Glycerol turnover in the nonpregnant and ketotic pregnant sheep

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Recent studies have shown that free glycerol is present in small amounts in the blood of various animal species (12, 14, 18, 22, 35, 36) and that its concentration tends to follow that of the plasma free fatty acids (FFA). This is particularly the case after injection of hormones such as insulin, glucagon, or adrenal medullary hormones and can be considered as evidence that the principal site of glycerol production is in adipose tissue. Smaller amounts of glycerol could still conceivably be produced in other areas of the body, however. In this regard, if only the blood concentration of a given metabolite is known, knowledge of its metabolism is incomplete since the blood concentration at any given time is determined by the balance between the rate of the metabolite's production and utilization and thus either a change in the rate of production or rate of utilization could alter the concentration in the body fluids.

Isotope-dilution methods have been used to measure turnover (production and utilization) rates of glycerol (18, 21, 22), FFA (3, 4, 21, 37), glucose (3, 7, 9, 16), ketone bodies (10, 11, 28), and other metabolites in the whole animal and, in addition, the rates of turnover of some of these metabolites have been compared with their respective plasma concentrations. Armstrong et al. (4) have concluded that the plasma concentration of FFA in the dog is regulated by the rate of FFA production or release from the body tissues and that the plasma concentration is the controlling factor for FFA utilization. Furthermore, studies in this laboratory (10, 11) have shown that a similar phenomenon exists for ketone bodies in the ketotic sheep but only up to a plasma total ketone body concentration of about 20 mg/100 ml. At higher plasma concentrations the utilization of ketone bodies does not seem to increase. In certain instances the turnover and plasma concentration of glucose are linearly related but these relationships are known to be affected by various physiological states such as pregnancy (8), lactation (9), or the rate of insulin and other endocrine secretions (16). There are only limited published data on glycerol turnover, however, which specifically relate to the control of its concentration in the body fluids over an extensive range or variety of physiological conditions. These latter studies on glycerol turnover were performed in exercising man and in fasted and diabetic dogs given glucose, insulin, nicotinic acid, or norepinephrine (21, 22).

Previous studies have shown that the twin-pregnant sheep is susceptible to a severe hypoglycemia and ketosis during the last several weeks of pregnancy and, in fact, starvation can reproduce most features of this so-called ovine pregnancy toxemia (26). Plasma concentrations of FFA have been shown to be elevated in ketosis of ruminants (2, 27, 33) but there are no published data on glycerol concentrations.

The present study was undertaken to measure the rates of glycerol turnover together with plasma concentrations of glycerol and other metabolites in fed and fasted sheep and also in twin-pregnant sheep suffering...
from hypoglycemic ketosis. In addition, the effects of norepinephrine, insulin, and glucose on glycerol turnover and its concentration in plasma were investigated in both normal and ketotic sheep. This study, therefore, would help clarify the relationship of glycerol turnover to concentration over a wide range of physiological conditions and provide further information on the hormonal control of fat mobilization particularly as applied to starvation and ketosis in the ruminant animal.

**METHODS**

**Animals and Diet**

The experimental animals were sheared, Corriedale-type, mature ewes. Pregnant sheep were selected on the basis of their breeding dates so that they were in the last 1–3 weeks of pregnancy. At least 1 week before an experiment, abdominal radiographs were taken and only those pregnant animals carrying twin fetuses were used. The sheep were then housed in individual laboratory pens in a controlled environment room at 19 ± 1°C and were thoroughly accustomed to handling.

The diet for nonpregnant ewes consisted of about 800 g of either loose or pelleted alfalfa hay (19% protein) per day. The twin-pregnant ewes were fed this same hay ad libitum but in addition were given about 200 g of a grain mixture (16% protein) twice each day. Water and a salt lick were available at all times. Hypoglycemia and ketosis were produced by fasting the twin-pregnant sheep for 3–5 days. Nonpregnant sheep also were fasted for the same period of time but, as previously described, did not become significantly hypoglycemic or ketotic (7, 25).

**Experimental Procedure**

Details of the general experimental procedure and calculations for 14C experiments have been described in previous reports (7, 9, 10). In this study, randomly 14C-labeled glycerol was given as a constant intravenous infusion into a jugular vein for at least 5 hr and at a rate of 10–20 μCi (46 ml)/hr. The glycerol was dissolved in sterile 0.9% NaCl and, except where indicated, only negligible weights of the labeled glycerol were infused (<0.1 mmole/hr). A priming dose was not necessary in these glycerol-14C trials, as was the case in previous glucose turnover experiments (7, 9), because of the rapid turnover or attainment of constant plasma glycerol concentrations and specific activities.

