THE EFFECT OF INSULIN UPON THE KETONE METABOLISM OF NORMAL AND DIABETIC CATS*

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Until recently the views prevalent in the literature with respect to ketone formation in the normal and diabetic organism were as follows: The long fatty acid carbon chains are split by successive $\beta$ oxidation, 2 carbons at a time, with the formation of intermediate shorter fatty acids and acetic acid (Knoop hypothesis). Normally this process continues to completion, but in the absence of sufficient carbohydrate stores (fasting), or without insulin as an obligatory carbohydrate catalyst (diabetes), the process stops at the 4-carbon ketone stage. Each molecule of fatty acid gives rise to 1 molecule of ketone. An obligatory chemical coupling of the oxidation of carbohydrate and ketones was assumed. Failing carbohydrate oxidation, ketones cannot be utilized by the peripheral tissues; hence they are excreted in toto.

Ketone Utilization by Peripheral Tissues—Since 1928, however, considerable evidence has accumulated in the literature, necessitating complete revision of these concepts. For example, Snapper and Gruenbaum (1928), in perfusion experiments on striated muscle of normal animals, found a considerable disappearance of circulating ketones. Chaikoff and Soskin (1928), from a study of the rate of disappearance of ketones from the blood following injection of acetoacetate, concluded that ketones are utilized by the muscles of the diabetic as well as the normal eviscerated dog. Later Friedemann (1936) and Mirsky and Broh-

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Kahn (1937) performed similar experiments and came to the same conclusion. Barnes, MacKay, Moe, and Visscher (1938) demonstrated a disappearance of β-hydroxybutyrate from heart-lung preparations of normal dogs and goats. Recently Blixenkrone-Møller (1938, a, b) showed in a convincing manner, by perfusion experiments on the hind limbs of normal and diabetic cats, that there was an active utilization of ketones by muscle. This was markedly increased in the contracting muscle, and Blixenkrone-Møller (1938, b) concluded that ketone oxidation might furnish a considerable fraction of the total energy requirements. Toeniessen and Brinkman (1938), from similar perfusion experiments in the normal rabbit, also concluded that ketone bodies formed in the liver are burned in the muscles.

**Hypothesis of Multiple Alternate Oxidation of Fatty Acids—**As early as 1916, Hurtley rejected the Knoop hypothesis of successive β oxidation as an explanation of the mechanism of production of ketones in the diabetic. Large amounts of the intermediate fatty acids (butyric, caproic, etc.) should be formed in a diabetic excreting 70 gm. of ketones a day. But Hurtley found no significant amounts of butyric acid in the blood or tissues in such a case. He therefore proposed the hypothesis that the fatty acid is attacked at alternate carbon atoms simultaneously along the whole length of the carbon chain according to the scheme, . . .CH₂·CH₂·CH₂·CH₂·CH₂·CH₂·CH₂·CH₂ . . . = . . .CO·CH₂·CO·CH₂·CO·CH₂ . . .

Jowett and Quastel (1935) studied the rate of formation of ketones from fatty acids by liver slices. They also abandoned the successive β oxidation hypothesis and adopted that of Hurtley, which they called the “multiple alternate oxidation” hypothesis. According to it, the fatty acids undergo an oxidation throughout the entire fatty chain, alternate carbon atoms being affected. As a result, the entire molecule breaks down into acetoacetic acid and perhaps other acid products.

Deuel, Hallman, Butts, and Murray (1936) studied the rate of excretion of ketone bodies in rats after feeding ethyl esters of fatty acids. Their results indicated that in the case of palmitic, stearic, and oleic acids at least 3 molecules of ketones were formed per molecule of fatty acid oxidized, and they therefore supported the hypothesis of multiple alternate oxidation.

Blixenkrone-Møller (1938, a) compared the oxygen consumption
of perfused livers from diabetic cats with the total ketone production. It was possible to explain the low oxygen to ketone ratio only by assuming that 4 molecules of ketones were formed per molecule of fatty acid oxidized.

In the experiments reported in this paper we present evidence bearing on these problems. We have determined the rate of formation of ketone bodies and the oxygen consumption of surviving liver slices of normal and diabetic cats. We have also determined directly the utilization of ketones by muscle mince of normal and diabetic cats. In addition, we have experiments showing the influence, in vitro, of carbohydrates with and without insulin upon ketone formation by the liver of diabetic cats.

EXPERIMENTAL

Animals—The diabetic cats were depancreatized under nembutal anesthesia and used, as a rule, 48 hours later. The completeness of pancreatectomy was confirmed by autopsy and by the degree of glycosuria.

Preparation of Tissue. Liver—After a preliminary period of 1.0 hour (for the collection of urine) the abdomen was opened under nembutal anesthesia, and the aorta was severed. The liver was placed in ice-cold saline and weighed. Samples were taken for total fats, glycogen, ketones, etc. Slices (0.40 ± 0.05 mm.) were prepared with a double razor blade cutter. Averaging 200 mg., they were placed in conical Warburg vessels together with 3.0 cc. of appropriate medium and 100 per cent oxygen. The respiration period was 2.0 hours. The total oxygen consumption and CO₂ production determined by the customary Warburg technique (38°) are given as micromoles per gm. of wet liver.

Muscle—Portions of muscle as free as possible from tendinous tissue were quickly dissected out, collected on cracked ice, and minced (in the cold room at 0°) by the Latapie mincer. Weighed portions of this mince were transferred to Warburg vessels containing the appropriate media, through which 100 per cent O₂ was passed for 1 minute, and transferred to the bath at 38° for measurement of oxygen consumption, etc.

