Abnormalities of hepatic carbohydrate metabolism in diabetic animals have been repeatedly described. We have previously reported that the presence of diabetes, following either alloxan administration or partial pancreatectomy, results, in rat liver slices, in a diminished glucose uptake, an almost completely inhibited glycogen deposition from glucose, and in a markedly increased formation of glucose from pyruvate, from glycogen, from fructose, and from unknown precursors (1, 2).

The primary site of the metabolic defect in diabetes is not known, though much evidence points to the initial steps of glucose utilization. Cori, working mainly with muscle, showed that decreased glucose phosphorylation was a major metabolic lesion resulting from insulin deficiency (3). Levine et al. suggested that insulin increases the permeability of the muscle cell surface to glucose (4), and Park added further support to this concept (5). Chernick and Chaikoff postulated a "block" between glucose and glucose-6-phosphate in liver slices from alloxan-diabetic rats (6) and recent studies from this laboratory have confirmed and extended their findings (2). The comparative utilization and metabolic fate of glucose and fructose, present together in the medium and alternately labeled with C\textsuperscript{14}, enabled us to obtain a quantitative estimation of the system transforming extracellular free glucose into intracellular phosphorylated glucose. Liver slices from diabetic rats phosphorylated glucose at one-tenth to one-fourth the normal rate.

When insulin is administered \textit{in vivo}, metabolic effects on the isolated liver can be demonstrated. Thus the administration of insulin to diabetic rats for 1 to 4 days before sacrifice has been shown to restore the measured
diabetic metabolic abnormalities to normal (6–8). Knowledge of the biochemical sequence of events during this correction of diabetic metabolism by insulin might add information on the primary site or sites of insulin action. We have attempted to study this biochemical sequence of events within the limits of the measurements available to us.

Insulin was administered to diabetic rats for varying lengths of time before sacrifice; slices of their livers were incubated with labeled glucose, fructose, pyruvate, and acetate and analyzed. In addition, the diaphragms of these animals were incubated with labeled glucose and analyzed. It should be noted that, as on previous occasions, all incubations were carried out in a system designed to preserve a normal intracellular cationic environment (9).

Materials and Methods

Animals—Male albino rats of the Wistar strain, raised in our own colony, weighing between 250 and 350 gm. were used. They were fed ad libitum unless otherwise noted. The method employed in producing alloxan diabetes, as well as the criteria used to establish the presence of diabetes, has been previously described (1).

Medium and Substrates—Unless otherwise noted, the media used contained, in millimoles per liter, K⁺ = 110, Mg⁺⁺ = 20, Ca⁺⁺ = 10, HCO₃⁻ = 40, Cl⁻ = 130; in the flasks containing acetate or pyruvate, Cl⁻ = 90 and CH₃COO⁻ = 40 or CH₃COO⁻ = 40. For the incubation of diaphragm, K⁺ = 110 was replaced by Na⁺ = 110 mmoles per liter. The solutions were equilibrated with 5 per cent CO₂-95 per cent O₂, giving a pH in the presence of liver slices varying between 7.4 and 7.5. C¹⁴-labeled glucose¹ and fructose¹ were uniformly labeled in all carbons. Pyruvate¹ was labeled in the α position and acetate¹ was carboxyl-labeled. Each flask contained one substrate only, glucose and fructose in the concentration of 20 mmoles per liter and pyruvate and acetate in the concentration of 40 mmoles per liter.

Insulin—Glucagon-free insulin, specially prepared by the Nordisk Insulin Laboratorium and containing 26 units per mg., was used whenever insulin was added in vitro or given intravenously and subeutaneously within 3 hours of sacrifice. Squibb commercial protamine zinc insulin was used whenever a long acting preparation was needed.