In some experiments, the effects of l-norepinephrine, insulin,1 or glucose on glycerol turnover and plasma concentrations of various metabolites were studied by infusing the compound 2 hr after beginning the infusion of labeled glycerol. In these experiments, however, the utilization of glycerol was not always equal to the production since the plasma glycerol concentration was being altered by the infused hormone or glucose. The term “production” rate, therefore, is used for these latter experiments instead of the term “turnover” rate. This concept has been used and discussed in an earlier study on acetocetate production in the sheep (10). Specific details of the present calculations are presented in a later section.

In all experiments, heparinized blood samples were collected at 30-min intervals from a previously implanted jugular vein catheter (9). They were then immediately chilled in ice and centrifuged at 5°C for collection of plasma. Sodium oxalate and NaF were added to the blood samples to be used for glucose and ketone body determinations.

**Chemical Methods**

**Glycerol.** Plasma concentrations of glycerol were determined by modifications of the glycerol dehydrogenase method of Hagen and Hagen (19). Fifteen milliliters of plasma were deproteinized by the addition of 2 N perchloric acid. After centrifugation and filtration, 13 ml of the filtrate were brought to pH 8.5 with 2 N KOH and the volume noted. After chilling for 1 hr, the precipitated K perchlorate was removed by centrifugation at 5°C. Duplicate 1-ml portions of this neutralized filtrate were then used for the enzymatic assay of glycerol (19) and the remainder saved for the assay of glycerol-14C as noted below. The glycerol dehydrogenase was obtained commercially (Nutritional Biochemicals Corp., Cleveland).

**Glycerol-14C.** Randomly 14C-labeled glycerol (New England Nuclear Corp., Boston) was used in all experiments. The specific activity of the glycerol was measured by isolation and purification as the glycerol tribenzoate derivative by modifications of the procedure of Abraham and Hassid (1). Carrier glycerol (1 mmole) was added to each neutralized plasma filtrate (14 ml) to increase the weight of precipitate. Recoveries, performed on each sample, were in the order of 20–40% of the theoretical weight. Corrections for this loss were always made on each sample by the following calculation: $14C$ (actual μC) in the 14-ml aliquot – (observed μC 14C in final precipitate) X (100/ % recovery). The specific activity of plasma glycerol was then calculated by dividing the actual microcuries of 14C in the 14-ml aliquot by the quantity of glycerol carbon originally present in this aliquot, i.e., as determined by the glycerol dehydrogenase method.

Much of the glycerol-14C was converted to plasma glucose by the body and it was found that this labeled glucose interfered with the usual glycerol tribenzoate preparation (1). Several modifications of this method therefore were devised (Table 1). It was found that glucose was most effectively removed by a double treatment of the 14 ml of neutralized filtrate, and added carrier glycerol, with 1 ml CuSO4 and 1 g Ca(OH)2 for 0.5 hr (6) and subsequent removal of the end products by ion-exchange resins (Amberlite IR-43 and IR-120 after conversion to the acidic form with 1 N HCl). Three

1 Glucagon-free, crystalline, beef, insulin; lot PJ-4609; kindly donated by Dr. W. W. Bromer, Lilly Research Laboratories, Indianapolis, Ind.
milligrams of nonlabeled glucose were added between the first and second CuSO₄-lime-resin treatments in order to dilute any glucose-¹⁴C remaining after the first step. Table 1 shows that this method was satisfactory in that it removed all but minute amounts of glucose-¹⁴C in the final product and that recoveries of glycerol-¹⁴C were still nearly complete (97 ± 2%).

