THE contributions of volatile fatty acid (VFA) to energy utilization or glucose production in the horse are at present unknown. The anatomical arrangement of the intestinal tract should permit a substantial fraction of glucose absorption per se, whereas there is no appreciable glucose absorption in ruminants fed high roughage diets (Bergman, 1963). However, end products of cellulose digestion arising from fermentation processes in the lower tract may be important in furnishing potential energy.

More data are now becoming available from digestibility studies in horses (Fonnesbeck et al., 1967; Fonnesbeck, 1968), but information on physiological and metabolic reaction is deficient. This study was therefore undertaken as a preliminary step to investigate the animal’s plasma glucose response to intravenous loading of glucose and VFA.

Experimental Procedure

Experimental animals were five Shetland ponies used in two 5 x 5 latin square designs. Treatments consisted of intravenous infusions of equimolar amounts of glucose, and the sodium salts of acetate, propionate and butyrate adjusted to pH 7.4. Isotonic saline served as the control. A level of 3.5 mM/kg body weight (Somers, 1969), was established for the average pony (200 kg), and the level to infuse was calculated by the formula:

\[
\frac{W_{75\text{kg}}}{200\text{kg}} \times 700 = \text{mM to infuse.}
\]

The first trials were performed after the animals had been fasted for 72 hours. Preliminary data indicated that fasting glucose levels reached a fairly constant value after a 72 hr. fast. In the second series, the animals were fed good quality hay ad libitum, but infusions were not started until 4 hours after the morning feeding.

All infusions were completed within a 5 min. period, and blood samples were drawn before, and at intervals for 2 hr. after the infusion. A jugular catheter was used to minimize excitement.

Blood was collected in heparinized, fluorinized tubes, chilled in ice, and centrifuged. The plasma was removed and frozen for subsequent analysis. Plasma glucose concentration was determined by the method of Dubowski (1962).

Statistical treatment consisted of an analysis of variance to determine significant changes in plasma glucose resulting from treatments other than glucose infusion.

As there was considerable variation in weight and conformation among the five ponies, two further tests were performed in an effort to quantitate these differences. Total body water (TBW) was estimated using an antipyrine method (Brodie et al., 1949), and liver function was estimated using bromsulphalein (BSP) (MacDonald, 1939).

Results and Discussion

Kinetics of intravenous glucose loading have been described as conforming to an exponential relation in man (Ikkos and Luft, 1957) and sheep (Reid, 1958). Ikkos and Luft (1957) suggested that a plot of the log of the absolute glucose concentration with respect to time allows the separation of a fast mixing component. They indicated that only after the 25th or 30th min. did the glucose concentration proceed according to a first order reaction. Reid (1958) used the log of the excess glucose values (absolute minus pre-injection) to calculate the rate of disappearance, but did not separate a mixing component.

It appears that the assumption cannot be made that a glucose load is cleared from an instantaneously and uniformly mixed pool. Nor do these data confirm a first order process using the log of the absolute glucose values, but appear to follow an exponential function of the type:

\[
\frac{dx_1}{dt} = -k_1x_1
\]

\[
\frac{dx_2}{dt} = -k_2x_2
\]
where $x_1$ and $x_2$ represent the amounts of excess glucose in the plasma and interstitial fluids respectively, and $k_1$ and $k_2$ represent the rates of transfer of excess glucose across the capillary and cell membranes, respectively.

When integrated for injection into the plasma, equation (1) becomes $G(t)=G_1 e^{-k_1 t}+G_2 e^{-k_2 t}+C$ (2), where $G_1$ and $G_2$ are the intercepts of the 2 components of the log excess glucose concentration and $C$ represents the pre-injection concentration.

The slopes of the components were determined by the method of least squares from the log excess glucose concentration (figure 1). The equations predicted from the curves (figure 1) agree with the mean experimentally obtained points (table 2) with correlation coefficients of $r=-0.997$ and 0.994 for the fasted and fed animals, respectively. It is therefore concluded that a two component equation best fits the data, the second component being the one of interest, and representing the rate at which the glucose excess is leaving an uniformly mixed pool.