Insulin Administration—In order to insure an immediate high and relatively constant insulin level in the diabetic animals from the time of injec-

¹ Uniformly labeled glucose and fructose were obtained from the Nuclear Instrument and Chemical Corporation, Chicago, Illinois. α-Carbon-labeled pyruvate and carboxyl-labeled acetate were prepared by Dr. Manfred Karnovsky of this laboratory.
tion to the time of sacrifice, even though these periods varied from 10 minutes to 48 hours, the following schedules of injection were adopted. Insulin administration was always begun by the intravenous injection of 10 units of crystalline, glucagon-free insulin. When the animals were sacrificed 10 minutes or 1 hour later, no other insulin was given. When they were to be sacrificed 6 hours after the beginning of insulin administration, an additional 5 units of glucagon-free crystalline insulin were administered subcutaneously 2 hours before sacrifice. When they were to be sacrificed after 24 or 48 hours of insulin action, 5 units of protamine zinc insulin were injected subcutaneously, together with the initial intravenous injection of crystalline insulin, and the injection of protamine zinc insulin was repeated every 12 hours; in addition 5 units of glucagon-free crystalline insulin were injected subcutaneously 2 hours before death.

Methods—Chemical methods and isotopic analyses used were similar to those previously described (1, 2, 9, 10). Fatty acids were isolated and prepared for isotopic analysis by the following modification of established procedures: The liver slices were subjected to alkaline hydrolysis and saponified with potassium hydroxide in 60 per cent ethanol, then extracted with petrol ether. The water-soluble residue was acidified to Congo red with hydrochloric acid, then reextracted with petrol ether. The petrol ether extract was washed with water until neutral, then evaporated to dryness in a boiling water bath. The residue was dissolved in alcohol, evaporated, redissolved in acetone-petroleum ether, and transferred to tared flasks, then evaporated and dried in vacuo over calcium chloride, potassium hydroxide, and paraffin. The final residue was weighed and a portion transferred to planchets, weighed, and counted. The average molecular weight of the fatty acids thus obtained was determined by titration and was found to correspond to an average of 12 carbon atoms per molecule.

Calculations—All calculations were identical with, or similar to, those previously described (1, 2, 9). From these we obtained glycogen synthesis from glucose, fructose, pyruvate, and acetate; CO₂ formation from glucose, fructose, pyruvate, and acetate; fructose disappearance from the medium and pyruvate disappearance from the medium. Also obtained were glucose uptake (1), glucose output (1), and total glucose phosphorylation (2); glucose formation from fructose, pyruvate, and acetate; incorporation of carbon from glucose, fructose, pyruvate, and acetate into fatty acids.

Results

Many attempts to demonstrate an effect of insulin on hepatic carbohydrate metabolism in vitro have been made in this laboratory. Although
it was thought in one series of experiments (11) that insulin, in vitro, had a
demonstrable effect on glucose uptake and glucose output, an extension of
the series has failed to confirm this preliminary conclusion. Indeed, as of
the present time, we have been unable to establish a consistent, repro-
ducible effect of insulin in vitro, and therefore no detailed account of these
experiments will be given. In brief, insulin has been added in concentra-
tions of up to 1 unit per ml. and, to insure good initial penetration of the
hormone, whole liver and single liver lobes were on occasion perfused with
insulin solutions before slicing. Measurements made included uptake of
glucose, fructose, and pyruvate, as well as glycogen deposition and CO₂
production from these substrates, total glucose output, glucose production
from fructose and pyruvate, and total glucose phosphorylation (2). In-

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Animals</th>
<th>Total glucose phosphorylation (per cent of normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Normal</td>
<td>100 ± 6.2</td>
</tr>
<tr>
<td>7</td>
<td>Diabetic</td>
<td>6 ± 1.8</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>7 ± 2.1</td>
</tr>
<tr>
<td>5</td>
<td>Diabetic, given insulin intravenously and subcutaneously for 24 to 72 hrs. before death</td>
<td>87 ± 16.3</td>
</tr>
</tbody>
</table>

sulin was added to liver slices from normal, from both mildly and severely
diabetic, and from adrenalectomized, hypophysectomized, and diabetic
hypophysectomized rats. In no instance was a consistently positive effect
obtained. Effects of Insulin Administered in Vivo Experiments were first per-
formed to confirm the return towards normal of diabetic, hepatic, carbo-
ydrate metabolism after 48 hours of insulin administration and to es-