Collection of Urine—The urinary ketone excretion was always measured exactly 1.0 hour before the animals were killed. The bladder was initially emptied by pressure upon the abdomen.
At the end of 1.0 hour, the bladder was again emptied in the same way, and the completeness of emptying was verified by inspection of the bladder after the abdomen was opened.

Buffered Medium—This was always 3.0 cc., and the composition (aside from additions indicated for the individual experiments in the tables) was 0.050 M sodium phosphate (pH 7.0), 0.130 M NaCl, 0.007 M KCl, and 0.005 M MgCl₂.

Insulin¹—The insulin used was Lilly's amorphous insulin of highest purity. It was zinc-free and assayed 22 units per mg. Appropriate solutions were made up at pH 7.0 and added to the vessels at the final concentration indicated.

Analysis of Ketones. Tissue and Warburg Vessel Contents—At the end of the period of equilibration, the Warburg vessels were chilled in ice water for 5 minutes and then, through the side sac, 0.5 cc. of a 2 per cent solution of sodium bisulfite was added to bind any acetone which had formed. The tissue plus the contents of the vessel was transferred quantitatively to a calibrated centrifuge tube and appropriate amounts of tungstic acid reagent were added to precipitate the proteins. Replicates of the centrifugate were then analyzed for ketones by a slightly modified Shipley and Long (1938) method, which we found excellently suited for the determination of amounts of ketones down to 0.05 to 0.1 micromole. Each sample was analyzed separately for acetoacetic acid and β-hydroxybutyric acid.

The determination of the acetone in the distillates was carried out by means of the Scott-Wilson reagent. Readings of the turbidity were made on the Evelyn photoelectric colorimeter, with a standard curve which was measured anew for each set of determinations.

All values of acetoacetate, β-hydroxybutyrate, or total ketones are reported as micromoles per gm. of wet tissue.

Calculation—To calculate from gm. of liver to kilos of cat we used our average value of 26.3 ± 1.5 gm. of liver per kilo of cat. To calculate from gm. of muscle to kilo of cat we assumed an average of 350 gm. of muscle per kilo of cat.

Ketone Formation by Liver Slices of Fasted Normal Cats—There was found (Table I) a considerable ketone formation by the liver. Since urinary excretion was zero in all cases during the pre-

¹ We are indebted to Dr. F. B. Peck of the Lilly Research Laboratories for generous supplies of insulin.
experimental period of 1.0 hour, we conclude that there was a considerable ketone utilization (240 ± 17 micromoles per kilo of cat per hour)\(^2\) by the peripheral tissues, presumably for the most part by the muscles.

**Table I**

**Basic Ketone Formation by Liver Slices and Ketone Utilization by Normal and Diabetic Cats**

Average weight of slices 200 mg.; 3.0 cc. of phosphate buffer (no added nutrient); 2.0 hours equilibration; 38°.

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Fasted</th>
<th>Liver weight</th>
<th>Ketone formation</th>
<th>Ketone excretion per kilo cat per hr.</th>
<th>Ketone utilization per kilo cat per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hrs.</td>
<td>kg.</td>
<td>gm.</td>
<td>per gm. liver per hr.</td>
<td>per kilo mg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal cats</td>
<td>99A</td>
<td>48</td>
<td>3.5</td>
<td>104</td>
<td>8.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>99B</td>
<td>72</td>
<td>2.0</td>
<td>43</td>
<td>13.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>99C</td>
<td>84</td>
<td>2.6</td>
<td>68</td>
<td>5.7</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9.7 ± 1.4</td>
<td></td>
<td>240 ± 17</td>
<td>0</td>
</tr>
</tbody>
</table>

|                    |         |            |                  |                                       |                                       |
| Diabetic cats (48 hrs. after pancreatectomy) | 31   | 48 | 2.6 | 64  | 36 | 885 | 330 | 655 |
|                    | 79   | 48 | 3.1 | 66  | 50 | 1065 | 105 | 960 |
|                    | 96A  | 48 | 2.8 | 84  | 70 | 2105 | 155 | 1950 |
|                    | 96B  | 48 | 2.1 | 62  | 55 | 1175 | 30  | 1145 |
|                    | 96C  | 48 | 2.5 | 74  | 27 | 810  | 0   | 810  |
|                    | 96D  | 48 | 3.1 | 77  | 33 | 825  | 80  | 790  |
|                    | 96E  | 48 | 2.4 | 58  | 53 | 1280 | 185 | 1095 |
|                    | 96F  | 48 | 2.7 | 82  | 55 | 1650 | 180 | 1470 |
| Mean               |        | 47 ± 6     | 1200 ± 150       | 130 ± 35 | 1100 ± 130 |

*Whenever in this or the subsequent tables a mean value with its standard error is given, it indicates that two or more separate samples of tissue were equilibrated and the mean value recorded.

**Basic Ketone Formation by Liver Slices from (48 Hour) Diabetic Cats**—The average ketone formation by the liver (1200 ± 150

\(\text{Standard error of mean} = \frac{1.25 \times \text{deviations from mean}}{n \sqrt{n - 1}}\)
micromoles per kilo of cat per hour) during the experimental period in vitro was about 10 times the mean urinary excretion during the 1.0 hour preexperimental period in vivo.

In the diabetic cat, in contrast with the normal cat, the enzyme system concerned with the oxidation of ketones may be assumed to be saturated when ketones are excreted, and ketone utilization is then probably maximal. The mean excess (1100 ± 150 micromoles per kilo per hour) of ketone formation over ketone excretion probably represents the maximum ketone utilization of the diabetic cat.

