EFFECT OF PLANE OF NUTRITION AND PREGNANCY ON GLUCOSE ENTRY RATES IN SHEEP

J. W. STEEL* and R. A. LENG*

Summary
Measurements were made of entry rates of glucose into the body pool of glucose at approximately 80, 100 and 140 days of pregnancy in sheep (a) allowed unrestricted access to feed, (b) moderately underfed, (c) severely underfed, and (d) starved for four days. From 80 to 140 days of pregnancy, glucose entry rates increased by 21%, 17% and 28% in sheep on (a), (b) and (c) planes of nutrition respectively. Starvation severely limited the ability of the ewe to meet the glucose demand of late pregnancy, indicating little mobilisation of body reserves.

I. INTRODUCTION
Pregnancy toxæmia occurs in sheep subjected to either prolonged moderate undernutrition or to a short period of starvation (Reid 1960). Affected animals exhibit hyperketonaemia and hypoglycaemia. The symptoms of pregnancy toxæmia have been ascribed to the low concentrations of blood glucose and consequent lack of glucose for brain metabolism (McClymont and Setchell 1955, 1956). Work in this laboratory has been directed towards a fuller understanding of the aetiology of pregnancy toxæmia by investigations into the interrelationships between the metabolism of glucose and ketone bodies. High ketone body levels associated with undernutrition or starvation in late pregnancy are due mainly to an increased rate of production rather than a decreased rate of utilization (Bergman and Kon 1964; Leng 1965). However, estimates of glucose entry rate in induced and spontaneous field cases of pregnancy toxæmia are variable (Kronfeld and Simesen 1961; Bergman 1963; Ford 1963, 1965) and may be confounded by differences in nutrition.

Since little glucose is absorbed from the gut, sheep given roughage rations have to depend largely on gluconeogenesis to meet their glucose requirements. Some workers have concluded that the ruminant is in a critical balance with respect to the supply of glucose precursors and that during pregnancy the sheep may have difficulty in meeting the glucose requirements of the foetus (Armstrong 1965). The investigations now reported were designed to determine the ability of the ewe to synthesise glucose during pregnancy and how this ability varies with quality and quantity of diet.

II. MATERIALS AND METHODS
(a) Experimental Animals
Merino ewes at 50-60 days post-mating were brought into the animal house. Groups of four animals were randomly selected and given daily rations of:
(i) Lucerne chaff ad libitum (Group 1)
(ii) 800 g lucerne chaff (Group 2)
(iii) 250 g lucerne chaff + 250 g wheaten chaff (Group 3).

*Department of Biochemistry and Nutrition, University of New England, Armidale, N.S.W.
Glucose entry rates were measured at approximately 80, 100 and 140 days of pregnancy. A catheter was inserted into the jugular vein of each animal on the day before an experiment. At 0900 h on the day of an experiment each animal was injected with 50 μg of [U-14C] glucose and blood samples were taken at regular intervals over the following 12 or 24 hours. The animals given lucerne chaff ad libitum were then starved for four days and termed Group 4, and the experiment was repeated.

(c) Isolation and Estimation of the Concentration and Specific Radioactivity of Plasma Glucose

Plasma glucose concentrations were determined using the glucose oxidase method (Huggett and Nixon 1957). Isolation of the pentaacetate derivative (Jones 1965) and counting by liquid scintillation spectrometry was used to estimate the specific radioactivity of glucose in plasma.

(d) Estimation of Glucose Entry Rates

Figure 1 shows a typical plot of log. specific radioactivity of plasma glucose with time following a single injection of 50 μg of [U-14C] glucose. This curve was resolved into three exponential components following an initial 15 to 20 minute period during which mixing processes were still evident. The curve was described by the following equation:

\[ SR_t = A_1e^{-m_1t} + A_2e^{-m_2t} + A_3e^{-m_3t} \]

where \( SR_t \) = specific radioactivity at time \( t \) (μg/mg carbon)

\( A_1, A_2, A_3 \) = zero time intercepts of the three components, and \( SR_0 \) is their sum.

\( m_1, m_2, m_3 \) = slopes of the three components.

