A METABOLIC LESION IN DIETARY NECROTIC LIVER DEGENERATION*

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Dietary necrotic liver degeneration is recognized now as a distinct disease entity; it is a deficiency of complex nature, which, so far, has been produced in rats (2, 3), mice (4), rabbits,1 and pigs (5, 6). The disease in rats is primarily characterized by acute massive necrosis of the liver which develops suddenly after an uneventful experimental period of several weeks duration. In most animals, the first attack of necrosis is fatal. Only 23 per cent of the animals dying from the disease show signs of earlier episodes of necrosis of the liver (7, 8). A detailed description and discussion of the various facets of this deficiency disease are found in the monograph on “Nutritional factors and liver diseases” (9).

Three distinct dietary factors have been identified as protective against necrotic liver degeneration, namely, cystine (2, 10), vitamin E (11), and Factor 3 (12). Any one of these three chemically different substances affords protection by itself (10).

Assuming that the acute attack of massive necrosis is the end-result of a degeneration of metabolism in the hepatic parenchyma, we have investigated in vitro the metabolism of tissues of rats on diets which produce liver necrosis. Slices from livers of such animals exhibit a peculiar metabolic lesion several weeks before hepatic necrosis appears. They are incapable of maintaining normal respiratory activity in the Warburg apparatus; oxygen consumption declines after 30 to 60 minutes at initially normal rates. In the following, this metabolic lesion is described, its relation to the three factors which specifically prevent dietary liver necrosis is demonstrated, its biochemical nature is partially defined, and it is compared to other experimentally induced types of hepatic damage.

EXPERIMENTAL

Care of Rats—Male, weanling Sprague-Dawley rats of the National Institutes of Health strain, 18 to 22 days old, were placed on a basal diet containing Torula yeast as the sole source of protein (13). This ration consists

* A preliminary report has appeared (1).

1 Hove, E. L., personal communication.
of *Torula* feed yeast 30, sucrose 59, vitamin E-free lard 5, salts 5 (14), vitamin mixture (in lactose) 1. It contains approximately 15 per cent protein, 66 per cent carbohydrate, and 6 per cent fat. Under our experimental conditions, the mean survival time of the Sprague-Dawley rat is about 45 days and the incidence of liver necrosis approximates 100 per cent (13).

The animals were maintained on the basal diet for 18 to 22 days; the diets of some were then supplemented with vitamin E (50 mg. per cent of dl-α-tocopherol acetate), sulfur-containing amino acids (0.5 or 1 per cent L-cystine, 1 per cent L-cysteine or 1.23 per cent DL-methionine), or a source of Factor 3, whereas others were continued on the basal ration. The level of vitamin E used is completely protective against the occurrence of dietary necrotic liver degeneration (10, 11). The amounts of sulfur amino acids and Factor 3 employed were selected to prolong the survival time of the rats, although they did not always completely protect against the deficiency disease. Therefore, dosage effects were observed with respect to both dietary necrotic liver degeneration and the respiratory lesion reported here.

**Procedures in Vitro; Slices**—Rats were sacrificed by decapitation and their livers rapidly excised and placed in cold isotonic phosphate buffer. Slices were prepared free-hand, blotted free of excess moisture, and weighed. Slices were transferred to the main compartment of a Warburg vessel that contained 3 ml. of oxygenated Krebs-Ringer-phosphate buffer, pH 7.4 (15). Unless otherwise noted, the buffer contained 0.01 M glucose. The center well contained 0.2 ml. of 30 per cent KOH and a roll of filter paper. The gas phase was replaced with O$_2$ and the flasks were equilibrated in the constant temperature bath (37.5°C) for 10 minutes. Thereafter, readings of the manometers were made at 10 minute intervals for as long as 3 hours. After the period of incubation, tissue nitrogen was determined on the entire flask contents by the semimicro-Kjeldahl method.

Oxygen consumption was calculated in microliters of oxygen consumed per hour per 100 mg. of fresh weight ($Q_{O_2}$ (F 100)) or per mg. of tissue nitrogen ($Q_{O_2}$ (N)).