These experiments illustrate further that the urinary ketone excretion of normal or diabetic animals is unreliable as an index of the rate of ketone production.

Direct Determination of Ketone Utilization by Minced Muscle from Normal and Diabetic Cats—We were successful in demonstrating this in the case of acetoacetate in the following way. 0.2 gm. samples of muscle mince of normal or diabetic cats were equilibrated for 2.0 hours at 38° in Warburg vessels containing 3.0 cc. of phosphate buffer and known amounts of acetoacetate. Parallel samples of muscle mince were run without the addition of acetoacetate to measure the spontaneous formation of β-hydroxybutyrate which usually occurred, and for which a correction was made in the calculation. At the end of the period of equilibration, the samples were deproteinized and analyzed for acetoacetate and β-hydroxybutyrate. The muscle mass was assumed to be 350 gm. per kilo of cat and ketone utilization per kilo of cat was calculated accordingly from the value per gm. of muscle. By this direct method we found (Table II) a utilization of acetoacetate, which in the mean was 915 ± 265 micromoles per kilo per hour.

In similar experiments with added β-hydroxybutyrate, we were unable to demonstrate a diminution of total ketones (Table II). In point of fact, we found as a rule an increase, chiefly of β-hydroxybutyrate. There is evidence in the literature against the participation of the muscles in ketone formation. On the other hand, Krebs and Johnson (1937) have demonstrated the formation of ketones in muscle from pyruvic acid, implying that carbohydrate as well as fat may be a source of ketones. Our own experience and that of Krebs and Johnson indicate that it is not possible to exclude the muscles as a site of ketone formation.
Our experiments also raise the question whether acetoacetate is the sole form of ketone which is oxidized by muscle.

**Ketone Utilization by Diabetic Minced Muscle Simultaneously Equilibrated with Diabetic Liver Slices**—It is possible that the oxidation by muscle mince of β-hydroxybutyrate is limited to the naturally occurring L isomer. This we did not have available.

### Table II

*Direct Determination of Utilization of Ketones by Muscle Mince of Normal and Diabetic Cats*

200 mg. of mince; 3.0 cc. of phosphate buffer with added ketones; 2.0 hours; 38°.

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Class</th>
<th>Concentration of acetoacetate acid</th>
<th>Net change* of ketones per kilo cat per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mM per l.</td>
<td>micromoles</td>
</tr>
<tr>
<td>96A</td>
<td>Diabetic</td>
<td>5.0</td>
<td>−1600 ± 60</td>
</tr>
<tr>
<td>96C</td>
<td>&quot;</td>
<td>0.4</td>
<td>−570 ± 210</td>
</tr>
<tr>
<td>99A</td>
<td>Normal</td>
<td>2.0</td>
<td>−1680 ± 175</td>
</tr>
<tr>
<td>99B</td>
<td>&quot;</td>
<td>2.0</td>
<td>−315 ± 0</td>
</tr>
<tr>
<td>99C</td>
<td>&quot;</td>
<td>0.4</td>
<td>−100</td>
</tr>
<tr>
<td>Mean (9 samples)</td>
<td>−915 ± 265</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Class</th>
<th>β-Hydroxybutyric</th>
<th>Net change* of ketones per kilo cat per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mM per l.</td>
<td>micromoles</td>
</tr>
<tr>
<td>96A</td>
<td>Diabetic</td>
<td>5.0</td>
<td>−730 ± 420</td>
</tr>
<tr>
<td>96B</td>
<td>&quot;</td>
<td>2.0</td>
<td>+670 ± 210</td>
</tr>
<tr>
<td>96C</td>
<td>&quot;</td>
<td>0.4</td>
<td>−130</td>
</tr>
<tr>
<td>99A</td>
<td>Normal</td>
<td>2.0</td>
<td>+600 ± 700</td>
</tr>
<tr>
<td>99B</td>
<td>&quot;</td>
<td>2.0</td>
<td>+350 ± 140</td>
</tr>
<tr>
<td>99C</td>
<td>&quot;</td>
<td>0.4</td>
<td>+90</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>+140 ± 220</td>
</tr>
</tbody>
</table>

* Calculated from the change per gm. of muscle by assuming the muscle mass to be 350 gm. per kilo.

At any rate, we were able to demonstrate the oxidation of ketones by diabetic muscle mince by a third method; *viz.*, the simultaneous equilibration of diabetic liver and muscle. By this device the muscle would presumably be supplied with the natural ketones continuously formed by the diabetic liver and, to a certain extent, the conditions *in vivo* would be imitated. 0.2 gm. portions of diabetic muscle mince were placed in Warburg vessels together
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with 30 mg. slices of diabetic cat liver and 3.0 cc. of phosphate buffer. 100 per cent O₂ was passed into the vessels for 1 minute and the vessels equilibrated at 38° for 2.0 hours. In parallel vessels, similar portions of muscle and liver were equilibrated independently. The assumption was made that the rate of formation of ketones by the liver equilibrated with the muscle was the same as that of the liver equilibrated independently, and the calculations were made accordingly. We found (Table III) by this method a mean utilization of ketones of 2070 ± 430 micromoles per kilo per hour.

**Table III**

*Ketone Utilization by Muscle Mince of Diabetic Cat When Simultaneously Equilibrated with Diabetic Liver Slices As Source of Ketones*

200 mg. of muscle mince; 30 mg. of liver slices; 3.0 cc. of phosphate buffer; 2.0 hours; 38°.

