Abstract Changes of blood glucose, protein and lipid levels during fasting in sheep and rats, and the effects of intravenously injected glucose on plasma 11-OHCS, protein and lipid levels were studied in the fasting sheep. In both species, blood glucose level decreased for the first few days (P<0.05), then remained at a low level and finally showed a slight rebound. During fasting period plasma total protein level continued to increase up to 10 days in sheep (P<0.01), while it began to decrease on the 4th day of fasting in rats where decrease was significant only in the female on the 6th day (P<0.05). Changes in plasma total lipid level with the progress of fasting were similar to that of protein in both species. In sheep, major changes in lipids were found in the free fatty acid fraction (FFA) (P<0.01). Intravenous injection of glucose into the fasting sheep caused marked decreases in levels of plasma 11-OHCS (P<0.01), total plasma protein (P<0.01), total lipids (P<0.05) and FFA (P<0.01). Clear reciprocal changes found between levels of circulating 11-OHCS and blood glucose in the fasting sheep suggested that glucocorticoids seem to play an important role for the regulation of blood glucose in this species.

Materials and Methods

Experimental animals, sheep (Japanese Corriedale) and rats (Wistar strain), were fed at the Laboratory of Animal Breeding of Miyazaki University. About 16 months old wethers and about 2 months old rats were used. The mean body weights with standard deviation were 34.7±4.8 kg in sheep, 308.8±56.1 g in the male rats and 211.3±28.6 g in the female rats. Each animal was kept individually in a metal cage for rats and in a pen with sand bed for sheep. Rats were fed in an environment-controlled chamber (Koitotron), where the temperature was kept at 25°C and the relative humidity was 60 to 70%. Water was given ad libitum during fasting.

This study consisted of two experiments. In Exp. I, blood samples were taken from each
sheep (6 heads) on the 0, 2, 4, 6, 8, 10, 20 and 30th day after the removal of feeds, and from the different rats (24 males; 15 females) allotted to each particular day: 0, 2, 4, 6, 8 and 10th day of fasting. Blood glucose, plasma protein and lipid were determined in all samples.

In Exp. II, glucose solution (10 g%, w/v) was intravenously injected twice with the interval of one hour into the sheep fasted for 24 days. The dose for each time was 0.5 mM per kg of the body weight before fasting as was used by MCATEE and TRENKLE. It took 5 minutes to inject the dose of glucose. Blood samples to determine blood glucose, plasma 11-OHCS, protein and lipids, were taken at 0, 2, 3, 4, 6 and 28 hours after the first injection of glucose.

Blood glucose, plasma total lipid and protein were determined by SOMOGYI method, BRAGDON method and Tsukasa Protein Refractometer, respectively. Densitometric determination of plasma lipid fractions by TLC was carried out by the method of KANNO et al. Plasma 11-OHCS were determined by the method described previously.

Results

(Exp. I)

Blood glucose, plasma total protein and lipid levels in sheep and rats before the removal of feeds are shown in Table 1.

Changes of body weight, blood glucose, plasma total protein and lipid levels are shown in Figs. 1 and 2. Body weight gradually decreased during fasting in both sheep and rats as in the previous experiment. Blood glucose level decreased more rapidly for the first 2 days.

Table 1. Blood glucose, plasma total protein and lipid levels in sheep and rats before the removal of feeds

<table>
<thead>
<tr>
<th>Animal</th>
<th>Glucose (mg%)</th>
<th>Protein (g%)</th>
<th>Lipids (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep (6)</td>
<td>47.6±3.9*</td>
<td>5.90±0.33</td>
<td>251.9±15.8</td>
</tr>
<tr>
<td>Male (6)</td>
<td>99.7±10.8</td>
<td>6.53±0.35</td>
<td>283.2±63.1</td>
</tr>
<tr>
<td>Female(3)</td>
<td>89.2±12.2</td>
<td>7.27±0.25</td>
<td>487.4±18.2</td>
</tr>
</tbody>
</table>