The line of best fit was obtained by means of a computer programme.

The size of the glucose pool (P) with which the injected dose mixes is given by:

\[ P \ (g \ glucose) = \frac{\text{Injected dose} \times 2.5}{SR_0} \]

The rate of entry of glucose (E) into the body pool was then calculated from the equation:

\[ E \ (mg \ glucose/min) = (P \times SR_0 \times 1000) \left[ \frac{1}{m_1} + \frac{1}{m_2} + \frac{1}{m_3} \right] \]

The use of this method (Baker et al. 1959) for calculating these parameters has been justified elsewhere (Luick et al. 1968).

III. RESULTS

The average plasma glucose concentrations, glucose pool sizes and glucose entry rates for each group are shown in Table 1. During the last 40 days of pregnancy, there was a tendency for plasma glucose concentration to decline in
all groups. However, none of the classical signs of pregnancy toxaemia, for example loss of eye preservation reflex or coma, were seen in any group.

As pregnancy progressed there was an increase in glucose entry rate in fed sheep (Figure 2). This increase was more marked in sheep fed ad libitum than in either of the restricted groups. The intake of lucerne chaff by Group 1 also increased from 953 g at 67 days before lambing to 1103 and 1406 g at 46 and 8 days from lambing respectively. Glucose entry rate following 4 days starvation of the sheep
TABLE 1
Pool sizes and entry rates of glucose in pregnant sheep on different planes of nutrition.
(Results expressed as means ± standard errors.)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Expts.</th>
<th>Sheep Wt. (kg)</th>
<th>Days from lambing</th>
<th>Plasma Glucose Conc. (mg/100ml)</th>
<th>Glucose† Pool Size (mg glucose /kg)</th>
<th>Glucose† Entry Rate (mg glucose /kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>36.7</td>
<td>67</td>
<td>69.4±3.1</td>
<td>153.4±9.3</td>
<td>2.19±0.18</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>32.9</td>
<td>71</td>
<td>65.3±1.5</td>
<td>125.7±16.2</td>
<td>1.83±0.27</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>32.4</td>
<td>68</td>
<td>61.3±0.2</td>
<td>148.6±12.1</td>
<td>1.45±0.10</td>
</tr>
<tr>
<td>4*</td>
<td>4</td>
<td>32.5</td>
<td>63</td>
<td>46.5±4.9</td>
<td>105.1±14.8</td>
<td>1.12±0.03</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>38.2</td>
<td>46</td>
<td>72.0±1.4</td>
<td>147.2±13.7</td>
<td>2.22±0.07</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>33.0</td>
<td>52</td>
<td>66.6±2.0</td>
<td>137.3±12.8</td>
<td>1.95±0.19</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>31.5</td>
<td>47</td>
<td>50.6±3.7</td>
<td>120.4±37.2</td>
<td>1.60±0.37</td>
</tr>
<tr>
<td>4*</td>
<td>4</td>
<td>33.4</td>
<td>42</td>
<td>44.6±3.7</td>
<td>93.5±12.9</td>
<td>1.21±0.07</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>41.1</td>
<td>8</td>
<td>65.5±1.5</td>
<td>131.5±11.4</td>
<td>2.65±0.17</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>35.7</td>
<td>11</td>
<td>60.6±4.8</td>
<td>121.2±13.0</td>
<td>2.15±0.15</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>32.1</td>
<td>5</td>
<td>45.9±5.4</td>
<td>137.4±48.4</td>
<td>1.86±0.29</td>
</tr>
<tr>
<td>4*</td>
<td>3</td>
<td>35.5</td>
<td>5</td>
<td>31.1±2.8</td>
<td>60.0±4.5</td>
<td>1.06±0.04</td>
</tr>
</tbody>
</table>

*Group 1 animals following 4 days starvation.
†Pre-starvation liveweights were used for calculations on a per kg basis in Group 4.
given lucerne chaff ad libitum remained almost constant at each of the three stages of pregnancy examined.