After treatment with CuSO₄, lime, and resins, the samples were evaporated in beakers over mild heat and a stream of nitrogen and dried overnight in a desiccator. After cooling on ice, 1 ml of pyridine and 0.4 ml of benzoyl chloride were slowly added. After standing 2 days in a desiccator, the mass or oil was transferred to a separatory funnel with 30 ml of cold acidified water and extracted with about 40 ml of ethyl ether. The ether layer was then washed three times with 30 ml of saturated NaHCO₃ and two times with 30 ml of water. The ether extract was then evaporated in a centrifuge tube and the resulting oil dissolved in 2 ml of hot absolute methanol. After cooling at −10°C for 20 min the tubes were scratched with a stirring rod to crystalize the glycerol tribenzoate. The excess methanol was removed and the precipitate recrystallized from methanol as before. The precipitate was then washed with cold water, filtered, dried in a vacuum oven overnight at 40°C, weighed, and dissolved in 20 ml of scintillation fluid (11). The efficiency of counting was about 70% as expressed as microcuries per mole of carbon.

Free fatty acids, glucose, and ketone bodies. Plasma FFA concentrations were measured by the method of Trout et al. (39), plasma glucose by the glucose oxidase-peroxidase method ("Glucostat," Worthington Biochemical Corp., Freehold, N. J.), and plasma total ketone bodies by the method of Procos (31).

RESULTS

Experimental Conditions and Calculation of Results

A total of 26 glycerol-¹⁴C infusion experiments were performed and results typical of an individual experiment are illustrated in Fig. 1. Nearly constant plasma concentrations and specific activities were obtained during the 7-hr period of infusion and this shows that the animals were in a reasonably constant or steady-state condition. The specific activities and concentrations of glycerol, however, usually were the most variable (so within ±10%). All calculations of glycerol turnover were made on the basis of dilution of the ¹⁴C and in a similar manner as described previously for acetoacetate (10, 11).

Briefly, this consisted of the following: glycerol turnover or production rate (in mmoles/hr) = (µc glycerol infused/hr)/(µc/mole plasma glycerol carbon) x (3 or number of carbon atoms/glycerol molecule).

In order to test these glycerol experimental conditions more rigorously, the turnover of glycerol was measured in a fed and fasted sheep immediately before and during an intravenous infusion of 15 or 30 mmoles of exogenous (nonlabeled) glycerol per hour (Table 2). The glycerol concentrations again were nearly constant during these two periods of infusions and the stated values are the mean of the values obtained. The glucose concentrations increased slightly, presumably due to the glucogenic action of the bulk glycerol infused, but this would not affect the calculation of glycerol turnover in any way. The total glycerol turnover in these experiments increased by almost the same amount in both sheep so that the measured endogeneous glycerol turnover was within 3–5% of the initial rate.

In addition, further calculations of the data in Table 2 are in order. By assuming the volume of distribution of glycerol to be 65% of the body weight (24), the turnover time of glycerol in the body fluids can be calculated by dividing the total body glycerol (glycerol pool) by the rate of glycerol turnover. Values of only 15–18 min were obtained which correspond to a glycerol half-life of about...
11 min. On this basis, further calculations, similar to those of Armstrong et al. (4) for the turnover of FFA, reveal that the theoretical specific activity of the circulating glycerol, 0.5 hr after beginning an infusion of labeled glycerol, would be about 85% of its final or asymptotic value. After 1 hr, however, the specific activity would be about 98% of the final value. Analysis of samples taken 1 hr or more after the beginning of infusion thus would be expected to closely estimate true glycerol turnover. In the present study, samples were always taken after this 1-hr time interval of labeled glycerol infusion and never earlier than 0.5 hr after injection of a hormone or compound which could alter the rate of glycerol production or turnover (production and utilization). It must be emphasized, therefore, that a theoretical error of as much as 15% was present in these latter production rate measurements but that the theoretical error in the steady-state or turnover experiments would be expected to be minimal. This problem of rapid changes in glycerol production and plasma concentrations (or pool size) and the resultant temporary incomplete mixing of glycerol in blood with that of other fluid compartments has been discussed by Havel (21). These errors therefore exist but, nevertheless, the measured changes in glycerol production are still a valid index of hormone or metabolic action.

**TABLE 2. Effects of glycerol infusions on glycerol turnover and plasma concentrations**

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Wt. kg</th>
<th>Glycerol Infused</th>
<th>Plasma Conc.</th>
<th>Glycerol Turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmoles/hr</td>
<td>mmoles/ hr</td>
<td>Time, min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mmoles</td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>55</td>
<td>0.3</td>
<td>32</td>
<td>4.2</td>
</tr>
<tr>
<td>Fasted*</td>
<td>59</td>
<td>0.3</td>
<td>32</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* Glycerol-14C was infused for a total of 7.5 hr in each experiment, 2.5 hr with a tracer dose only, and 5 hr with carrier glycerol in the quantity indicated. * Total minus glycerol infused. * Assuming a volume of distribution of 65% of the body weight. * Tracer dose only (<0.1 m mole/hr). * Fasted 4 days.