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Utilization of ketones per kilo cat per hr.</th>
<th>O₂ consumption of liver + muscle per 2.0 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>micromoles</td>
<td>micromoles</td>
</tr>
<tr>
<td>96A</td>
<td>2800</td>
<td>64</td>
</tr>
<tr>
<td>96B</td>
<td>1820</td>
<td>31</td>
</tr>
<tr>
<td>96C</td>
<td>1580</td>
<td>34</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>2070 ± 430</strong></td>
<td></td>
</tr>
</tbody>
</table>

We also compared the observed oxygen uptake of the combined liver and muscle systems with that calculated from the oxygen uptake of the samples equilibrated independently. The agreement of the calculated with the observed values is an indication that the oxidations of the liver slice and muscle mince in the composite system were going on independently of each other.

**Comparison of Portal and Hepatic Blood Ketones**—To show that the rate of ketone formation by liver slices *in vitro* is essentially the same as that of the intact liver with normal circulation, we obtained blood samples simultaneously from the portal and hepatic veins of diabetic cats under nembutal anesthesia. These samples were analyzed for ketones.

The blood flow through the diabetic liver is quite variable, but as a sufficient approximation we can take the mean value de-
terminated by Schmid (1908) for the cat as 32 cc. per hour per gm. of liver. By the equation

\[
\frac{\text{Gm. liver}}{\text{Kilos of cat}} \times \text{ liver blood flow (cc. per gm. per hr.)} \\
\times \text{ portal-hepatic difference (micromoles per cc.)} = \text{ketone formation (micromoles per kilo of cat per hr.)}
\]

we calculated the rate of ketone formation \textit{in vivo} (Table IV). The mean value of 1265 ± 410 micromoles per kilo per hour is to be compared with our mean value obtained \textit{in vitro} of 1200 ± 150 micromoles per hour (Table I). The identity of these values is evidence that the rate of ketone formation by liver slices \textit{in vitro} is the same as the rate \textit{in vivo}.

We have calculated (Table V) from the data in the literature the values for ketone utilization obtained by different workers and different methods. These are in such good agreement that we can accept with assurance the value of 1300 micromoles per kilo per hour as representing the maximum basal ketone utilization.

**Molecular Ratio of Ketones Formed to Fatty Acid Oxidized by Liver of Diabetic Cats; Multiple Alternate Oxidation Hypothesis**—It is possible to make an estimate of the ratio from a knowledge of the molecular ratio of the oxygen uptake to the ketone formation. Three cases have to be considered.

1. If one assumes, according to the older views, that fatty acids...
TABLE V
Determinations in Literature of Ketone Utilization by Peripheral Tissues of Normal and Diabetic Animal

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean ketone utilization (micromoles per kg. per hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaikoff and Soskin (1928)</td>
<td></td>
</tr>
<tr>
<td>By measurement of rate of disappearance of injected ketones from blood of Diabetic eviscerated dogs</td>
<td>1200 ± 400</td>
</tr>
<tr>
<td>Normal eviscerated dogs</td>
<td>1400 ± 230</td>
</tr>
<tr>
<td>Blixenkrone-Moller (1938, b)</td>
<td></td>
</tr>
<tr>
<td>By comparison of ketone formation by perfused diabetic cat livers with prior urinary ketone excretion</td>
<td>1600 ± 330</td>
</tr>
<tr>
<td>By measurement of rate of disappearance of ketones from blood perfused through Resting hind limbs of normal and diabetic cats</td>
<td>1100 ± 160</td>
</tr>
<tr>
<td>Working &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>5400 ± 280*</td>
</tr>
<tr>
<td>Stadie, Zapp, and Lukens</td>
<td></td>
</tr>
<tr>
<td>By comparison of ketone formation by diabetic cat liver slices with prior urinary ketone excretion</td>
<td>1100 ± 150</td>
</tr>
<tr>
<td>By measurement of ketone utilization of normal and diabetic cat muscle mince in presence of added acetoacetate</td>
<td>915 ± 265</td>
</tr>
<tr>
<td>By measurement of ketone utilization by diabetic cat muscle mince simultaneously equilibrated with diabetic cat liver slice</td>
<td>2070 ± 430</td>
</tr>
<tr>
<td>By measurement of the portal-hepatic ketone difference of diabetic cats</td>
<td>1240 ± 410</td>
</tr>
<tr>
<td>Mean basal ketone utilization</td>
<td>1340 ± 130</td>
</tr>
</tbody>
</table>

* This value is excluded from the basal mean.

In the diabetic are oxidized by β oxidation to the ketone stage only, the reaction for the oxidation of a typical fatty acid (palmitic) is

\[ \text{C}_{16}\text{H}_{32}\text{O}_2 + 18.5\text{O}_2 = \text{C}_7\text{H}_6\text{O}_2 + 12\text{CO}_2 + 12\text{H}_2\text{O} \]

Molecular ratio of \( \text{O}_2 \) to ketones = 18.5:1; r.q. = 0.65

2. It is possible, however, that the oxidation in the liver is partial, yielding only 1 molecule of ketone, the balance of the molecule being oxidized to some other acid products, perhaps acetic acid. In that case, the reaction is

\[ \text{C}_{16}\text{H}_{32}\text{O}_2 + 6\text{O}_2 = \text{C}_4\text{H}_8\text{O}_2 + 6\text{CH}_3\text{COOH} \]