The rate of glucose disappearance differed markedly between the two treatments (figure 1, table 1) with half times of the fasted animals averaging 140 min. as compared to 36 min. for those fed ad libitum. The glucose appeared to return nearly to the pre-injection values by 120 min. post-injection in the fed animals, but not the fasted. The time required to return to within 10 mg of the pre-injection value may be calculated from equation 2, and was found to be 561 min. for the fasted and 163 min. for the fed animals.

The rates were also quite variable between animals on the same treatment. These differences were still apparent when calculated as fraction/hr./kg $^{76}$ (table 1). Moreover, there were no significant correlations ($P<.05$) between the rate of glucose disappearance and the other variables (table 3). However, correlations of $r=-0.84$ and $r=+0.76$ between TBW and fasted glucose tolerance, and BSP and fasted glucose tolerance may indicate that the effect of body composition and liver function parameters would in part account for the variations observed among animals.

The increased rate of glucose disappearance noted for the fed ponies has been noted in sheep (Reid, 1958), and has been ascribed to low levels of glucokinase activity. It might be expected that horses would exhibit a greater glucose tolerance than ruminants, because the horse probably derives a larger proportion of its energy from glucose. However, the half times reported by Reid (1958) were of the same magnitude for fasted and fed sheep as those reported here.

Other reports of glucose tolerance tests in horses have indicated somewhat faster disappearance rates for glucose with the glucose returning to fasting levels within 90 min., although preliminary data suggested that the return to normal may not always be this rapid (Tasker, Whiteman and Martin, 1966). The ration or length of fast in these animals was not pointed out, and it is possible that a greater tolerance would be present in animals accustomed to a high grain level in the ration. Reid (1958) found that diets other than roughage increased the rate of glucose disappearance in sheep. It appears that the horse may also be able to adapt in its capacity to metabolize glucose depending on the ration fed. Whether this adaption is mediated by hepatic or extrahepatic tissues is not known.

The average results shown in table 2 indicate that none of the VFA except propionate stimulated any significant glucose response. The stimulation from propionate was only significant ($P<0.05$) in the animals which had been fasted, with mean glucose concentration rising from 63.8 at 30 min. to 83.8 mg/100 ml at 120 minutes. The rise in glucose was exponential (mean $T morals of 15 min.), appearing 30 to 40 min. after infusion and sustaining a higher concentration for the remainder of the trial. Differences between animals on these treatments were not significant ($P<.05$).

Horino et al. (1968) failed to demonstrate a significant increase in plasma glucose in ruminants, but the amount of VFA infused was considerably less than used here and approached physiological levels. However, they established that propionate and butyrate infusions caused a marked secretion of insulin. If the same mechanism operates in the horse, it would not be surprising to find a lack of a glucose response to low levels of VFA infusion. The osmotic activity initially added to the extracellular compartment, ca. 7 mOsm/kg would account for a dilution of plasma glucose on the order of 8%. This may be the reason for the initial decrease below the isotonic control in both the fasted and fed animals. The continuous decrease seen in the control in the fed animals can probably be explained as a normal decline in blood sugar 5 to 6 hr. after feeding.

Infusions of hyperphysiological solutions of

PLASMA GLUCOSE CONCENTRATION IN PONIES

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propionate and butyrate have shown significant increases in glucose concentration in ruminants and may be more comparable to our data (Corse, 1968; Phillips and Black, 1966). Phillips and Black (1966) demonstrated marked increases in plasma glucose in either fasted or fed lambs from propionate or butyrate infusions of 2.5 mM/kg. The most marked response was caused by butyrate. Moreover, butyrate infusions to lambs made hypoglycemic with insulin caused a remission of convulsions, but the increase in plasma glucose was rapidly dissipated due to the high levels of insulin present. The response was considered to be mediated by a factor other than gluconeogenesis, i.e., glucagon. The mechanism by which butyrate stimulates an increase in plasma glucose appears to be ab-
TABLE 1. RATES OF DISAPPEARANCE OF GLUCOSE FROM THE CIRCULATION FOLLOWING AN INTRAVENOUS INFUSION

