Studies on Influence of Protein on the Liver Injury due to Chloroform and Carbon Tetrachloride

By

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There are many papers which reported the protective effects of nutrition against liver injury due to poisons. Goldschmidt1) observed that a high protein diet, previous to the anesthesia with chloroform, markedly reduced the incidence of hepatocellular necrosis in rats. Miller2) reported that chloroform anesthesia could be tolerated with little evidence of liver injury in well-fed healthy dogs. Howe3) showed higher survival rate in the rats on high protein diet than on low protein diet in the experiment of carbon tetrachloride poisoning. All these reports stressed the protective effects of high protein diet, which is one of the basis for the high protein therapy for liver diseases.

On the other hand, papers against these results have been frequently published. In the experiment of Drill et al.4), bromsulphalein retention test showed a greater degree of hepatic dysfunction in the dogs fed with a normal protein diet than those receiving a low protein high carbohydrate diet, and hepatocellular necrosis was not observed in the dogs fed with the low protein diet but was present in 3 of 6 dogs fed with the normal protein diet. According to Campbell and Kosterlitz5), the rats placed on a protein-free diet for 3 days showed the least liver damage, whereas those on a 18% casein diet showed the highest incidence of hydropic degeneration.

Thus the results are not always in accordance with each other. In the experiment of long term poisoning of carbon tetrachloride of the authors, fibrosis of the liver of rats was more extensive on a high protein diet than on a low protein diet6)-8). What caused such disaccordance of the results in connection with the influence of protein on liver injury? Is it due to the difference of poisons? Is it due to the difference of judging the results on the basis of death rate of experimental animals, pathological changes of the liver or liver function tests? Or is it due to the assumption as proposed by Campbell and Kosterlitz5) that low protein diet is effective
when the duration of protein deficiency is short whereas high protein diet is effective when the duration is long.

In order to answer these questions, rats were administered with chloroform and carbon tetrachloride after they were fed with low protein or high protein diet for various periods. Death rate and pathological findings of the liver and kidney were studied in these animals.

**Experimental**

**Method**

The composition of experimental diets employed is shown in Table I.

**Table I**

Composition of Experimental Diets

<table>
<thead>
<tr>
<th>Constituent</th>
<th>5% casein diet</th>
<th>30% casein diet</th>
<th>18% yeast diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Casein</td>
<td>5</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>85</td>
<td>60</td>
<td>72</td>
</tr>
<tr>
<td>Lard</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Osborne-Mendel's salt mixture</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Daily vitamin supplements: thiamin 50 µg., riboflavin 50 µg., pyridoxine 20 µg., Ca-pantothenate 100 µg., and nicotinamide 500 µg.

Rats were fed with 5% casein and 30% casein diet for 5 days, 2 weeks, 4 weeks and 8 weeks respectively, and thereafter administered with chloroform or carbon tetrachloride by stomach tube. Deaths were recorded every 12 hours. Three days after the administration of the poisons all surviving rats were killed in order to study the liver and kidney pathologically. Chloroform and carbon tetrachloride were administered at the level of 0.35-0.635 and 1.7-1.75 cc./kg. body weight respectively. Preliminary experiment established that this level of chloroform or carbon tetrachloride would kill about 50% of the rats on the high protein diet 3 days after the administration. However, comparison of death rate was occasionally unsatisfactory due to low mortality of rats. Chloroform was used as 20-40% solution of olive oil, while carbon tetrachloride was used alone.

Histologically the ratio of necrosis of liver cells to specimens was calculated. There may be a possibility that even under the same condition of sex of animals and amount of poison death rate and pathological changes of the liver may vary at different time of experiment. Therefore, comparisons were made only between two dietary groups which were fed
at the same time. In some experiments the difference of resistance to poison appeared to be influenced by body weight, and an additional experiment was undertaken to investigate this problem.

Results

Experiment of chloroform poisoning
The results are shown in Table II.

Table II
Influence of Protein on Liver Injury Due to Single Administration of Chloroform