Molecular ratio of \( \text{O}_2 \) to ketones = 6:1; r.q. = 0
3. According to the multiple alternate oxidation hypothesis, the entire molecule is simultaneously oxidized, yielding 4 molecules of ketones; the reaction is then

\[
C_{14}H_{25}O_3 + 5O_2 = 4C_4H_8O_2
\]

Molecular ratio of O₂ to ketones = 1.25:1; R.Q. = 0

In Table VI we give the observed values for the oxygen uptake and the ketone formation by liver slices of diabetic cats. In the mean, the molecular ratio of these two values is 1.68 ± 0.12. It must be remembered that there are oxidations other than ketone formation occurring in the liver which, if corrected for, would make the ratio still lower. For example, we have previously determined (Stadie, Lukens, and Zapp, 1940) that the mean rate of deamination by diabetic liver slices is 16.4 micromoles of urea per gm. per 2.0 hours. It is quite permissible, then, to correct the total oxygen uptake by this amount, in which case the mean ratio of O₂ to ketones would be 1.54:1. This ratio is sufficiently close to the one calculated according to the hypothesis of multiple alternate oxidation to warrant the conclusion that, in the diabetic cat liver, each fatty acid molecule on oxidation yields 4 molecules of ketones. This is in complete agreement with the conclusion of Blixenkrone-Møller (1938, a) on the basis of his experiments.

### Table VI

*Molecular Ratio of Oxygen Uptake to Ketone Formation by Liver Slices from Diabetic Cats*

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>O₂ per gm. per 2.0 hrs.</th>
<th>Ketone formation per gm. per 2.0 hrs.</th>
<th>Ratio, O₂ to ketones</th>
<th>R.Q.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>micromoles</td>
<td>micromoles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>157 ± 3</td>
<td>100</td>
<td>1.57</td>
<td>0.21</td>
</tr>
<tr>
<td>96A</td>
<td>192 ± 18</td>
<td>138</td>
<td>1.39</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>96B</td>
<td>201</td>
<td>110</td>
<td>1.83</td>
<td>0.39</td>
</tr>
<tr>
<td>96C</td>
<td>163</td>
<td>110</td>
<td>1.48</td>
<td>0.40</td>
</tr>
<tr>
<td>96D</td>
<td>149</td>
<td>67</td>
<td>2.22</td>
<td>0.40</td>
</tr>
<tr>
<td>96E</td>
<td>177</td>
<td>106</td>
<td>1.70</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean...</td>
<td>175 ± 8</td>
<td>105 ± 8</td>
<td>1.68 ± 0.12</td>
<td>0.32 ± 0.04</td>
</tr>
</tbody>
</table>
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on the rate of oxygen consumption and ketone formation in perfused diabetic cat livers.

It is to be noted further that the low R.Q. \((0.32 \pm 0.04)\) of diabetic cat liver slices is in accordance with the expectation that the major portion of the oxidations occurring in the diabetic liver do not produce \(\text{CO}_2\).

**Effect of Fructose with and without Insulin upon Ketone Formation by Diabetic Cat Liver**—Our experiments (Table VII) show the possibility of markedly influencing the ketone formation by diabetic liver slices with fructose with and without insulin. Most

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Basic ketone formation, no added nutrient, per gm. per 2.0 hrs.</th>
<th>10 mM fructose with and without insulin</th>
<th>0.002 M fumarate with and without insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>100 +11</td>
<td>-12 -39</td>
<td>-12 -49</td>
</tr>
<tr>
<td>96B</td>
<td>117</td>
<td>-20 -27</td>
<td>-49 -62</td>
</tr>
<tr>
<td>96C</td>
<td>55</td>
<td>+11 +6</td>
<td>+7 -15</td>
</tr>
<tr>
<td>96D</td>
<td>67</td>
<td>+5 +22</td>
<td>-15 -50</td>
</tr>
<tr>
<td>96E</td>
<td>106</td>
<td>+9 -12</td>
<td>-25 -42</td>
</tr>
</tbody>
</table>

**Table VII**

Effect of Insulin with and without Substrates upon Ketone Formation by Liver Slices from Diabetic Cats

200 mg. slices; phosphate buffer, 3.0 cc.; Lilly's Zn-free insulin, 1 unit per cc.; 2.0 hours; 38°.

marked is the effect with fructose + fumarate + insulin (-42 per cent), a finding in accordance with the hypothesis of Szent-Györgyi (1937) that the 4-carbon dicarboxylic acids may inhibit the formation of ketones in the diabetic liver.

**Effect of Time of Equilibration upon Action of Insulin on Ketone Formation of Diabetic Cat Liver Slices**—The inhibiting action of insulin upon the ketone formation of diabetic cat liver slices can be considerably enhanced if a longer time than 2 hours is allowed for its action. We equilibrated three different samples of liver slices from the same diabetic cat. The phosphate buffer contained
(a) no additions, (b) 0.010 M fructose, or (c) 0.010 M fructose + 1 unit per cc. of insulin. At intervals of 1 hour the slices were transferred to similar fresh media. The media from each respiratory period were then analyzed for total ketones. The summations of the ketone formation are plotted in Fig. 1. In all cases the oxygen uptake continued essentially at the initial rate for 4 hours. This indicated the continued viability of the liver cells. The progressive fall in the rate of ketone formation is greatest in the case of fructose + insulin, and at the end of the 4 hour period the ratios of ketone formation in the three cases were 1:0.87:0.55.