Effects of the latter type can easily be shown in mus-
cle (12), whereas Chernick and Chaikoff had noted that subcutaneous
insulin administration to three diabetic rats for 1 to 3 hours before sacrifice
did not restore glucose oxidation to CO₂ or glucose incorporation into fatty
acids to normal (7). In our experiments, no early insulin effects on he-
patic metabolism could be found, and the data concerning total glucose
phosphorylation (2), the most sensitive index of glucose utilization avail-
able in our system, are summarized in Table I. It is evident that hepatic
glucose phosphorylation was markedly decreased in diabetic animals, and furthermore that 24 to 72 hours of insulin administration restored glucose phosphorylation to near normal activity. The intravenous administration of 5 to 10 units of insulin 10 to 70 minutes before sacrifice, however, did not affect the rate of glucose phosphorylation, even though this dose was sufficient to produce a drop in blood glucose from a mean of 447 mg. per cent to a mean of 231 mg. per cent between the time of insulin ad-

**Table II**

**Glycogen Deposition from Various Substrates by Rat Liver Slices and by Rat Diaphragm at Varying Time Intervals after Insulin Administration**

All values expressed as micromoles of glucose equivalents per gm. of wet liver per 90 minutes.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animals</th>
<th>Duration of insulin administration</th>
<th>Liver glycogen from</th>
<th>Muscle glycogen from glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min.</td>
<td>Glucose</td>
<td>Fructose</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>0</td>
<td>22.2</td>
<td>36.2</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0</td>
<td>17.4</td>
<td>27.4</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0</td>
<td>14.0</td>
<td>24.0</td>
</tr>
<tr>
<td>4</td>
<td>Diabetic</td>
<td>0</td>
<td>0.1</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0</td>
<td>0.1</td>
<td>1.4</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0</td>
<td>0.1</td>
<td>3.8</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>10</td>
<td>0.1</td>
<td>5.7</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>10</td>
<td>0.1</td>
<td>5.0</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>1</td>
<td>0.2</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>1</td>
<td>0.2</td>
<td>4.9</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>1</td>
<td>0.2</td>
<td>6.7</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>6</td>
<td>0.5</td>
<td>3.3</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>6</td>
<td>0.4</td>
<td>8.1</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>24</td>
<td>12.5</td>
<td>12.7</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>48</td>
<td>23.4</td>
<td>15.0</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>48</td>
<td>28.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

ministration and sacrifice. The further addition of insulin in vitro after slicing was without effect. It might further be noted that, similarly, no early insulin effects on liver slices from five normal animals could be demonstrated.

A more complete survey of the sequence of insulin effects in time seemed indicated and sixteen experiments were carried out. The results obtained are shown in Tables II, III, and IV. Some comments are warranted.

As shown in Table II, significant amounts of glycogen were synthesized from glucose, fructose, and pyruvate, but not from acetate. The presence of diabetes resulted in a markedly decreased hepatic glycogen synthesis
from all substrates, glycogen synthesis from glucose, however, being affected to a much more striking degree. The administration of insulin was without effect on glycogen synthesis from glucose up to 1 hour after insulin administration, but showed minimal effects after 6 hours, and restored glycogen synthesis to normal between 24 and 48 hours after the first insulin injection. The ratio, glycogen from glucose to glycogen from fructose, was much less than unity in the diabetic rat and remained so up to 24 hours after insulin administration, when it attained unity; it exceeded unity after. The same is true for the ratio of glycogen from glucose to glycogen from pyruvate, although to a different degree. The glycogen synthesis by muscle will be discussed later.