**Liver Homogenates**—Livers were homogenized for 2 minutes by the Potter-Elvehjem procedure in an ice-cold isotonic medium consisting of 9 volumes of 0.154 M KCl and 1 volume of 0.01 M potassium phosphate buffer, pH 7.4. The final concentration of tissue in the homogenate was 10 per cent. 1 ml. of the suspension (100 mg. of liver) was added to a Warburg vessel containing 2 ml. of the medium. The basal medium consisted of the 9:1 KCl-PO$_4$ buffer, to which the following substances were added (final concentrations): MgSO$_4$ 3 × 10$^{-3}$ M, cytochrome c 4 × 10$^{-5}$ M, and glucose 1 × 10$^{-3}$ M. The following substances, when added, were present at 2 × 10$^{-3}$ M final concentration: adenosine triphosphate (ATP), adenosine diphosphate, adenylic acid, diphosphopyridine nucleotide (DPN), and, at
5 \times 10^{-4} \text{M} \text{ concentration, fumarate, succinate, malate, lactate, } \alpha \text{-ketoglutarate, pyruvate, and coenzyme A. The gas phase was air. The flasks were equilibrated for 10 minutes at 37.5^\circ } \text{ before readings were started.}

\section*{Results}

\textit{Decline in } \textit{O}_2 \textit{ Consumption of Liver Slices; Protective Effect of Vitamin E—}

Oxygen consumption of liver slices from rats fed the basal Torula yeast diet is compared with that from animals fed the vitamin E-supplemented diet and a stock diet. In Table I, the rats are grouped with respect to period on the diet or to body weight. Animals fed the basal diet are further divided into those having normal livers, or sufficient intact liver tissue to allow for normal slices, and those having such extensive necrosis that no normal slices were obtainable. \textit{Q}_02 (F 100) values are calculated at 30 minute intervals for 2 hours of incubation. As was to be expected, the grossly necrotic slices consumed oxygen at very slow rates (Table I, Fig. 1). Therefore, in subsequent experiments with livers of rats fed the basal diet, normal tissue and normal slices were used exclusively, unless specifically mentioned.\textsuperscript{2} The \textit{O}_2 consumption of liver slices from animals fed the three different diets was essentially the same for the first 30 minutes of incubation; thereafter, the respiration of slices from rats fed the basal diet declined, while slices from animals fed the vitamin E-supplemented diet, or the stock ration, continued to respire at the initial rate for at least 2 hours. Only slices from the group on the basal diet showed the decline. There appeared to be some relationship between the period on the basal diet and the severity of this respiratory lesion. However, twenty out of twenty-two livers from the group fed the basal ration for only 21 to 29 days showed a 2 hour respiratory decline of more than 25 per cent, whereas none of the livers of rats fed the vitamin E-supplemented diet, or the stock diet, declined to this extent during the incubation period.

The typical time-course of \textit{O}_2 consumption for livers of the three different groups is presented in Fig. 1. Frequency distribution of the \textit{Q}_02 (F 100) values for the first and the last 30 minute intervals is shown in Fig. 2. It is clear that the initial rates of \textit{O}_2 consumption are practically identical in all three groups. However, as the period of incubation continues, only slices from rats on the basal diet failed to maintain the initial respiratory activity. After 2 hours of incubation, only 10 per cent of the values of the group on the basal diet overlapped with the lowest values of the groups receiving

\textsuperscript{2} Histological examination of representative slices confirmed repeatedly the absence of necrotic cells in slices from normal areas of deficient livers, even when other distinctly necrotic areas were present in the liver. We are indebted to Dr. George L. Fite, National Institutes of Health, for histological examination of tissues throughout these studies.
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Some overlapping was to be expected, since, under our experimental conditions, a small percentage of the rats fed the basal Torula diet may survive for as long as 70 to 80 days before succumbing to liver necrosis (13).

The nitrogen content of liver slices from rats fed the basal diet was somewhat less than that from rats of almost equal weight fed the vitamin E-supplemented diet or the stock diet (2.3, 2.6, and 2.7 per cent, respectively).