Ketone Formation by Liver Slices from Diabetic Cat Previously Injected With Insulin—The reversal toward normal of the diabetic type of metabolism of the isolated liver slice may also be accomplished by the prior injection of adequate doses of insulin, provided sufficient time elapses after the injection. For this purpose we used cats 48 hours after pancreatectomy. Initial samples of blood from the ear vein were taken for glucose and ketones. The cats were then injected subcutaneously with divided doses of Lilly's amorphous insulin, and frequent blood
sugar determinations were made by the Shaffer-Hartmann-Somogyi method (Peters and Van Slyke, 1932) until the desired level was reached. We found by experience that doses of 30 to 40 units of insulin and a lapse of 4 to 6 hours were necessary in order to accomplish this. The cats were then anesthetized with nembutal as in our previous experiments, and liver slices were prepared and equilibrated for 2.0 hours in phosphate buffer which contained no additional insulin. In some cases substrates were added. At the end of the period, the contents of the Warburg vessels and the tissue were analyzed for total ketones. The data are presented in Table VIII.

We emphasize the following points. (1) When the doses of insulin or the time elapsed was small, the subsequent rate of formation of ketones (Cat 102A) was not less than the mean value (46 ± 6 micromoles per gm. of liver per hour) found in the cases of untreated diabetic cats. However, the effect of added substrates, e.g. fructose, glucose, or d-lactate, in diminishing ketone formation is quite apparent, being as much as −32 per cent in the last instance. (2) With adequate dosage of insulin and sufficient time, the effect of the insulin is quite marked. The mean rate of

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Cat weight kg.</th>
<th>Liver weight gm.</th>
<th>Blood sugar mg. per cent</th>
<th>Initial mg. per cent</th>
<th>Final mg. per cent</th>
<th>Insulin given units</th>
<th>Insulin action hrs.</th>
<th>Basic ketone formation* Per gm. liver per hr.</th>
<th>Per cent change with 10 mm fructose</th>
<th>10 mm glucose</th>
<th>10 mm d-lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>102A†</td>
<td>2.7</td>
<td>82</td>
<td>264</td>
<td>104</td>
<td>6</td>
<td>1.5</td>
<td>53 ± 14</td>
<td>1650 ± 440</td>
<td>−19 −15 −32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102B</td>
<td>2.6</td>
<td>79</td>
<td>243</td>
<td>138</td>
<td>40</td>
<td>5.8</td>
<td>17 ± 2</td>
<td>495 ± 40</td>
<td>0 +13 +6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102C</td>
<td>2.1</td>
<td>58</td>
<td>226</td>
<td>60</td>
<td>35</td>
<td>6.0</td>
<td>17 ± 0.5</td>
<td>460 ± 7</td>
<td>−32 0 −39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102D</td>
<td>1.9</td>
<td>46</td>
<td>338</td>
<td>110</td>
<td>30</td>
<td>4.5</td>
<td>18 ± 4.5</td>
<td>435 ± 110</td>
<td>−22 −35 −49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17 ± 1</td>
<td>460 ± 18</td>
<td>−18 −9 −28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values in this column are means of two separate samples of liver slices.
† Excluded from the mean of this series because the time of action and amount of insulin were insufficient.
ketone formation of the three treated cases is $17 \pm 1$ micromole per gm. of liver per hour or $37 \pm 4$ per cent of that of untreated cases. (3) The additional effect of the added substrates is also evident; ketone formation was further reduced (with two exceptions) by 20 to 50 per cent. (4) The restoration toward a normal rate of ketone formation may precede the storage of glycogen in the liver and the decrease of the usual high liver fat. In Cat 102B the liver glycogen was 0.05 per cent and the liver fats 18 per cent, essentially that of untreated cats. In Cat 102C, in which the blood sugar was allowed to fall to quite low levels (60 mg. per cent), the liver glycogen was raised to 3.2 per cent. Nevertheless the ketone formation in these two cases was found to be essentially the same. We take this to mean that insulin

**Table IX**

*Ketone Formation by Liver Slices from Houssay Cat*

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Hypophysectomy</th>
<th>Pancreectomy</th>
<th>Experiment</th>
<th>Cat weight</th>
<th>Liver weight</th>
<th>Blood sugar</th>
<th>Liver glycogen</th>
<th>Liver ketone formation per kilo cat per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>105A</td>
<td>June 8</td>
<td>June 14</td>
<td>June 19</td>
<td>3.4</td>
<td>50</td>
<td>60</td>
<td>0.04</td>
<td>68</td>
</tr>
<tr>
<td>105B</td>
<td>Sept. 7</td>
<td>Sept. 18</td>
<td>Sept. 20</td>
<td>2.1</td>
<td>49</td>
<td>0.02</td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>

may act independently to suppress ketone formation and to aid in the storage of glycogen. The view that the storage of glycogen is an obligatory first step in the suppression of ketone formation is not in accordance with these observations.