*: Mean±standard deviation; numbers of animals are given in parentheses

Table 2. Effect of fasting on plasma lipid fraction levels in sheep

<table>
<thead>
<tr>
<th>Day of fasting</th>
<th>Cholesterol ester</th>
<th>Triglyceride</th>
<th>Free fatty acids</th>
<th>Cholesterol</th>
<th>Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>57±21</td>
<td>47±21</td>
<td>10±7*</td>
<td>46±7</td>
<td>92±7</td>
</tr>
<tr>
<td>2</td>
<td>39±11</td>
<td>35±7</td>
<td>71±30</td>
<td>50±14</td>
<td>75±16</td>
</tr>
<tr>
<td>4</td>
<td>46±14</td>
<td>36±19</td>
<td>83±32</td>
<td>50±16</td>
<td>80±13</td>
</tr>
<tr>
<td>6</td>
<td>45±16</td>
<td>37±23</td>
<td>101±30</td>
<td>68±13</td>
<td>74±22</td>
</tr>
<tr>
<td>8</td>
<td>54±20</td>
<td>28±16</td>
<td>109±17</td>
<td>71±9</td>
<td>73±17</td>
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<tr>
<td>10</td>
<td>58±15</td>
<td>30±8</td>
<td>104±15</td>
<td>64±11</td>
<td>84±22</td>
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<td>15</td>
<td>55±7</td>
<td>21±6</td>
<td>84±17</td>
<td>63±9</td>
<td>83±16</td>
</tr>
<tr>
<td>20</td>
<td>42±5</td>
<td>29±12</td>
<td>107±21</td>
<td>63±13</td>
<td>81±24</td>
</tr>
</tbody>
</table>

(Unit: mg%) a and b: Differences between means in a column with the different letter are significant (P<0.01).

Each value is the mean with S.D. of four determinations.
Glucocorticoids and Blood Glucose Level in the Fasted Animals

Fig. 1. Changes of body weight, blood glucose, plasma total protein and lipid levels during fasting in sheep
* Mean of six determinations, expressed as percentage for the value before the removal of feeds.

in rats and 4 days in sheep after the removal of feeds than did body weight. The mean blood glucose level at the end of each period was significantly lower than the control level (P<0.05). From that time, the blood glucose level was maintained up to the end of fasting, or even elevated at the time.

The change of plasma total protein level in sheep was contrasted with that in rats. The protein level increased up to 10 days of fasting in sheep (P<0.01), but began to decrease on the 4th day after the removal of feeds in rats and its decrease became significant on the 6th day in the female (P<0.05).

Plasma total lipid level in sheep began to increase on the 2nd day of fasting and reached a maximum on the 10th day (P<0.05). But the level seemed to decrease gradually thereafter (Fig. 1). FFA increased markedly by fasting (P<0.01), while triglyceride somewhat decreased as shown in Table 2. In rats plasma total lipid level decreased rapidly (Fig. 2).

(Exp. II)

The mean plasma 11-OHCS level with standard deviation prior to glucose injection was 4.50±1.08 µg/100 ml and it was significantly higher than the level before the fasting; 3.00±0.95 µg/100 ml (P<0.05).
When glucose was injected into the sheep fasted for 24 days, the blood glucose level changed as shown in Fig. 3. The increase of blood glucose level was accompanied with the significant decline of plasma 11-OHCS level (P<0.01). The blood glucose at 28 hours after the injection declined to the level before the injection, accompanied with the recovery of 11-OHCS level.

Also, plasma total protein (P<0.01) and lipid (P<0.05) levels decreased after the glucose injection (Fig. 4). Their decreases seemed to occur later than that of plasma.
Glucocorticoids and Blood Glucose Level in the Fasted Animals

In the lipid fractions, only FFA decreased significantly (P<0.01) as shown in Table 3.

Discussion

In general, the amount of carbohydrate which is present in a body is very small; occurring much less than 1% of the body at any given moment. But, it is constantly being formed and broken down in metabolism and thus performs a multitude of vital functions\textsuperscript{14). A great amount of glucose was absorbed from the gut in most of animals, while only little part of carbohydrate in feeds was absorbed as glucose in ruminants\textsuperscript{6,11}. The glucose utilization expressed as a function of metabolic body size (W\textsuperscript{3/4}) in ruminants was comparable to that in non-ruminants according to Baxter et al\textsuperscript{4). Therefore, one could assume a hypothesis that gluconeogenic action of glucocorticoids was of particular importance in ruminants.\textsuperscript{8}