<table>
<thead>
<tr>
<th>Duration of feeding before administration of CHCl₃</th>
<th>Sex of rats</th>
<th>CHCl₃ administered, cc/kg.</th>
<th>Diet</th>
<th>Initial body weight, g.</th>
<th>Body weight at the time of administration of CHCl₃, g.</th>
<th>Death rate after administration, %</th>
<th>Per cent of rats showing liver necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>F 0.635</td>
<td>5% casein</td>
<td>14</td>
<td>139.9</td>
<td>134.4</td>
<td>11.1</td>
<td>0 14 36* 36 43 57</td>
</tr>
<tr>
<td>2 weeks</td>
<td>F 0.635</td>
<td>5% casein</td>
<td>11</td>
<td>146.2</td>
<td>141.6</td>
<td>11.2</td>
<td>0 36* 64* 73 73 73</td>
</tr>
<tr>
<td></td>
<td>F 0.35</td>
<td>5% casein</td>
<td>15</td>
<td>135.2</td>
<td>121.6</td>
<td>10.4</td>
<td>0 7 7 13 27 27</td>
</tr>
<tr>
<td>4 &quot;</td>
<td>F 0.35</td>
<td>5% casein</td>
<td>14</td>
<td>83.3</td>
<td>132.2</td>
<td>11.1</td>
<td>0 0 0 0 0 8</td>
</tr>
<tr>
<td>8 &quot;</td>
<td>F 0.35</td>
<td>30% casein</td>
<td>12</td>
<td>145.1</td>
<td>140.1</td>
<td>11.0</td>
<td>0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

* The difference between 5% casein diet group and 30% casein diet group is statistically significant.

Death rate: In the experiments of 5 day feeding and 2 week feeding, death rate of 5% casein group was higher than that of 30% casein group 1 or 1.5 days after the administration. The difference was statistically significant (P<0.05). In the experiments of 4 week feeding and 8 week feeding, no significant difference was observed between both dietary groups.

Pathology of the liver: Macroscopically, the liver was generally swollen, soft and friable, and presented a mottled appearance with yellowish-gray necrotic areas and reddish-brown hemorrhagic areas. Histological studies revealed coagulation necrosis with hemorrhage and round cell infiltration in the central and middle part of hepatic lobules, which occasionally extended all over the hepatic lobules. Sometimes fatty metamor-
phosis of liver cells was noticed at the periphery of the hepatic lobules, and hydropic degeneration of liver cells and congestion of blood were more or less observed in all groups.

The difference of frequency of hepatocellular necrosis between 5% casein and 30% casein groups was statistically significant in the experiments of 4 week feeding and 8 week feeding (P<0.05), while there was no significant difference in the experiments of 5 day feeding and 2 week feeding.

Pathology of the kidney: The kidney was swollen, friable, tarnished, and reddish-gray in color. Histologically, the epithelium of renal tubules showed cloudy swelling and hydropic degeneration, including coagulation necrosis of the epithelium of convoluted tubules and hyaline and granular casts in the lumen of tubules. No changes was, however, noticed in Bowman's capsule and glomerulus. On the whole, the degree of injury of the kidney were slighter than that of the liver.

The frequency of coagulation necrosis of the epithelium of renal tubules was significantly lower in 5% casein group than in 30% casein group in the experiment of 2 week feeding, whereas no statistically significant difference was observed in the other experiments.

Thus, in the experiment of chloroform poisoning, liver injury was severer on the low protein diet than on the high protein diet when the duration of protein deficiency was long. The death rate failed to run parallel with the degree of liver and kidney injury.

*Experiment of carbon tetrachloride poisoning*

The results are shown in Table III.

Death rate: In the experiments of 5 day feeding and 2 week feeding both dietary groups showed low death rate without statistically significant difference. In the experiment of 4 week feeding the death rate of 5% casein group 1.5 or 3 days after the administration of carbon tetrachloride was lower than that of 30% casein group. The difference was statistically significant (P<0.05). In the experiment of 8 week feeding the death rate of 5% casein group 1 day after the administration was significantly higher than that of 30% casein group (P<0.05).

Pathology of the liver: The changes of the liver was, macroscopically and histologically, almost the same as observed in chloroform poisoning. In the experiment of 4 week feeding, the frequency of liver necrosis was significantly lower in 5% casein group or 18% yeast group than in 30% casein group (P<0.05), whereas no significant difference was observed in the experiments of 5 days feeding, 2 week feeding, and 8 week feeding.

Pathology of the kidney: The kidney showed almost the same change as in the chloroform poisoning, but the degree of injury was slighter.
Influence of Protein on Liver Injury

**TABLE III**

Influence of Protein on Liver Injury Due to Single Administration of Carbon Tetrachloride