### Table I

*Respiratory Decline in Liver Slices and Its Prevention by Vitamin E*

<table>
<thead>
<tr>
<th>Days on diet†</th>
<th>Body weight</th>
<th>No. of rats</th>
<th>Incidence of necrosis</th>
<th>Liver slices</th>
<th>QO₁(F 100)§</th>
<th>Decline in respiration, a-dX 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>per cent</td>
<td></td>
<td></td>
<td>a-d</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td>65-100</td>
<td>14</td>
<td>0</td>
<td>Normal</td>
<td>2.7</td>
<td>222</td>
</tr>
<tr>
<td>40-50</td>
<td>150-200</td>
<td>11</td>
<td>0</td>
<td></td>
<td>3.1</td>
<td>242</td>
</tr>
<tr>
<td>21-29</td>
<td>69</td>
<td>22</td>
<td>41</td>
<td>Normal</td>
<td>2.2</td>
<td>224</td>
</tr>
<tr>
<td>30-39</td>
<td>74</td>
<td>10</td>
<td>70</td>
<td></td>
<td>2.3</td>
<td>210</td>
</tr>
<tr>
<td>40-49</td>
<td>79</td>
<td>32</td>
<td>78</td>
<td></td>
<td>2.3</td>
<td>227</td>
</tr>
<tr>
<td>50-70</td>
<td>82</td>
<td>27</td>
<td>84</td>
<td></td>
<td>2.3</td>
<td>227</td>
</tr>
<tr>
<td>30-60</td>
<td>81</td>
<td>10</td>
<td>100</td>
<td>Necrotic</td>
<td>2.2</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal diet for 3 wks.; then 50 mg. % α-tocopherol acetate added</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-30‖</td>
<td>92</td>
<td>12</td>
<td>0</td>
<td>Normal</td>
<td>2.6</td>
<td>237</td>
</tr>
<tr>
<td>40-70‖</td>
<td>141</td>
<td>9</td>
<td>0</td>
<td></td>
<td>2.6</td>
<td>200</td>
</tr>
<tr>
<td>90-180‖</td>
<td>168</td>
<td>12</td>
<td>0</td>
<td></td>
<td>2.6</td>
<td>216</td>
</tr>
</tbody>
</table>

* Male Sprague-Dawley rats; about 100 mg. of liver slices in 3 ml. of Krebs Ringer-phosphate medium; glucose 0.01 M; gas phase, O₂; temperature of incubation, 37.5°.
† Weanling rats, 18 to 20 days old, when started on the experiments.
‡ Number of rats showing signs of necrosis, either current or past. With the exception of the group so marked, all experiments were performed with slices from "normal" areas of liver; i.e., areas in which necrosis was not present.
§ QO₁ (F 100) = oxygen consumption (in microliters) per hour per 100 mg. of liver slices, calculated at 30 minute intervals of incubation. Averages of duplicate determinations.
‖ Days after 3 weeks on the basal diet.
The $Q_{o_2}$ (N) for the first 30 minute period was 91, 91, and 84 for the three groups of rats.

The average glycogen content of prenecrotic livers from animals on the basal diet did not differ from that of vitamin E-supplemented animals. Both values were within normal limits.

$O_2$ Consumption of Kidney and Diaphragm of Rats Fed Basal Diet—A series of experiments was conducted to determine the tissue specificity of the alteration in oxidative metabolism demonstrated for the liver of the rats fed the basal diet. Slices of liver and kidney and thin sheets of diaphragm were obtained from animals fed either the basal diet, the vitamin E-supplemented diet, or the stock diet and were incubated in Krebs-phosphate buffer as described above. The rats fed the basal diet alone were divided into two groups: those with livers essentially normal in appearance or showing minimal necrosis or scarring ("normal" slices), and those with severe hepatic necrosis in which necrotic areas even appeared in the slices. Rats in the former group were strong and active, whereas those of the latter group were weak and inactive.

\* Changing from the sodium salt medium of Krebs to the potassium-rich medium of Buchanan, Hastings, and Nesbett (16) tended to maintain the glycogen content of the slices during incubation, but did not affect the course of the respiratory lesion. Lowering the incubation temperature to 28°, while decreasing the rate of respiration and the rapidity of the decline, did not essentially alter the deranged pattern of respiration of deficient slices.
weak or moribund from liver disease. The results presented in Table II are calculated from data obtained after 30 and 60 minutes of incubation.