The presence of diabetes also resulted in a markedly decreased CO$_2$ production from glucose, whereas CO$_2$ production from fructose, pyruvate, and acetate remained unaffected (Table III). The administration of insulin was without effect up to 1 hour, showed minimal effects after 6 hours, and marked effects 48 hours after the beginning of insulin injection. As further shown in Table III, there were striking changes in the incorpora-

**Table III**

_Incorporation of Carbon from Various Substrates into CO$_2$ and Fatty Acids by Liver Slices at Varying Time Intervals after Insulin Administration_

All values expressed as micromoles of carbon per gm. of wet liver per 90 minutes.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animals</th>
<th>Duration of insulin administration</th>
<th>CO$_2$ from</th>
<th>Fatty acid carbon from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glucose</td>
<td>Fructose</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>min.</td>
<td>51</td>
<td>119</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>0</td>
<td>58</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>0</td>
<td>38</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>Diabetic</td>
<td></td>
<td>6</td>
<td>137</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>0</td>
<td>9</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>1</td>
<td>4</td>
<td>87</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>10</td>
<td>6</td>
<td>79</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>hrs.</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>1</td>
<td>14</td>
<td>63</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>1</td>
<td>7</td>
<td>107</td>
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<td>12</td>
<td>&quot;</td>
<td>6</td>
<td>23</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>6</td>
<td>11</td>
<td>95</td>
</tr>
<tr>
<td>14</td>
<td>&quot;</td>
<td>24</td>
<td>100</td>
<td>174</td>
</tr>
<tr>
<td>15</td>
<td>&quot;</td>
<td>48</td>
<td>87</td>
<td>97</td>
</tr>
<tr>
<td>16</td>
<td>&quot;</td>
<td>48</td>
<td>92</td>
<td>101</td>
</tr>
</tbody>
</table>
tion of substrate carbon into fatty acids. Diabetes resulted in a marked decrease of fatty acid synthesis from all sources, although the synthesis from glucose was decreased to a particularly marked degree. No significant insulin effect could be detected before 24 hours after the beginning of insulin administration. After 48 hours fatty acid synthesis from all substrates was not only normal but considerably more active than normal.

**Table IV**

Fructose Utilization, Total Glucose Phosphorylation, and Glucose Formation from Various Substrates by Rat Liver Slices at Varying Time Intervals after Insulin Administration

All values expressed as micromoles of fructose or glucose per gm. of wet liver per 90 minutes.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animals</th>
<th>Duration of insulin administration (min.)</th>
<th>Fructose utilized</th>
<th>Glucose phosphorylation Max.</th>
<th>Glucose from</th>
<th>Fructose</th>
<th>Pyruvate</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>0</td>
<td>144</td>
<td>89</td>
<td>56</td>
<td>72</td>
<td>55</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>0</td>
<td>139</td>
<td>88</td>
<td>53</td>
<td>70</td>
<td>57</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>0</td>
<td>138</td>
<td>80</td>
<td>37</td>
<td>59</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Diabetic</td>
<td>0</td>
<td>108</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>77</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>0</td>
<td>100</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>66</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>0</td>
<td>112</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>61</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>10</td>
<td>113</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>99</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>10</td>
<td>138</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>82</td>
<td>35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animals</th>
<th>Duration of insulin administration (hrs.)</th>
<th>Fructose utilized</th>
<th>Glucose phosphorylation Max.</th>
<th>Glucose from</th>
<th>Fructose</th>
<th>Pyruvate</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>&quot;</td>
<td>1</td>
<td>110</td>
<td>11</td>
<td>7</td>
<td>9</td>
<td>75</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>&quot;</td>
<td>1</td>
<td>119</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>65</td>
<td>35</td>
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<td>11</td>
<td>&quot;</td>
<td>1</td>
<td>136</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>63</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>6</td>
<td>145</td>
<td>22</td>
<td>16</td>
<td>19</td>
<td>102</td>
<td>34</td>
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<td>13</td>
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<td>6</td>
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<td>7</td>
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<td>&quot;</td>
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<td>115</td>
<td>178</td>
<td>141</td>
<td>160</td>
<td>76</td>
<td>9</td>
</tr>
<tr>
<td>16</td>
<td>&quot;</td>
<td>48</td>
<td>122</td>
<td>149</td>
<td>111</td>
<td>130</td>
<td>69</td>
<td>4</td>
</tr>
</tbody>
</table>

this being particularly true for fatty acid synthesis from pyruvate and from glucose.