<table>
<thead>
<tr>
<th>Duration of feeding before administration of CCl₄</th>
<th>Sex of rats</th>
<th>CCl₄ administered, cc/kg.</th>
<th>Diet</th>
<th>No. of rats</th>
<th>Initial body weight, g.</th>
<th>Body weight at the time of administration of CCl₄, g.</th>
<th>Daily food intake, g.</th>
<th>Dath rate after administration, %</th>
<th>Per cent of rats showing liver necrosis</th>
<th>Per cent of rats showing proximal tubular necrosis of the kidney</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>F</td>
<td>1.75</td>
<td>5% casein</td>
<td>13</td>
<td>140.8</td>
<td>138.1</td>
<td>11.1</td>
<td>0</td>
<td>8</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30% casein</td>
<td>14</td>
<td>154.6</td>
<td>14</td>
<td>161.9</td>
<td>11.3</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>2 weeks</td>
<td>F</td>
<td>1.75</td>
<td>5% casein</td>
<td>11</td>
<td>135.9</td>
<td>138.5</td>
<td>11.4</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>30% casein</td>
<td>10</td>
<td>147.3</td>
<td>10</td>
<td>168.4</td>
<td>11.9</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>4 days</td>
<td>M</td>
<td>1.7</td>
<td>5% casein</td>
<td>14</td>
<td>62.7</td>
<td>58.2</td>
<td>5.9</td>
<td>7</td>
<td>14</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>30% casein</td>
<td>15</td>
<td>63.0</td>
<td>15</td>
<td>155.3</td>
<td>10.9</td>
<td>7</td>
<td>7</td>
<td>80<em>80</em>87<em>87</em></td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>18% yeast</td>
<td>15</td>
<td>64.7</td>
<td>15</td>
<td>88.3</td>
<td>9.1</td>
<td>13</td>
<td>13</td>
<td>27</td>
<td>7</td>
<td>33</td>
</tr>
<tr>
<td>8 days</td>
<td>M</td>
<td>1.6</td>
<td>5% casein</td>
<td>4</td>
<td>54.1</td>
<td>59.6</td>
<td>5.9</td>
<td>50</td>
<td>75</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30% casein</td>
<td>7</td>
<td>64.8</td>
<td>7</td>
<td>197.6</td>
<td>11.6</td>
<td>0</td>
<td>0</td>
<td>57</td>
<td>57</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>18% yeast</td>
<td>6</td>
<td>67.5</td>
<td>6</td>
<td>122.3</td>
<td>10.2</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

* The difference between 30% casein diet group and 5% casein or 18% yeast diet group is statistically significant.

Coagulation necrosis of the epithelium of tubules was observed in only 2 animals of the 5% casein group in the experiments of 4 week feeding and 8 week feeding. The frequency of coagulation necrosis, hydropic degeneration, and cloudy swelling showed no significant difference between 2 dietary groups.

Thus the results of carbon tetrachloride poisoning was different from those of chloroform poisoning. In the experiment of 4 week feeding, liver injury was milder and death rate was lower on the low protein diet than on high protein diet. Such tendencies was, however, not observed in the experiment of 8 week feeding.

In the experiment, in which the influence of body weight on the liver injury due to carbon tetrachloride was investigated, the frequency of liver necrosis of the rats in the group with an average body weight of 58.6 g. was significantly higher than that in the group with an average body weight of 172.1 g. (P=0.0042) (Table IV).

**DISCUSSION**

In the experiments it was noticed that liver injury of the low protein...
Influence of Body Weight on Liver Injury Due to Single Administration of Carbon Tetrachloride

* The difference between large body weight group and small body weight group is statistically significant.

** The composition of the 10% casein diet is as follows: casein 10%, sucrose 80%, lard 5%, cod liver oil 1%, and Osborne-Mendel's salt mixture 4%. Vitamins are added to supply the following amounts daily: thiamin 50 μg., riboflavin 50 μg., pyridoxine 20 μg., Ca-pantothenate 100 μg. and nicotinamide 500 μg.

<table>
<thead>
<tr>
<th>Duration of feeding before administration of CCl₄</th>
<th>Sex of rats</th>
<th>No. of rats</th>
<th>Initial body weight, g.</th>
<th>Body weight at the time of administration of CCl₄, g.</th>
<th>Diet</th>
<th>Daily food intake, g.</th>
<th>Death rate after administration, %</th>
<th>Per cent of rats showing liver necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>F</td>
<td>12</td>
<td>175.6</td>
<td>172.1</td>
<td>10%</td>
<td>12.0</td>
<td>0 0 0 0 0 0 0 0</td>
<td>21 0 0 0 21*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>57.2</td>
<td>58.6</td>
<td>10%</td>
<td>8.0</td>
<td>0 0 6 12 12 12</td>
<td>54 18 6 76*</td>
</tr>
</tbody>
</table>

* The difference between large body weight group and small body weight group is statistically significant.