![Graph](http://www.jbc.org/)

**Fig. 2.** Cumulative frequency distribution of $Q_{02} (F 100)$ values for liver slices calculated at 0 to 30 and 90 to 120 minutes of incubation. For the experimental conditions see Table I. The figures in parentheses denote the number of rats.

**Table II**

Oxidative Metabolism of Liver, Kidney, and Diaphragm in Dietary Necrotic Liver Degeneration*

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of rats</th>
<th>Rat weight gm.</th>
<th>Gross appearance</th>
<th>$Q_{02}$ (F 100), average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rat</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stock†</td>
<td>9</td>
<td>121</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Basal Torula yeast</td>
<td>12</td>
<td>82</td>
<td>Normal</td>
<td>to some necrosis</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>60</td>
<td>Moribund</td>
<td>Massive necrosis†</td>
</tr>
<tr>
<td>Basal + vitamin E (50 mg. %)</td>
<td>8</td>
<td>112</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

* For the experimental conditions see Table I.
† Age controls and weight controls have been combined.
‡ Liver slices contained necrotic tissue; other tissues normal in appearance.

The pattern of oxygen consumption of kidney and diaphragm was similar for rats on all three diets. This finding contrasts with that for liver.
slight diminution of O₂ consumption may be present in kidneys of animals on the basal diet. However, this finding is not consistent; it may be correlated to pathological changes seen in the kidneys of rats fed the basal diet (13).

In moribund animals with pronounced gross changes of the liver, the respiration of kidney and diaphragm is still approximately normal, even though O₂ consumption of the necrotic liver amounts to only 20 per cent of that of non-necrotic controls (Table II). A small depression of the O₂ consumption of diaphragm may also occur in this terminal condition.

### Table III

**Effect of Addition of Sulfur Amino Acids to Basal Diet on Respiratory Decline of Liver Slices**

<table>
<thead>
<tr>
<th>Supplement after 3 wks. on basal diet</th>
<th>Deaths with necrosis</th>
<th>Liver necrosis at sacrifice</th>
<th>Q₀₂ (F 100) average</th>
<th>Decline in respiration, ( \frac{\text{min}}{\text{sec}} \times 100 ) per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kind</td>
<td>Concentration</td>
<td>Weeks</td>
<td>Weight gain per wk.</td>
<td>None</td>
</tr>
<tr>
<td>------</td>
<td>---------------</td>
<td>-------</td>
<td>---------------------</td>
<td>-----</td>
</tr>
<tr>
<td>None</td>
<td>0.5</td>
<td>1-4</td>
<td>8</td>
<td>5/10</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.5</td>
<td>10</td>
<td>13</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>10</td>
<td>13</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3</td>
<td>16</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4</td>
<td>18</td>
<td>0/5</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>1.0</td>
<td>4</td>
<td>13</td>
<td>3/7</td>
</tr>
<tr>
<td>Dl-Methionine</td>
<td>1.23</td>
<td>2</td>
<td>23</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>1.23</td>
<td>3</td>
<td>18</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>1.23</td>
<td>4</td>
<td>20</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* For the experimental conditions see Table I.
† Rats dead with necrosis versus total in group.
†† One rat liver severely necrotic; necrosis in area taken for slices Q₀₂ (F 100) = 77 at 30 minutes, not included in averages.

**Sulfur Amino Acids and Respiratory Decline**—In dietary necrotic liver degeneration, methionine, homocystine, and cysteine have shown only about 25 to 35 per cent of the activity of equivalent amounts of cystine (10, 17). Supplementation of the basal diet with 0.5 per cent cystine markedly prolongs the average survival time and reduces the incidence of dietary necrotic liver degeneration (13). In the current series (Table III), only two of the twenty rats receiving the 0.5 per cent cystine supplement developed gross necrosis during the 68 day experimental period. However, without exception, the respiration of the liver slices declined during the period of incubation. Increasing the supplementary cystine to 1 per cent almost completely prevented both the metabolic and the histological lesions of the
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Liver. Respiration of livers of rats which received 1 per cent cystine in no instance declined more than 20 per cent; the average decline was approximately 10 per cent after 2 hours of incubation.