**Effects of Norepinephrine**

The effects of infusions of L-norepinephrine were studied in a series of seven experiments on normal non-pregnant sheep (Figs. 2 and 3, Table 4). Figure 2 illustrates the results of five of these experiments and shows the effects of graded doses of the norepinephrine on the plasma concentrations of glycerol, FFA, and glucose. The effects of norepinephrine infusions on plasma glycerol and FFA concentrations were approximately the same at the two highest infusion rates employed (0.5 and 1.0 μg/kg per min) which suggest a maximal response of the adipose tissue to norepinephrine. This was not the case with glucose, however (Fig. 2). In all experiments, the concentration of plasma glucose, and presumably the utilization of glucose, increased and this may have been the cause of the gradual decrease in the glycerol and FFA concentrations during the continued

**TABLE 3. Glucose turnover and plasma concentrations in fed and fasted nonpregnant and ketotic twin-pregnant sheep**

<table>
<thead>
<tr>
<th>Sheep</th>
<th>N</th>
<th>Wt. kg</th>
<th>Plasma Concentration</th>
<th>Glycerol Turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mmole/hr</td>
<td>m mole/ hr</td>
</tr>
<tr>
<td>Nonpregnant, fed</td>
<td>5</td>
<td>39±2</td>
<td>38±4</td>
<td>230±25</td>
</tr>
<tr>
<td>Nonpregnant, fasted*</td>
<td>7</td>
<td>59±2</td>
<td>88±10</td>
<td>938±145</td>
</tr>
<tr>
<td>Pregnant, ketotic*</td>
<td>6</td>
<td>73±7</td>
<td>100±12</td>
<td>1,461±156</td>
</tr>
<tr>
<td>Pregnant, ketotic*</td>
<td>6</td>
<td>70±2</td>
<td>132±7</td>
<td>1,462±52</td>
</tr>
</tbody>
</table>

Values are means ± SEM. N = no. of animals. * Fasted 3–5 days.
administration of submaximal dosages of norepinephrine (0.05–0.2 μg).

Figure 3 illustrates one of two additional and more detailed experiments in which a submaximal amount of norepinephrine was infused. The rate of glycerol production was measured by the infusion of glycerol-4C and the determination of plasma glycerol specific activities. It can be seen (Fig. 3; Table 4) that the concentration of glycerol largely reflected the rate of glycerol production by the body rather than its rate of utilization. In addition, the production and concentration of glycerol declined earlier or faster than did the concentration of FFA. This latter finding also is evident in Fig. 2. It is of further interest to note that the plasma ketone body concentrations increased in proportion to the plasma FFA but that there was a lag time of roughly 0.5 hr between their maximal changes (Fig. 3).

Effects of Insulin and Glucose

These results are summarized in Table 4 and a typical experiment is illustrated as Fig. 4. Insulin and glucose infusions are included in this same section since both are known to increase glucose utilization and since all of these experiments were performed on hypoglycemic and ketotic pregnant sheep. Both insulin and glucose proportionately decreased the rate of glycerol production and its concentration in plasma together with decreasing the concentrations of FFA and ketone bodies. During prolonged insulin infusion (Fig. 4), however, a rebound in glycerol production and plasma concentrations of glycerol, FFA, and ketone bodies occurred which probably was due to the effects of the marked hypoglycemia, possible decreased glucose utilization, and subsequent release of adrenal medullary hormones. The production and concentration of glycerol again seemed to increase more rapidly than did the FFA concentration and, further, there again was a lag time of about 0.5 hr between the maximal concentration changes of plasma FFA and ketone bodies.