<table>
<thead>
<tr>
<th>Pony</th>
<th>Wt (kg)</th>
<th>T½/2 (min.)</th>
<th>k* (hr.)</th>
<th>k/hr./kg²/4</th>
<th>Wt (kg)</th>
<th>T½/2 (min.)</th>
<th>k* (hr.)</th>
<th>k/hr./kg²/4</th>
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<td>A</td>
<td>225</td>
<td>126.0</td>
<td>.33</td>
<td>.006</td>
<td>209</td>
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<td>.024</td>
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<tr>
<td>B</td>
<td>203</td>
<td>106.6</td>
<td>.39</td>
<td>.007</td>
<td>203</td>
<td>21.2</td>
<td>1.96</td>
<td>.036</td>
</tr>
<tr>
<td>C</td>
<td>155</td>
<td>93.6</td>
<td>.44</td>
<td>.010</td>
<td>144</td>
<td>53.7</td>
<td>.77</td>
<td>.019</td>
</tr>
<tr>
<td>D</td>
<td>247</td>
<td>277.0</td>
<td>.15</td>
<td>.002</td>
<td>244</td>
<td>51.0</td>
<td>.82</td>
<td>.013</td>
</tr>
<tr>
<td>E</td>
<td>128</td>
<td>97.6</td>
<td>.43</td>
<td>.011</td>
<td>127</td>
<td>20.5</td>
<td>2.03</td>
<td>.054</td>
</tr>
</tbody>
</table>

Mean 140.2 .35 .007 35.5 1.38 .029
C.V. 55.3 34.2 50.9 44.9 43.5 56.0

* Fraction of excess glucose cleared from the plasma per hour. (Calculated from the slope of the second component-log excess glucose concentration).

sent in the horse, although Lieb, Baker and Crawford (1969) reported marked portal—
carotid differences in both propionate and
butyrate following cecal infusions. In addition,
propionate appears to cause an increase
in glucose in the horse which is exponential
and maintains a peak concentration, whereas
in the ruminant the return to normal is rapid.
The increases found in dairy cattle from simi-
lar loads of propionate conformed to a linear
regression (Corse, 1968).

These data suggest that changes in plasma
glucose in the horse are mediated at least in
part by different mechanisms than in the
ruminants. It is evident that more work is
needed in obtaining information on glucose
homeostasis, as well as quantitative data con-
cerning glucose contribution from cellulose
end products in the cecum and colon. Studies
using isotope dilution techniques are necessary
as the osmotic effect or a possible insulinogenic
effect resulting from intravenous loading may
obscure any potential glucogenic mechanism.

TABLE 2. EFFECT OF GLUCOSE, SALINE AND VFA INFUSIONS ON BLOOD GLUCOSE LEVELS IN FASTED AND FED PONIES

<table>
<thead>
<tr>
<th>Infusion</th>
<th>0 ⁴</th>
<th>5 ⁴</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
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</tr>
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<td>56.0</td>
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<td>80.7</td>
<td>83.6</td>
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<td>65.4</td>
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<td>62.1</td>
<td>61.3</td>
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<td>Glucose</td>
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<td>575.8</td>
<td>446.3</td>
<td>335.1</td>
<td>276.1</td>
<td>269.9</td>
<td>250.6</td>
<td>217.7</td>
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</table>

⁴ Values represent the mean of five animals+S.E.
⁵ Values at time zero represent mean of three values taken at 10, 5 and 0 min. pre-injection.
⁶ Values at 5 min. represent time from beginning of infusion.
Summary

Factors affecting glucose tolerance and the effect of volatile fatty acids on plasma glucose were studied with five ponies in two 5 x 5 Latin square trials. The treatments were equimolar infusions of glucose, acetate, propionate, butyrate and isonicotinic acid in fed or fasted ponies.

Animals fasted for 72 hr. exhibited a markedly lower glucose tolerance than those fed ad libitum. Propionate appeared to be the only VFA stimulating a significant glucose response in the fasted animals, but no response was noted in the fed animals. The data suggest that length of fast is an important variable in evaluating glucose tolerance in the horse, and that plasma glucose control may be mediated in part by different mechanisms than in the ruminant species.

Reference