Supplementation of the basal Torula diet with 1 per cent L-cysteine or 1.23 per cent DL-methionine promoted growth about as well as 1 per cent L-cystine, but, unlike cystine, did not fully protect against the pathologic and metabolic signs of dietary necrotic liver degeneration (Table III).

**Table IV**

Effect of Addition of Factor 3 to Basal Diet on Respiratory Decline of Liver Slices*

Groups of ten rats were fed the diets for 3 weeks.†

<table>
<thead>
<tr>
<th>Supplement after 3 wks. on basal diet</th>
<th>Concentration§gm.</th>
<th>Weight gain per wk.</th>
<th>Qtr (F 100), average</th>
<th>Decline in respiration, a — b</th>
<th>No. of livers showing no decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Brewers' yeast autolysate</td>
<td>64</td>
<td>13</td>
<td>228</td>
<td>185</td>
<td>19</td>
</tr>
<tr>
<td>B. Crude tissue powder</td>
<td>59</td>
<td>9</td>
<td>220</td>
<td>171</td>
<td>22</td>
</tr>
<tr>
<td>C. Factor 3 preparation from B</td>
<td>29</td>
<td>10</td>
<td>234</td>
<td>142</td>
<td>30</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>5</td>
<td>258</td>
<td>92</td>
<td>64</td>
</tr>
</tbody>
</table>

* For the experimental procedures see Table I.
† All rats fed Factor 3 concentrates survived and none showed signs of hepatic degeneration. Twenty rats were continued on the basal diet. In the second 3 week period, ten died with signs of necrosis, and ten survived, of which eight had areas of hepatic necrosis when sacrificed for respiration studies.
‡ Units per 100 gm. of diet determined (18) by ability of substance to prolong survival and prevent necrosis in rats fed the basal diet. 1 unit is that amount of protective substance which doubles the survival time when supplemented daily.
§ Respiration for 90 to 120 minutes was within 10 per cent of the initial rate.

Factor 3 and Respiratory Decline—In Table IV are summarized the effects of the addition of sources of Factor 3 on the respiratory lesion. Assay of potency of the preparations was made by the procedure previously reported (18). Supplements A and B almost completely prevented the occurrence of respiratory decline, whereas Supplement C was only half as effective with respect to both Factor 3 activity and the metabolic effect. No livers of rats fed Factor 3 were found to contain areas of necrosis at the time of sacrifice. The results with sulfur amino acids and Factor 3 fractions suggest that somewhat more of the effective substances is necessary to protect the liver from the metabolic defect than is necessary to protect it from necrosis per se.

Effect of Substrates on Decline in Respiration of Liver Slices—The effects of the addition of various metabolic intermediates on oxygen uptake of the
slices were investigated in an attempt to delineate the site of the respiratory lesion. After 20 to 30 minutes of control respiration, substrates were added from the side arm to the main compartment of the Warburg vessel. The amounts added were such that the final concentration of substrate was 0.01 M. Eight to sixteen flasks, each containing 100 mg. of slices, were prepared from each liver. This provided for duplicate comparisons of four to eight substrates on the same organ. The results, summarized in Fig. 3, are presented as the average percentages of the control respiration measured at 10 minute intervals for 90 minutes of incubation. Composite curves of the

![Graph](http://www.jbc.org/) Fig. 3. Effects of various substrates on O$_2$ uptake of liver slices. Initial 30 minute period of control respiration set equal to 100. For the experimental conditions see Table I. The points for lactate and oxalacetate are not recorded separately for the group on the basal diet; the results were practically identical with those presented for pyruvate.

values obtained from five experiments are presented. O$_2$ uptake during the control period was similar for all the samples from a single liver. Lactate, oxalacetate, pyruvate, fumarate, isocitrate, and cis-aconitate each significantly increased the oxygen uptake of normal liver slices. However, these substrates neither prevented nor markedly altered the decline with time of the respiratory rate of deficient liver slices. Of the substrates tested, only succinate and isocitrate significantly and consistently altered the pattern of respiration of such liver slices. They produced a temporary stimulation of O$_2$ uptake, which merely reflected the integrity of the specific dehydrogenase systems (19, 20) and which is not similar to the more prolonged stimulation of O$_2$ consumption produced in normal liver slices.

