 6</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>2.0</td>
<td>35 ± 4</td>
<td>26 ± 3*</td>
</tr>
</tbody>
</table>

(Table 3). Thus galactosamine does not appear to alter intercellular compartmentation of glucose synthesis in livers from normal rats. In livers from diabetic rats, lactate addition increased O₂ uptake to the same extent as in livers from normal rats, but only in perportal regions. Since no extra O₂ for glucose synthesis was observed in pericentral areas, it is concluded that glucose is synthesized from lactate exclusively in periporal regions of the liver lobule in diabetes. In pericentral regions, marked differences were observed between livers from normal and diabetic rats. O₂ uptake increased in livers from normal rats, but decreased in livers from diabetic rats when lactate was infused (Table 2). How can these differences be explained?

Glucose synthesis, as measured with miniature O₂ electrodes, was identical in periporal regions in livers from normal and diabetic rats (Table 2); however, lactate uptake and glucose output were markedly lower in diabetics (Tables 2 and 3). Since O₂ uptake in pericentral regions declines in livers from diabetic rats, it is concluded that some of the glucose produced in periporal regions is metabolized in pericentral regions to lactate. Metabolism of glucose to lactate would produce 2 mol of ATP per mol of glucose metabolized to lactate, thereby decreasing the O₂ requirements in pericentral regions and leading to the observed decrease in O₂ uptake in the diabetic liver (Table 2). The extra lactate produced from metabolism of glucose most likely accounts for the apparent lower rates of lactate uptake observed in the diabetic liver (Table 3).

Indeed, Katz & McGarry (1984) hypothesized that blood glucose is taken up by hepatocytes in periporal regions of the liver and metabolized via glycolysis to lactate, which then circulates via the blood back to periporal regions where it can be converted via gluconeogenesis into glucose and ultimately into glycogen. In support of this idea, Matsumura & Thurman (1984) showed that changes in O₂ partial pressure correlated with the kinetics of glucose production after infusion of lactate, and concluded that glycolysis occurred predominantly in periporal regions of the liver lobule. If the ‘glucose paradox’ hypothesis is correct, part of the glucose produced in periporal regions would not appear in the effluent perfusate in our experiments, since it was metabolized in pericentral regions. Indeed, output of glucose was about 50% less in livers from diabetic than normal rats (Table 2). Therefore, it is concluded that glucose produced in periporal areas is converted into lactate in livers from diabetic rats, data consistent with the glucose paradox hypothesis.

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The data from this study, supporting the hypothesis that glucose is synthesized from lactate in periporal regions, yet newly formed glucose is converted into lactate in pericentral areas in diabetes, is convincing. Why has this intercellular metabolism been overlooked in previous studies? The answer may be in the choice of experimental models. In previous work with isolated hepatocytes, glucose output was greater in cells from diabetic rats than in those from control rats (Wagle & Ingebritsen, 1975; Lardy et al., 1965). Hepatocytes are mixtures of periporal and pericentral cells. Because of the mixing of cell types from various regions, specialized functions which occur in one region of the liver lobule may have been overlooked in previous work. More recent work has demonstrated higher rates of glucose synthesis in hepatocytes isolated from periporal regions than from pericentral regions (Quistoff & et al., 1985); however, studies with cells isolated from specific regions of the liver lobule from diabetic rats have not yet been performed. Insight into this problem comes from the observation that specific pathways of carbohydrate metabolism follow the natural O₂ gradient in the liver. Gluconeogenesis predominates in periporal regions during perfusions in the anterograde direction, yet shifts rapidly to pericentral areas when the direction of flow (i.e. the area receiving high O₂ partial pressure) is changed (Matsumura et al., 1984). Similarly, glycogenolysis predominates in pericentral regions of the liver lobule when flow is in the normal anterograde direction, yet predominates in periporal regions when the O₂ gradient is reversed (Matsumura & Thurman, 1983). Thus the functions of glucose synthesis or breakdown appear to be defined in some unknown manner by processes which correlate with the O₂ gradient across the liver lobule. Since hepatocytes are incubated at similar O₂ partial pressure, all cells may act similarly and intercellular metabolism may not be observed.

The concept of intercellular futile cycling was presented by Katz & McGarry (1984) as the ‘glucose paradox’. Evidence for this interesting idea, however, has been scant. Why the diabetic liver carries out this energy-wasteful futile cycling of carbohydrates is unclear. It is possible that a homeostatic mechanism dependent on glucagon or glucagon/insulin ratio has gone awry. In support of this concept, Kinugasa & Thurman (1986) observed that glucagon stimulated gluconeogenesis exclusively in periporal regions of the liver lobule in livers from normal rats. In perfusions in the retrograde direction, the effect of glucagon was shifted to pericentral regions. Thus the effect of glucagon on gluconeogenesis also followed the O₂ gradient in livers from normal rats. Gluconeogenesis may occur exclusively in periporal regions of the liver lobule in livers from diabetic rats because of the high glucagon: insulin ratio characteristic.
of the diabetic state. The chronic effect of this high ratio may also explain why glucose is metabolized to lactate in pericentral regions in diabetes.

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REFERENCES


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