The data concerning hexose balance are shown in Table IV. Fructose utilization proceeded at approximately the same rate throughout. Total glucose phosphorylation, on the other hand, was almost negligible in the diabetic liver and was but gradually restored after insulin administration. Glucose synthesis from pyruvate was considerably more active in diabetic than in normal liver. It is of particular interest to note that this excessive gluconeogenesis from pyruvate was still present 24 hours after the beginning.
of insulin administration and only reached normal activity or better between 24 and 48 hours after the first insulin injection.

In summary then, the administration of insulin to the diabetic animals resulted in an almost complete return to normal of all abnormal measurements within 24 to 48 hours. Indeed, some measurements (total glucose phosphorylation, CO₂ production from glucose, glucose synthesis from pyruvate, and fatty acid synthesis from all substrates) were overcorrected, thus indicating some insulin overdosage. The most striking observation, however, concerned the shorter periods of insulin administration. No insulin effects of any kind on hepatic carbohydrate metabolism were apparent at 10 minutes and 1 hour after the intravenous injection of insulin (although at 10 minutes the blood glucose level had already fallen from a mean of 435 mg. per cent to a mean of 357 mg. per cent, and at 1 hour from a mean of 426 mg. per cent to a mean of 217 mg. per cent). 6 hours after the beginning of insulin administration, there were suggestive but quantitatively small effects on glycogen synthesis from glucose, on CO₂ formation from glucose, and on total glucose phosphorylation. Glucose formation from pyruvate was still in the diabetic range 24 hours after the beginning of insulin administration.

The sequence of events in hepatic tissue after insulin administration was in marked contrast to the situation in striated muscle. Stadie et al. (12, 13) had previously shown that marked insulin effects result from exposure of diaphragm to insulin for a few minutes and Bleehen and Fisher (14) have demonstrated insulin effects on the perfused rat heart within 15 minutes of the addition of insulin to the system. Similarly, when the diaphragms of those animals, in which the hepatic metabolic changes have been described, were removed and incubated, maximal insulin effects were demonstrated within 10 to 60 minutes of insulin administration. The measurements of glycogen synthesis from glucose are shown in Table II. It is evident that glycogen synthesis from glucose was only moderately decreased in the diabetic muscle, and that insulin administration produced a marked increase in glycogen synthesis over and above normal values. Insulin effects were particularly marked 10 minutes and 1 hour after insulin administration; they were not apparent after 24 to 48 hours.

DISCUSSION

The effects of insulin on isolated liver and muscle, with respect to time, have been illustrated by three curves (Fig. 1).

Curve A represents the effect of insulin on the synthesis of glycogen from glucose by diaphragm. It is apparent that normal levels of glycogen synthesis from glucose are reached within minutes after the first administration of insulin, and that maximal insulin effects are reached within 10 to 60 minutes.
Curve B demonstrates the effect of insulin on total glucose phosphorylation by liver slices. This effect of insulin becomes apparent only gradually, normal values being reached between 6 and 24 hours and maximal values at 24 and 48 hours after the beginning of insulin administration. Similarly plotted curves representing hepatic glycogen synthesis from glucose and CO₂ formation from glucose follow the same general pattern.

**Fig. 1.** Biochemical sequence of events after insulin administration to diabetic rats. Curve A, values expressed in micromoles of glucose equivalents per gm. of liver per 90 minutes. The dotted line represents the mean normal value. *Glyc.* = glycogen. Curve B, values expressed in micromoles of glucose per gm. of liver per 90 minutes. *G-6-P* = glucose-6-phosphate. Curve C, values expressed in micromoles of carbon per gm. of liver per 90 minutes. *F. A.* = fatty acids.

Curve C shows the effect of insulin on the synthesis of fatty acids from pyruvate. It would seem that the effect of insulin on fatty acid synthesis reaches its maximum somewhat later than the effect of insulin on total glucose phosphorylation. It is of interest to note that in adipose tissue the marked increase in lipogenesis from glucose which follows insulin administration (15) is similarly preceded by an increased glycogen deposition (16). The latter effect was shown to be, at least in part, a direct, local effect of the hormone on adipose tissue.