Relationship of Glycerol Turnover to Plasma Glycerol Concentration

Figure 5 graphically represents all of the individual data of this study (Tables 2, 3, and 4) with regard to glycerol turnover per unit metabolic size and plasma glycerol concentration. The regression line of this scatter diagram, as calculated by the method of least squares, shows a linear relationship of the glycerol turnover to glycerol concentration over the entire glycerol concentration range studied (30–300 μM). There was a slight suggestion that glucose, insulin, or norepinephrine (closed circles, Fig. 5) may have altered the slope or intercept of this regression line but no statistically significant differences could be found between a regression line for these values alone and a line when these values were removed. A single regression for all of the data therefore was calculated and presented.
TABLE 4. Effects of norepinephrine, insulin, and glucose on glycerol production by nonpregnant or ketotic twin-pregnant sheep

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Status of Animal</th>
<th>Wt, kg</th>
<th>Exptl Conditionsa</th>
<th>Min of Glycerol-14C Infusionb</th>
<th>Plasma Concnc</th>
<th>Glycerol Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glycerol</td>
<td>FFA</td>
</tr>
<tr>
<td>15</td>
<td>Nonpregnant, fed</td>
<td>67</td>
<td>Control</td>
<td>60-120</td>
<td>53</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norepi</td>
<td>150-180</td>
<td>304</td>
<td>2,190</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norepi</td>
<td>210-240</td>
<td>131</td>
<td>2,575</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norepi</td>
<td>270-300</td>
<td>40</td>
<td>1,012</td>
</tr>
<tr>
<td>97</td>
<td>Nonpregnant, fasted</td>
<td>59</td>
<td>Control</td>
<td>360-420</td>
<td>35</td>
<td>186</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Norepi</td>
<td>30-130</td>
<td>67</td>
<td>472</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Norepi</td>
<td>180-210</td>
<td>238</td>
<td>2,340</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Norepi</td>
<td>240-270</td>
<td>58</td>
<td>2,070</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After Norepi</td>
<td>300-330</td>
<td>65</td>
<td>1,500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>390-450</td>
<td>47</td>
<td>433</td>
</tr>
<tr>
<td>76</td>
<td>Pregnant, ketotic</td>
<td>61</td>
<td>Control</td>
<td>60-120</td>
<td>100</td>
<td>1,407</td>
</tr>
<tr>
<td>92</td>
<td>Pregnant, ketotic</td>
<td>70</td>
<td>Insulin</td>
<td>150-180</td>
<td>62</td>
<td>1,085</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>60-120</td>
<td>53</td>
<td>1,108</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Insulin</td>
<td>150-210</td>
<td>41</td>
<td>482</td>
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<tr>
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<td>After Ins</td>
<td>270-330</td>
<td>86</td>
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<tr>
<td>76</td>
<td>Pregnant, ketotic</td>
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<td>Control</td>
<td>60-120</td>
<td>133</td>
<td>1,383</td>
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<td>Glucose</td>
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<td>801</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>After Norepi</td>
<td>60-120</td>
<td>63</td>
<td>1,613</td>
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<tr>
<td>22</td>
<td>Pregnant, ketotic</td>
<td>70</td>
<td>Control</td>
<td>150-270</td>
<td>93</td>
<td>1,105</td>
</tr>
</tbody>
</table>

- a Infusion of norepinephrine, glucagon-free insulin, or glucose was started 2 min after end of control period.
- b Glycerol-U-14C was infused at a constant rate of 20 μC/hr.
- c Norepinephrine, 0.25 μg/kg per min.
- d Fasted 4 days.
- e Glucagon-free insulin, 0.5 U/kg as a primer dose plus 0.2 U/kg per hr.
- f Glucose, 200 mg/kg as a primer dose plus 75 mg/kg per hr.

FIG. 4. Effects of glucagon-free insulin on glycerol production and plasma concentrations of glycerol, FFA, ketone bodies, and glucose in a pregnant ketotic sheep. A dose of 0.5 U/kg was given as a primer followed by a constant infusion of 0.2 U/kg per hr. Further details on this sheep are given in Table 4.

DISCUSSION

It is well known that glycerol is released into the bloodstream along with FFA. In addition, the plasma concentration of glycerol in humans (13, 14, 20, 21, 35), and in various animal species (12, 19, 36, 41) is known to be small (<100 μM or 1 mg/100 ml) but that it increases during starvation and diabetes. The rate of turnover of glycerol also has been shown to increase during...
exercise or diabetes or after the injection of norepinephrine (21, 22) but there are only limited published data on glycerol turnover as specifically related to plasma concentration over a wide range of physiological conditions. Furthermore this is the first report on glycerol metabolism in the normal and hypoglycemic ketogenic ruminant. Moller and Black (30), however, have published a preliminary communication on glycerol turnover in the lactating phloridzinized goat.