**Ketone Formation by Liver of Houssay Cat**—The amelioration of the diabetic status in the hypophysectomized-depancreatized cat is too well known to require comment. This reversal was strikingly demonstrated by a study of the ketone formation by the liver slices of two Houssay cats (Table IX). From the data it is seen that the ketone formation of the liver is less than normal. This experiment emphasizes the possibility that insulin does not react directly in a regulatory fashion with the enzyme system concerned in the oxidation of fatty acids, but indirectly by acting as antagonist to the ketogenic hormone of the pituitary.
Insulin Effect on Ketone Metabolism

Total Respiration and Non-Ketone Respiration of Liver of Normal and Diabetic Cats—We have previously discussed the evidence indicating that the oxidation of a typical fatty acid (palmitic) in the liver follows the equation

\[
C_{16}H_{34}O_2 \text{ (palmitic acid)} + 5O_2 \rightarrow 4C_4H_8O_2 \text{ (\(\beta\)-hydroxybutyric acid)}
\]

We may therefore assume that, on the average, 1.25 moles of oxygen are required for the formation of 1 mole of ketone. If fatty acids are the sole precursors of ketones in the liver, it is possible to correct the total oxygen consumption of liver slices by subtracting the oxygen required for ketone formation, \((\text{i.e.}, 1.25 \times \text{ketones formed})\) to obtain the non-ketone oxygen consumption. Since ketone formation is presumably not accompanied by CO₂ formation, the total CO₂ divided by the non-ketone O₂ gives the non-ketone R.Q. These calculations for our series of normal cats, untreated diabetic cats, and insulin-treated cats show some contrasting aspects (Table X).

The mean oxygen consumption of the untreated diabetic cat liver slices was significantly higher than the normal mean. In the treated cats the mean oxygen consumption was restored toward normal.

The chief function of the diabetic liver is the partial oxidation of fatty acids to ketones: only 42/185 or 22 ± 5 per cent of the total oxygen uptake is concerned with oxidations other than ketone formation. This is in sharp contrast with livers from normal fasted and treated diabetic cats, in which 86 ± 8 per cent and 77 ± 3 per cent respectively of the total oxygen consumption are thus concerned.

In the diabetic liver the non-ketone respiration is associated with a high respiratory quotient (1.36 ± 0.34). The significance of this is a matter of conjecture.

In the treated diabetic cases there are indications that the metabolic status of the liver slice is intermediate between the normal and the untreated. For example, the ketone formation is low, the non-ketone oxygen is essentially normal, but the CO₂ is not correspondingly elevated; \((\text{i.e.}, \text{the non-ketone R.Q. is low})\). We have no further experimental data to aid in interpreting the significance of these findings. Our surmise, however, is that in the livers from insulin-treated diabetic cats partial oxidations of fatty
acids still make up the major portion of the hepatic metabolism, but that the oxidations do not produce ketones.

Possibility of Production of Carbohydrates from Fats by Diabetic Liver—The overproduction hypothesis of diabetes postulates that fats in large quantities are converted by the liver into carbohydrates. We have previously discussed evidence (Stadie, Lukens, and Zapp, 1940) on the rate of formation of carbohydrate by diabetic livers, which fails to support this hypothesis. The data in Table X force us to the conclusion that it is untenable for

Table X
Non-Ketone Respiration of Liver Slices from Fasted Normal, Untreated Diabetic, and Insulin-Treated Diabetic Cats

200 mg. slices; 3.0 cc. of phosphate buffer; 2.0 hours; 38°.

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>O₂ uptake per gm. per 2 hrs.</th>
<th>Total R.Q.</th>
<th>Non-ketone O₂ per gm. per 2 hrs.</th>
<th>Total CO₂ per gm. per 2 hrs.</th>
<th>Non-ketone R.Q.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fasted cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>99A</td>
<td>130 ± 13</td>
<td>0.70 ± 0.02</td>
<td>118</td>
<td>97</td>
<td>0.82</td>
</tr>
<tr>
<td>99B</td>
<td>144 ± 0</td>
<td>0.67 ± 0.01</td>
<td>111</td>
<td>97</td>
<td>0.87</td>
</tr>
<tr>
<td>99C</td>
<td>151</td>
<td>0.49</td>
<td>137</td>
<td>74</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean</td>
<td>142 ± 3</td>
<td>0.64 ± 0.03</td>
<td>122 ± 9</td>
<td>89 ± 9</td>
<td>0.81 ± 0.05</td>
</tr>
<tr>
<td>Untreated diabetic cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>157 ± 3</td>
<td>0.21</td>
<td>32</td>
<td>33</td>
<td>1.03</td>
</tr>
<tr>
<td>96A</td>
<td>192 ± 18</td>
<td>0.33 ± 0.04</td>
<td>21</td>
<td>64</td>
<td>3.04</td>
</tr>
<tr>
<td>96B</td>
<td>201</td>
<td>0.30</td>
<td>63</td>
<td>78</td>
<td>1.24</td>
</tr>
<tr>
<td>96C</td>
<td>163</td>
<td>0.40</td>
<td>25</td>
<td>65</td>
<td>2.60</td>
</tr>
<tr>
<td>96D</td>
<td>149</td>
<td>0.40</td>
<td>65</td>
<td>60</td>
<td>0.92</td>
</tr>
<tr>
<td>96E</td>
<td>177</td>
<td>0.22</td>
<td>44</td>
<td>39</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean</td>
<td>185 ± 8</td>
<td>0.32 ± 0.04</td>
<td>42 ± 9</td>
<td>57 ± 8</td>
<td>1.36 ± 0.34</td>
</tr>
<tr>
<td>Insulin-treated diabetic cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102B</td>
<td>167 ± 14</td>
<td>0.34 ± 0.03</td>
<td>126</td>
<td>57</td>
<td>0.45</td>
</tr>
<tr>
<td>102C</td>
<td>150 ± 6</td>
<td>0.51 ± 0.01</td>
<td>117</td>
<td>77</td>
<td>0.66</td>
</tr>
<tr>
<td>102D</td>
<td>152 ± 2</td>
<td>0.46 ± 0.04</td>
<td>117</td>
<td>70</td>
<td>0.60</td>
</tr>
<tr>
<td>Mean</td>
<td>156 ± 3</td>
<td>0.44 ± 0.06</td>
<td>120 ± 4</td>
<td>68 ± 7</td>
<td>0.57 ± 0.07</td>
</tr>
</tbody>
</table>
the following reasons. The long fatty acids (16 carbon atoms or more) occurring in the liver are initially essentially unoxidized. Therefore, for the conversion of the carbon atoms of the fatty acid into a hexose \((\text{C}_6\text{H}_{12}\text{O}_6)\), all (i.e. 3) moles of oxygen per mole of hexose must be supplied ultimately by respiratory oxygen. Accordingly, there could be formed a maximum of carbohydrate per micromole of \(\text{O}_2\) of \(0.180/3 = 0.06\) mg. per micromole of \(\text{O}_2\). The total oxygen available for carbohydrate formation from