Gluconeogenesis, the process by which non-carbohydrate precursors are converted to glucose, is of especial importance during fasting or when low carbohydrate diets are consumed\textsuperscript{25}. Then, the responses of plasma 11-OHCS levels to fasting were investigated in sheep, rabbits and rats in the previous study\textsuperscript{18). From our results of the present and the past studies, the change of blood glucose level after the removal of feeds seemed to be associated with that of plasma 11-OHCS level in sheep. A sufficient supply of glucose to the fasted sheep seemed to result in relaxation of hyperadrenocortical activity due to fasting. Moreover, cortisol given to sheep throughout fasting period of 10 days increased blood glucose level\textsuperscript{2). These results suggested that glucocorticoids were of particular importance for the maintenance of blood glucose level during fasting in sheep.\textsuperscript{18}

Plasma 11-OHCS level did not increase significantly during fasting in rats\textsuperscript{18). However, blood glucose level was maintained up to 8 days as in sheep. Some hormones other than glucocorticoids may act for the maintenance of blood glucose level in rats during fasting, though the participations of glucocorticoids in the maintenance cannot be absolutely ruled out. For example, epinephrine\textsuperscript{5,6,16), glucagon\textsuperscript{5,6,8,9,22) etc., also have the gluconeogenic action. Moreover, Yalow et al\textsuperscript{26) suggested that both sensitivity and secretion rate of insulin in rats were reduced by fasting. This insensitivity of insulin seems to be due to alterations in secretion of adrenal medullary hormones according to Feldman\textsuperscript{7). These hormones may -either independently or co-operatively act to maintain blood glucose level in rats.\textsuperscript{31}

With regard to the energy source during fasting in sheep, the interesting changes of plasma FFA were found in this study. The increase of FFA due to fasting has been shown in sheep\textsuperscript{6,12) and in dogs\textsuperscript{21). Trenkle\textsuperscript{24) reported that an intravenous injection of glucose into fasting sheep decreased the level of plasma FFA within 1 hour. These results agree with the results of present experiments.

FFA level in plasma increases with the general need for energy, and the utilization of the fatty acids is proportional to its plasma level in sheep\textsuperscript{17). The increase of plasma FFA level in sheep seems to represent active fatty acid mobilization. The mobilized fatty acids may be utilized in the extra neural tissues as the source of energy\textsuperscript{10,21), and may spare glucose. In facts Bassett et al.\textsuperscript{21) reported that fasting diminished the glucose utilization rate in sheep. It is suggested from the present results that plasma 11-OHCS may also indirectly contribute to the maintenance of blood glucose level through fat mobilization in sheep.\textsuperscript{17}

Blood glucose was not depressed below a certain critical level in spite of the prolonged
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fasting in both sheep and rats. However, the mechanism for the maintenance of blood glucose level could be different between the two animal species tested. Moreover, the present and the previous studies indicate that glucocorticoids would play more important roles in the mechanism in sheep than in rats.

References

2) BASSETT, J.M., Metabolism 17: 644-652. 1968.
絶食動物における血糖量の維持とグルココルチコイド

佐々木義之・熊崎一雄・池田 修

宮崎大学農学部，宮崎市 880

ヒッジおよびラットにおける絶食中の血糖，血漿中たんぱく質および脂質量の変化（実験I）および絶食ヒッジにおけるグルコース静脈注射（絶食前の体重1kg, 当り1.0 mM）の血漿中グルココルチコイド（11-OHCS）の变化および脂質量に対する影響（実験II）を調べた。実験I

絶食により，血漿中グルコース量が著しく減少した（P<0.05）。絶食ラットにグルコースを静脈注射すると，絶食により増加していた血漿中11-OHCS量が有意に減少した（P<0.01）。この時，血漿中たんぱく質（P<0.01）および総脂質（P<0.05）も減少し，とくに総脂質の減少は主として遊離脂肪酸の減少（P<0.01）によるものであった。前報1および本報に述べた成績から，ヒッジにおいてもラットにおいても，絶食中血糖量は一定のレベルを維持され，それに果たすグルココルチコイドの役割はラットよりもヒッジの方がより大きいものと推察された。