Studies with Liver Homogenates—Representative results, each derived from three to five individual experiments with liver homogenates of de-
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ficient and of normal animals, are illustrated in Fig. 4. In agreement with Wenner, Dunn, and Weinhouse (21), it was observed that supplementation of normal liver homogenates with ATP is relatively unimportant and that DPN is essential for the maintenance of rapid rates of oxygen uptake in liver homogenates. The addition of ATP to the DPN-fortified homogenate resulted in small, but consistent, increases of oxygen consumption. Metabolites such as fumarate, succinate, malate, lactate, α-ketoglutarate, and pyruvate, when added to the ATP- and DPN-fortified system, in-

![Diagram](http://www.jbc.org)  
**Fig. 4.** Oxygen consumption of liver homogenates. Each Warburg vessel contained 1 ml. of a 10 per cent homogenate in KCl-PO₄ buffer (see the text) and 2 ml. of the buffer with the substances indicated; gas phase, air; 37.5°. Curve 1, homogenate alone; Curve 2, same as for Curve 1 + MgSO₄, glucose, and cytochrome c; Curve 3, as for Curve 2 + ATP; Curve 4, as for Curve 2 + DPN; Curve 5, as for Curve 4 + nicotinamide; Curve 6, as for Curve 5 + ATP; Curve 7, as for Curve 6 + α-ketoglutarate; Curve 8, as for Curve 7 + coenzyme A; Curve 9, as for Curve 6 + lactate; Curve 10, as for Curve 9 + coenzyme A. See the text for concentrations.

creased oxygen uptake only to a small extent. Coenzyme A in any substrate combination tested produced little or no additional respiration.

The pattern of oxygen utilization by homogenates of deficient livers under these varying conditions was not significantly different. The progressive respiratory failure of the deficient liver slice could not be duplicated in the homogenate prepared from the same liver. However, it should be borne in mind that the duration of respiration of homogenates is much shorter than that of slices and is determined by the kind and amount of supplements added to the medium. The respiratory rates of fortified homogenates were approximately twice that of slices prepared from the same liver. In a typical experiment with liver from a rat fed the basal diet, the Qₒₒ (F 100) values for slices at 30, 60, and 90 minutes were 232, 175, and 135, respectively; the
homogenate, fortified by addition to the medium of DPN and nicotinamide, gave values of 464, 460, and 399.

Homogenates of normal and deficient livers were also found to be similar in their anaerobic glycolytic metabolism, as indicated by amounts and patterns of CO₂ liberation. These homogenates were fortified with ATP, DPN, nicotinamide, fructose diphosphate, and pyruvate, as suggested by Novikoff et al. (22).

Hepatic Damage from Other Causes—Several groups of ten male Sprague-Dawley rats each were fed diets reported to produce necrotic or fatty changes in hepatic cells. At several intervals rats were killed, and the effect of dietary treatment on the oxygen consumption of liver slices was tested as before. Only the three following groups need be considered in detail.

High fat-low protein diet (60 per cent lard, 10 per cent casein, 22 per cent sucrose, 5 per cent salts (14), 1 per cent vitamin mixture,⁴ and vitamins A and D in corn oil, 2 per cent⁵).

5 per cent cystine diet (15 per cent casein, 5 per cent cystine, 5 per cent lard, 69 per cent sucrose, 1 per cent vitamins (13), and 5 per cent salts (14)). Histological examination after 3 days on the diet revealed massive portal necrosis. About half of the rats died within 4 days. In some experiments, 50 mg. of dl-α-tocopherol were added per 100 gm. of diet (the amount used to protect rats on the Torula yeast diet from dietary necrotic liver degeneration).

0.6 per cent ethionine diet (18 per cent casein, 73.5 per cent sucrose, 0.5 per cent dl-ethionine, 1 per cent vitamins (13), 5 per cent salts (14), and vitamins A and D in corn oil, 2 per cent⁶). Histological examination after 4 or 9 days on the diet revealed moderately heavy deposition of fat in hepatic cells but few other cellular abnormalities.