Since glycerol is known to be a potent glucogenic compound, a potential error in the measurement of glycerol turnover rates is contamination of the plasma glycerol by labeled glucose. This error was eliminated in the present study by destruction of the glucose with CuSO₄ and Ca(OH)₂ and subsequent removal of the endl products with ion exchange resins. Recovery of labeled glycerol was still complete in that the glycerol-¹⁴C was recovered in the same proportion as added nonlabeled glycerol (Table 1). This problem of glucose-¹⁴C contamination apparently was not recognized in earlier studies (18) on glycerol turnover. However, Havel and Carlson (22) and Winkler et al. (41) have eliminated glucose as a contaminating agent by the use of other methods involving a conversion of the beta carbon of the glycerol to formic acid or removal of the glucose by the Fischer-Kiliani procedure.

The results of this investigation show that the turnover of glycerol in both normal and hyperglycerolemic animals was rapid. By comparison, Havel and Carlson (21, 22) obtained fractional turnover rates in both human beings and dogs of 0.04-0.09/min which correspond to a turnover time of the free glycerol pool of only 11-25 min. The present results of 15-18 min in the sheep, which were obtained by assuming a glycerol distribution volume of 65% of the body weight (24), thus are in close agreement with those of the previous investigators and no species difference seemed to be present. The mean concentration of glycerol in the plasma of the normal fed sheep (38 μM), however, was lower than the values of about 40-100 μM in postabsorptive humans (13, 14, 20, 35) and the mean value of 128 μM in unanesthetized dogs (41). Only when completely fasted for 3-5 days did the sheep of this study seem to have comparable plasma glycerol concentrations (mean, 88 μM) to other species. The ketotic and hypoglycemic sheep, however, had higher plasma glycerol concentrations (Table 3).

The rate of turnover of free glycerol in the body fluids was considerable during hypoglycemia and ketosis and, when expressed on the basis of metabolic size, the rate of glycerol turnover was linearly related to its concentration in plasma (Fig. 5). These results seemed true over the entire glycerol concentration range studied (30-300 μM) and which also included fed or fasted conditions, hypoglycemia, or the infusion of various hormones, glycerol, or glucose. It can be concluded, therefore, that the turnover rate of glycerol and its concentration in the blood stream are directly related under a variety of physiological conditions and this would mean that the concentration of glycerol is directly dependent on the rate of glycerol production and that the rate of glycerol utilization, in turn, is regulated by glycerol concentration. A similar phenomenon, but only in the normal dog infused with bulk glycerol, has been reported in a preliminary communication by Winkler et al. (41). Thus, the direct relationships of glycerol production, concentration, and utilization are similar to those reported for FFA (4) and for moderate concentrations of ketone bodies (10). No evidence was obtained in these studies, as recently proposed (20), that a certain agent or physiological state can change the utilization of glycerol without first altering its production and therefore its concentration.

The effects of norepinephrine on increasing the concentration of glycerol or FFA in several species of animals and in man are well documented in the literature (4, 14, 17, 19, 21, 27, 32, 35, 38) and the present results confirm these findings for the ruminant. These data show a close relationship between glycerol and FFA and further show that norepinephrine directly stimulates lipolysis of triglycerides and subsequent release of glycerol and FFA from the adipose tissue. The main effects of norepinephrine do not, therefore, appear to be on the reesterification or lipogenic processes, and this action of norepinephrine in the ruminant seems identical to its action in other mammals (22). In the present experiments on the ruminant an infusion rate of 0.5 μg/kg per min apparently elicits a maximal response of the adipose tissue since higher infusion rates produced nearly identical glycerol and FFA concentrations (Fig. 2). No maximal response of the blood glucose was obtained, however, since the blood glucose concentration progressively increased as the norepinephrine infusion rate was increased.

When submaximal amounts of norepinephrine were infused, the increase in fat mobilization, as evidenced by the increase in glycerol and FFA concentrations and production, was not sustained (Figs. 2 and 3). A possible explanation for this phenomenon is that norepinephrine gradually reduced the rate of blood flow through the adipose tissue. A more likely explanation is that the utilization of glucose by adipose tissue increased as a result of the marked rise in blood glucose concentration. Glucose injections are known to decrease fat mobilization and could thus overwhelm the effect of sustained norepinephrine infusion. Havel and Goldfien (23) have reported a similar transient action of epinephrine on FFA mobilization in the dog. Norepinephrine infusions in the dog, however, did not cause an increase in blood glucose and therefore resulted in a sustained elevation of the plasma FFA. Thus, it is possible that this represents a species difference in the response of blood glucose to norepinephrine.