** The composition of the 10% casein diet is as follows: casein 10%, sucrose 80%, lard 5%, cod liver oil 1%, and Osborne-Mendel's salt mixture 4%. Vitamins are added to supply the following amounts daily: thiamin 50 μg., riboflavin 50 μg., pyridoxine 20 μg., Ca-pantothenate 100 μg. and nicotinamide 500 μg.

group which was administered with chloroform was severer than that of the high protein group. On the contrary, liver injury of the low protein group which was administered with carbon tetrachloride was slighter than that of the high protein group in the experiment of 4 week feeding. Accordingly, chloroform and carbon tetrachloride poisoning showed quite different results.

Since a remarkable difference of body weight was observed between the two dietary groups which received carbon tetrachloride in the experiments of 4 week feeding and 8 week feeding, there seems to be a possibility that such a difference may be attributed to lower sensitivity to carbon tetrachloride of low protein group with light body weight. However, the experiment, which was undertaken to investigate this problem, revealed that the liver injury of light body weight group was, on the contrary to the conjecture, severer than that of the heavy body weight group. Taking the above-mentioned results into consideration, the liver injury of the low protein group which received carbon tetrachloride would be severer than that of the high protein group even in the experiment of 8 week feeding, if the influence of body weight were excluded.

When the results of the present investigation are compared with the former reports, they accord with Miller's report that liver injury of low
protein diet group which was administered with chloroform was severer than that of high protein group when protein depletion was over a certain limit. In the experiment of chloroform poisoning the difference between both dietary groups was not noticed when protein deficiency was slight. This result does not support Campbell's assumption that low protein diet gives the higher protection against liver injury if given for short periods before the administration of poisons whereas prolonged protein deficiency renders the liver highly susceptible to injury.

In the experiment of carbon tetrachloride poisoning, liver injury of the low protein group was milder than that of the high protein group when it was fed for 4 weeks. This observation supports more Drill's report than Hove's, and is in accordance with the previous reports of the present authors on prolonged carbon tetrachloride poisoning.

On the basis of death rate, Hove admitted the protective effect of high protein diet on carbon tetrachloride poisoning. However, the experiments indicate that death rate fails to accord with the patho-histological changes of liver cells. Judging results only on death rate may be inappropriate for the index for the estimation of the degree of liver injury.

According to Campbell and Kosterlitz, carbon tetrachloride liver injury of the rats which were fed with no protein for 3 days was milder than that of the rats fed with 18% or 54% casein diet. The present experiment showed that liver injury of the rats on low protein diet was milder than that on the high protein diet in the experiment of 4 week feeding, but not in the experiments of 5 day feeding and 2 week feeding. The difference may be attributed to the difference that 5% casein diet was used in the present experiment while no protein was included in Campbell's diet.

Thus the studies of the authors on the influence of protein on the liver injury due to poisons show that there is difference between chloroform administration and carbon tetrachloride administration, namely, if the protein depletion is over a certain limit, high protein diet is protective against the liver injury due to chloroform poisoning, while low protein diet is protective against the liver injury due to carbon tetrachloride. When the former reports are reviewed on the basis of this result, the disaccordance of the former reports can be comparatively well explained.

It is an interesting question why chloroform and carbon tetrachloride, chemically very related to each other, would cause such a difference. A further investigation is expected on this line.

SUMMARY AND CONCLUSIONS

1. Rats were fed with 5% and 30% casein diet for 5 days, 2 weeks, 4 weeks, and 8 weeks, and were given chloroform or carbon tetrachloride
by stomach tube. Three days after the administration, they were killed in order to study the liver and kidney pathologically. The difference due to nutrition was investigated.

2. In the experiment of chloroform poisoning, the incidence of liver necrosis in the rats which were fed with 5% casein diet for 4 or 8 weeks before the administration of chloroform was significantly higher than that in the rats which were fed with 30% casein diet. In the experiment of carbon tetrachloride poisoning, on the other hand, the incidence of liver necrosis of the rats which were fed with 5% casein diet for 4 weeks was significantly lower than that of the rats which were fed with 30% casein diet.

3. Kidney injury was generally milder than liver injury, and it was milder in the experiment of carbon tetrachloride poisoning than in that of chloroform poisoning. The incidence of necrosis of the epithelium of renal tubules was significantly lower in the rats which were fed with 5% casein diet for 2 weeks before the administration of carbon tetrachloride than in those which were fed with 30% casein diet.

4. Death rate after the administration of the poisons failed to run parallel with the degree of liver injury and was considered to be inappropriate for the indicator for the estimation of the degree of liver injury after the administration of poisons.

Death rate was also not proportional to the degree of kidney injury.

5. The disaccordance of the former reports regarding the influence of protein on liver injury due to poisons can be comparatively well understood by this experiment, if it is explained on the difference of used poisons.

References

7) Idem, ibid, 1956, 45, 858.