The O₂ consumption recorded in Table V is expressed as the total consumed during the 1st and the 2nd hour of incubation per 100 mg. of slices or per mg. of slice nitrogen. Only respiration of liver slices from rats fed the basal diet declined significantly during the 1st hour.

It was estimated that as much as 40 per cent of the hepatic cells were necrotic in some of the livers from rats fed the 5 per cent cystine diet. Respiration of such livers was not, however, lower than in normal controls. Addition of vitamin E to the cystine diet did not significantly alter the respiration. After 11 days, fatty infiltration and fibrosis of the livers were quite marked, which is reflected in Qₒₑ values.

Ennor (23) has made similar observations on livers of rats poisoned with

⁴ The vitamin mixture used was the same as that reported previously (13), except for the omission of choline.

⁵ Vitamin A 275 U. S. P. units and vitamin D 55 U. S. P. units per gm. of oil.
carbon tetrachloride or phosphorus. Even in the presence of considerable necrosis, he found liver respiration, on a defatted, dry weight basis, to be normal or greater. No decline in respiration with time was reported. Oxidation of fatty acids was likewise unimpaired in the poisoned livers.

These various types of liver injury failed to affect the ability of liver slices to maintain their initial rate of respiration for 2 hours in vitro. Only in the livers of rats on the ration producing dietary necrotic liver degeneration was there marked decline of oxygen consumption.

**TABLE V**

Dietary Liver Damage and Oxygen Consumption*

<table>
<thead>
<tr>
<th>Diet†</th>
<th>No. of rats</th>
<th>Liver slices</th>
<th>Qo (F 100)‡</th>
<th>Qo (N)</th>
<th>Decline in respiration ( \frac{a - b}{a} \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pathology</td>
<td>N content</td>
<td>0-60 min.</td>
<td>60-120 min.</td>
</tr>
<tr>
<td>Stock</td>
<td>40</td>
<td>Normal</td>
<td>3.5</td>
<td>280</td>
<td>272</td>
</tr>
<tr>
<td>Basal Torula yeast</td>
<td>30</td>
<td>Very fatty</td>
<td>2.3</td>
<td>189</td>
<td>179</td>
</tr>
<tr>
<td>High fat-low protein</td>
<td>4</td>
<td>Fatty, some necrosis</td>
<td>3.3</td>
<td>333</td>
<td>272</td>
</tr>
<tr>
<td>0.5% ethionine</td>
<td>11</td>
<td>Extensive portal necrosis</td>
<td>3.2</td>
<td>245</td>
<td>230</td>
</tr>
<tr>
<td>5% cystine</td>
<td>3</td>
<td>Necrosis, fibrosis, fatty infiltration</td>
<td>2.9</td>
<td>257</td>
<td>230</td>
</tr>
</tbody>
</table>

* For the experimental conditions see Table I.
† Rats maintained on the stock diet for 5 weeks after weaning and then fed experimental diet. The basal Torula diet was fed from weaning.
‡ Oxygen consumption for 1st hour and 2nd hour of respiration.

**DISCUSSION**

Slices from the livers of rats fed a vitamin E-free Torula yeast diet, which produces dietary necrotic liver degeneration, failed to maintain their initially normal respiratory rate, whereas liver slices from control rats respired for 2 to 3 hours without appreciable decline in the rate of oxygen uptake. Failure was evident after half an hour of incubation and antedated the histopathological changes of dietary necrotic liver degeneration by several weeks. The respiratory lesion was demonstrable in liver, but not consistently in kidney or diaphragm. By addition of appropriate substrates to the medium, it was shown that the metabolic lesion could not be characterized
in terms of defects in any of the specific enzymes of the glycolytic or tricarboxylic acid cycles. Evidence was obtained of the integrity of the succinate and isocitrate dehydrogenase enzymes and of the cytochrome systems to which they are linked. None of the substrates tested prevented the ultimate decline in O$_2$ uptake that distinguishes this hepatic defect from those described for diabetes (24) and fasting (25). The conversion of acetate to CO$_2$, fatty acids, or ketone bodies is likewise impaired in deficient livers (26).