Sheep fed lucerne chaff ad libitum showed a decline in pool size with time of pregnancy whereas in Groups 2 and 3 no trend was evident. Starvation markedly reduced the size of the glucose pool, this effect becoming more apparent in late pregnancy.

IV. DISCUSSION

These studies show that glucose entry rate in the pregnant ewe is dependent on the plane of nutrition. With the roughage rations used, it is unlikely that much glucose was absorbed from the alimentary tract and consequently entry rate values largely represent the synthesis of glucose from non-carbohydrate precursors, i.e. gluconeogenesis. It appears, therefore, that even on a fixed food intake, the pregnant ewe is capable of increasing the rate of gluconeogenesis. The marked increase in glucose entry rate for animals in Group 1 may be mainly due to an increased feed intake and hence a greater supply of precursors.

It has been suggested that pregnant sheep have a limited capacity to supply sufficient glucose for maternal and foetal requirements (Armstrong 1965). The placental perfusion experiments of Huggett (1961) indicated that a single sheep foetus near term removed about 20 mg hexose/min from the maternal circulation. Glucose entry rate increased by approximately 12 mg/min in sheep on the lowest intake over the period 79-142 days of pregnancy. In Groups 1 and 2, the increases were 28 mg/min and 17 mg/min respectively for a similar period. As all animals produced single lambs, these increases in glucose entry rate suggest that even the severely underfed ewes (Group 3) were probably supplying sufficient hexose to the foetus without severely impairing the supply of glucose to maternal tissues.

The results for starved sheep indicate that when the ewe is subjected to starvation in late pregnancy there is insufficient mobilisation of endogenous precursors of glucose, such as glycerol and amino acids, to maintain an adequate supply of glucose to both maternal and foetal tissues. On a whole animal basis, the mean increase in glucose entry rate between 84 and 143 days of pregnancy was only 3 mg/min for this group. Therefore, it appears that pregnancy toxæmia may have resulted if the period of starvation in late pregnancy had been extended.

These studies appear to be the first to investigate the ability of the ewe to meet the demands of the foetus for glucose through pregnancy on defined planes of nutrition. Bergman (1963) has shown that on similar rations ewes in late pregnancy have higher glucose entry rates than non-pregnant ewes; Ford’s (1963) work indicates that non-pregnant ewes have higher glucose entry rates than ewes in late pregnancy. It is difficult to compare the results of these two workers with our own because they used different rations and did not report the intake of feed.

The precursors of glucose in the fed sheep are thought to be mainly propionate produced in the rumen, amino acids from endogenous and exogenous sources, and glycerol from mobilised fat deposits. Little is known of the quantitative contribution of amino acids and glycerol to glucose in the fed sheep. However Leng, Steel and Luick (1967) showed that 54% of the glucose entering the body pool of non-pregnant sheep fed 800 g lucerne chaff was arising from rumen propionate. Only one-third of the propionate produced in the rumen was converted to glucose. It is evident that some of the increment in glucose entry rate in Groups 2 and 3 could
be due to an increased conversion of rumin propionate to glucose, and the contribution of glycerol and amino acids may also become more important. These considerations may also apply, to a lesser extent, to animals fed roughage rations ad libitum. In starvation one might expect the only sources of glucose to be glycerol arising from mobilisation of adipose tissue triglycerides, amino acids from tissue protein degradation and glycogen from carbohydrate stores. The results presented in this paper suggest that there is no change in the ability of ewes starved for four days to meet their requirements for glucose from these sources as pregnancy progresses.

Investigations are at present in progress to determine the major sources of glucose in pregnant sheep under the conditions of the experiments reported here.

If the critical factor in the induction of pregnancy toxaemia is hypoglycaemia affecting brain function, it appears that the ability of the ewe to avoid this disorder may be determined by several factors including (a) the number and size of foetuses, (b) the supply of glucogenic substrates absorbed from the gastro-intestinal tract, and (c) the capacity for gluconeogenesis in undernutrition or starvation.

V. ACKNOWLEDGMENTS

We are indebted to the Australian Wool Board and the University of New England for financial support for this project. The skilled technical assistance of Mr. S. Dawson is gratefully acknowledged.

VI. REFERENCES