Glucose and insulin injections, presumably by the process of increasing the utilization of glucose, have been reported to decrease the concentration of glycerol in the body fluids (14, 20, 35). Data on their effects on actual glycerol release or production by the adipose tissue in vivo are limited (21). The rate of glycerol
production by adipose tissue in vitro is not usually altered by insulin or glucose, so it is usually stated that these agents affect only the reesterification process (lipogenesis) in adipose tissue rather than the lipolytic process (17, 37). An exception to this, however, is that insulin has been found to have direct antilipolytic effects in the absence of albumin and furthermore that these effects are independent of its actions on glucose metabolism (23). Since glycerokinase apparently is absent in adipose tissue, it is thought that glycerol cannot be utilized by the adipose tissue but must be synthesized from glucose or an equivalent substrate (40). The present results in ketotic sheep nevertheless show that glycerol production was decreased during the infusion of glucose or insulin. These results also are in agreement with those of Carlson and Oro (14), who studied the combined effects of glucose and insulin on the release of glycerol by the abdominal adipose tissue of the dog, and those of Havel and Carlson (21, 22) who studied the effects of these agents on glycerol turnover in both the intact normal and diabetic dog. It is concluded, therefore, that conditions that increase glucose utilization must be able to decrease the rate of lipolysis in vivo even though a similar action has not yet been demonstrated in vitro.

During prolonged insulin infusion a rebound (Fig. 4) in glycerol production and glycerol and FFA concentrations occurred. This probably was the result of the severe hypoglycemia with possible decreased glucose utilization by the brain, or even adipose tissue, and subsequent release of epinephrine and norepinephrine from the adrenal medulla and sympathetic nerve endings. This assumption seems likely since it has been shown that epinephrine is released during insulin hypoglycemia in the sheep (15, 34) and, in addition, since the effects of insulin hypoglycemia on plasma FFA can be eliminated by the use of sympathoadrenergic blocking agents (5).

Although the concentrations of glycerol and FFA usually increased or decreased with one another, the concentrations of glycerol changed earlier or faster than did the concentration of FFA. This was mostly evident during norepinephrine infusions (Figs. 2 and 3) but was also suggested during the infusion of glucose or insulin. Furthermore, with the maximal rates of norepinephrine infusion (Fig. 2), the glycerol concentrations decreased even though the FFA concentrations remained elevated at a nearly constant level. This finding was unexpected since the turnover or replacement time of glycerol (15–18 min) is longer than that of FFA (2–3 min (4, 21)). One would, therefore, expect the FFA to disappear faster than the glycerol. The most likely explanation for this finding would seem to be that the triglycerides are not completely hydrolyzed in the adipose tissue with continued stimulation and, therefore, FFA would be released in a ratio greater than the initial anticipated ratio of 3:1. An alternative explanation is that ruminant adipose tissue differs from other species in that it can utilize free glycerol. Evidence for only a partial hydrolysis of triglycerides in other species is still fragmentary but has been suggested by the work of Havel (21).

It is also of interest to compare the concentration of ketone bodies with that of the FFA. The total ketone bodies immediately increased or decreased with plasma FFA but there was a lag time of roughly 0.5 hr between their maximal changes (Figs. 3 and 4). These findings were observed in six different experiments. This period, then, presumably represented the time required for hepatic penetration and metabolism of the FFA to ketone bodies but also probably represented the time for dilution and mixing of the ketone bodies in the body fluids. FFA are known to be precursors of ketone bodies (29) but the time course of their metabolism has not heretofore been illustrated in the living animal.

In summary, the total production and utilization (turnover) of free glycerol was grossly elevated during hypoglycemic ketosis and this increased turnover of glycerol was closely correlated with its concentration in plasma. Since the turnover of glucose is decreased during hypoglycemia in the sheep (7) the decreased glucose utilization could trigger the mobilization of glycerol and FFA either by a direct action on the adipose tissue itself or by stimulation of the nervous system and its associated pituitary gland. If FFA liberation was excessive, marked ketosis would result. Although glycerol is known to be a glucogenic compound, its liberation from adipose tissue is apparently not sufficient to sustain the body's need for glucose.

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REFERENCES

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