Homogenates of deficient livers, on the other hand, failed to reveal the metabolic lesion and consumed oxygen in a normal manner. The foregoing indicates that the primary lesion in prenecrotic livers is not located in one of the main streams of substrate utilization, but rather belongs to an essential, subsidiary mechanism. Results with homogenates tend to indicate that the generation of an adenine nucleotide may be primarily involved.

Addition to the basal diet of vitamin E, cystine, or a source of Factor 3 prevented the development of the metabolic defects that produced respiratory decline. These substances were also effective in preventing dietary necrotic liver degeneration, which is inevitable in rats fed the basal diet alone. The amounts of protective substances necessary to prevent the occurrence of the metabolic defect appeared to be somewhat greater than the amounts that prevented necrosis.

While difficult to prove with the data at hand, the results strongly suggest a relationship between the earlier metabolic changes and the necrosis that follows. Apparently, conditions in vitro bring into the open a degenerative metabolic process already under way in deficient livers. The final low metabolic activity represents a biochemical, but not yet histological, "necrobiosis" of the liver slices.

Dietary necrotic liver degeneration and the metabolic defect are prevented by the presence in the diet of adequate amounts of either vitamin E, cystine, or Factor 3 alone. In vitamin E deficiency, a usual metabolic finding is elevated oxygen consumption of isolated muscle strips before or during the phase of neuromuscular degeneration (27). These substances were also effective in preventing dietary necrotic liver degeneration, which is inevitable in rats fed the basal diet alone. The amounts of protective substances necessary to prevent the occurrence of the metabolic defect appeared to be somewhat greater than the amounts that prevented necrosis.

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bated with liver slices. The delayed inhibitory action of these drugs may be due to their slow rate of penetration into the cells (29), or to their conversion into inhibitory degradation products (30), or to the uncoupling of oxidative phosphorylation (31). The first two cases do not seem to apply to the metabolism of prenecrotic liver in dietary necrotic liver degeneration.

The view that failure in oxidative phosphorylation may be involved in the progressive decline in respiration gains credence from the observation that O₂ uptake is normal in fortified homogenates of deficient livers. This suggestion is under investigation. Indications that the primary metabolic lesion in dietary necrotic liver degeneration may be located close to the citric acid cycle had been obtained from earlier experiments (10): A serious incompatibility of fat can be demonstrated in prenecrotic animals. Administration of 2 gm. of fat precipitates the terminal phase of the disease (32). The sequence of events during this final phase, in turn, shows a complete breakdown of the carbohydrate balance (33). It was also observed that the citric acid level in such livers could be greatly elevated by sodium monofluoroacetate (10).

SUMMARY

1. Slices of livers from rats fed a vitamin E-free Torula yeast diet that produces necrotic liver degeneration consume oxygen at a normal rate for approximately 30 minutes. Thereafter, respiration declines until the oxygen uptake at 90 to 120 minutes of incubation is only 30 to 50 per cent of normal.

2. This metabolic defect is routinely observed in livers which are histologically normal, several weeks before the onset of necrosis.

3. Kidney and diaphragm of such animals show normal O₂ consumption patterns.

4. Substances which prevent or cure dietary necrotic liver degeneration, such as vitamin E, cystine, and Factor 3, also prevent the occurrence of the metabolic defect in liver slices. In the case of Factor 3 preparations, the observed degree of protection against the metabolic lesion was found to parallel the necrosis-preventing activity.

5. Addition of succinate or isocitrate to deficient liver slices caused a brief stimulation of oxygen uptake; other substrates tested were essentially without effect on the deranged pattern of respiration.

6. Respiratory patterns of liver homogenates from deficient rats did not differ significantly from those of normal rats under various conditions of fortification with coenzymes and substrates.

7. The characteristic pattern of the metabolic defect, normal respiration followed by a marked decline, is not observed in livers of rats fed a high fat-low protein diet, 5 per cent cystine, or ethionine nor in those poisoned with
phosphorus or carbon tetrachloride, although histological changes in the livers may be very severe.

BIBLIOGRAPHY

A METABOLIC LESION IN DIETARY NECROTIC LIVER DEGENERATION
Sidney S. Chernick, Janet G. Moe, Gerald P. Rodnan and Klaus Schwarz


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