The most striking observation illustrated by these curves is the complete lack of early insulin effects on hepatic tissue, as opposed to the early and dramatic response of muscle. While this observation only applies to the metabolic functions measured, these included the utilization of four differ-
ent substrates, glucose, fructose, pyruvate, and acetate, their incorpora-
tion into glycogen, glucose, and fatty acids, and their oxidation to CO₂.
Furthermore, it is difficult to question the adequacy of the insulin dosage
used, in view of the marked effect obtained on both blood glucose levels
and diaphragm. Three interpretations may be considered: (a) The
metabolic functions measured may not have detected the primary site of
insulin action on hepatic tissue. While this interpretation cannot be dis-
carded, there is no positive evidence to support it. (b) Insulin was de-
stroyed by hepatic tissue and therefore could not affect its metabolism.
It is difficult to explain, however, why the destruction of insulin should be
effective for a limited period of time only. (c) Insulin exerted immediate
metabolic effects on the periphery only, mainly on muscle and perhaps
adipose tissue (15, 16), while its effects on hepatic metabolism were slow
and gradual, representing either a different type of hormonal action or a
metabolic adaptation to the new demands created by the peripheral
changes. This last interpretation seems to us the most likely one, and a
brief discussion in the light of previous work may be warranted.

Convincing evidence of direct insulin effects on liver is scarce. Bouc-
kaert and de Duve (17), however, measured in dogs the amount of glucose
which had to be infused intravenously in order to maintain a constant blood
glucose level in the presence of “maximal” insulin stimulation. After
hepatectomy this glucose requirement decreased by about two-thirds.
This was interpreted as indicating that about two-thirds of the total insulin
effect on glucose disappearance was due to increased hepatic glucose up-
take. This quite generally accepted interpretation was recently ques-
tioned by Lang, Goldstein, and Levine (18), who demonstrated that,
under the conditions of Bouckaert and de Duve’s experiments, hepatec-
tomy is followed by decreased peripheral glucose uptake, and that it is not
necessary to assume a direct hepatic insulin effect.

Bloch and Kramer (19), and later Brady and Gurin (20), as well as
Haugaard and Stadie (21), have reported that insulin in vitro increases the
synthesis of fatty acids from labeled acetate by liver slices from normal
rats. However, no such insulin effect on liver slices from diabetic rats
could be demonstrated in vitro. The present results are in accord with
the latter observation.

At the present time it would seem reasonable, therefore, to retain the
hypothesis that insulin exerts immediate metabolic effects at the periphery
only. These metabolic effects reach their maximum so promptly that a
physical action of insulin at the cell surface or activating effects on one or
several superficially placed enzymes are suggested. On the other hand,
the delayed and gradual effect of insulin on hepatic metabolism provides
sufficient time for metabolic adaptation, including synthesis of new en-
zyme molecules, to occur. This could be the result of a direct, though slow, hormonal effect, quite different in type from the immediate peripheral action; it could also, however, represent adaptation to changes in substrate concentrations resulting from the changes in peripheral metabolism.

**SUMMARY**

1. Alterations of carbohydrate metabolism in liver slices and in diaphragm, after the administration of insulin to diabetic rats for varying periods of time, have been studied. The liver slices were incubated in a system designed to preserve a normal intracellular cationic environment.

2. The effects of insulin on diaphragm were apparent within a few minutes of its intravenous injection.

3. In contrast, no early insulin effects on hepatic carbohydrate metabolism could be demonstrated. Only minimal effects were noted 6 hours after the beginning of insulin administration, while normal values were reached after 24 to 48 hours. No consistent effects of insulin added in vitro could be shown.

4. The measurements made included the utilization of labeled glucose, fructose, pyruvate, and acetate, and the incorporation of each of these substrates into glycogen, glucose, CO₂, and fatty acids. The effects of insulin on lipogenesis and on gluconeogenesis appeared somewhat later than those on glucose phosphorylation.

5. These findings were thought to be consistent with the interpretation of immediate metabolic effects of insulin at the periphery only. The gradual effects on hepatic metabolism could be the result either of a direct, but slow, hormonal effect or of a metabolic adaptation to the new demands created by the peripheral changes.

**BIBLIOGRAPHY**

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