<table>
<thead>
<tr>
<th>Condition of cat</th>
<th>Cat No.</th>
<th>Acetoacetate, per cent of total ketones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, fasted</td>
<td>99A</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>99B</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>99C</td>
<td>57</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>62 ± 4</td>
</tr>
<tr>
<td>Diabetic</td>
<td>79</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>96A</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>96C</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>96D</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>96E</td>
<td>45</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Insulin-treated diabetic</td>
<td>102A</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>102B</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>102C</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>102D</td>
<td>46</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>46 ± 6</td>
</tr>
</tbody>
</table>

fat is the non-ketone oxygen. In our series of diabetic cat livers, the mean non ketone oxygen was 21 micromoles per gm. per hour, or, since the average weight of the livers of the cats was 26 gm. per kilo, the approximate maximum possible carbohydrate formation from fatty acids was \(0.06 \times 21 \times 26 = 33\) mg. per kilo of cat per hour. This is only a small fraction (16 per cent) of the total glucose (200 ± 30 mg. per kilo per hour) excreted by our diabetic cats in the preexperimental 1.0 hour period. But
our experiments show further that this small amount of non-ketone oxygen was entirely used up in the production of CO₂ (non-ketone R.Q. = 1.34 ± 0.34). In other words, there was no oxygen available in the metabolism of the diabetic liver slice for the oxidation of fatty acids to carbohydrate.

Proportion of Acetoacetate and β-Hydroxybutyrate to Total Ketone Formation by Liver—There are reasons for believing that the enzyme system concerned with the oxidation of fatty acids in the liver is adjusted so as to produce the two forms of ketones in fairly constant ratio to each other. This is brought out in Table XI. The total ketone formation in the diabetic cat is roughly divided between the two ketones in the ratio of 1:1. The higher value of acetoacetate in the normal cats is probably not significantly different from that of the diabetics.

We wish to express our thanks to Mildred S. Wright for her helpful criticisms and assistance in the experiments.

SUMMARY

1. With liver slices from fasted normal and diabetic cats, the rate of formation of ketones in vitro was determined. The urinary ketone excretion in a preliminary 1.0 hour period was also determined.

2. Ketone utilization by the muscles was calculated from measurements of (a) excess hepatic ketone formation over urinary ketone excretion, (b) oxidation of added acetoacetate by muscle mince equilibrated in vitro, (c) simultaneous equilibration of muscle mince with diabetic liver slices as source of ketones, (d) measurement of portal-hepatic blood ketone difference. An average value of 1300 micromoles per kilo of cat per hour was found for the utilization of ketones by muscle.

Utilization in the diabetic was found to be essentially the same as that in the normal cat. This demonstrates that the oxidation of ketones by muscle is independent of the presence of insulin.

3. No utilization of dl-β-hydroxybutyrate added to muscle mince could be demonstrated, either in normal or diabetic cats. This is a possible indication that acetoacetate is the sole form of ketone oxidized by muscle. There was found in four of six cases an increase both of β-hydroxybutyrate and total ketones, evidence
that muscle as well as liver may be a source of ketones in the diabetic.

4. The molecular ratio of oxygen consumed to ketone produced from fatty acids by the liver was found to be close to the value of 1.25 required by the multiple alternate oxidation hypothesis. This is evidence that the higher fatty acids yield not 1 but 4 molecules of ketones per molecule of fatty acid oxidized.

5. On the basis of the observed O₂ to ketone ratio, it is possible to calculate the non-ketone respiration of the liver and contrast its characteristics in the fasted normal, the diabetic, and the insulin-treated diabetic cat.

6. The non-ketone oxygen available for the possible oxidation of fatty acids to carbohydrates was found to be essentially zero. We conclude, therefore, that the hypothesis of the overproduction of carbohydrates from fats in diabetes is untenable.

7. Insulin in the presence of fructose, fumarate, and d-lactate inhibited the formation of ketones by the diabetic liver. This effect was enhanced by prolonged equilibration of slices with insulin-containing media.

8. Prior insulin treatment of diabetic cats restored the metabolism of the liver slice to a status intermediate between the normal and the diabetic. There was found a marked reduction of ketone formation and a type of non-ketone respiration suggesting partial oxidation of fatty acids beyond the ketone stage.

9. In Houssay cats we found that the ketone formation by the liver was essentially that of the fasted normal cat. This suggests that insulin controls ketone formation indirectly by acting antagonistically to the ketogenic pituitary hormone.

BIBLIOGRAPHY

Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry, Methods, Baltimore, 467 (1932).
THE EFFECT OF INSULIN UPON THE KETONE METABOLISM OF NORMAL AND DIABETIC CATS
William C. Stadie, John A. Zapp, Jr. and Francis D. W. Lukens