Glucose-Lactate Interrelationships: Effect of Ethanol

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ABSTRACT The effect of ethanol on the interrelationship of lactate and glucose metabolism was investigated in eight human volunteers. Lactate and glucose kinetics and intervconversion rates were determined by the sequential administration of L-(+) lactate-U-^{14}C and glucose-1-^{14}C over an 8 hr period. After a 12 hr fast, the glucose turnover and recycling rates were 94.0 ±3.8 (SEM) and 13.7 ±1.1 mg/kg per hr, respectively. Approximately 50% of the glucose turnover or 40.7 ±2.1 mg/kg per hr was converted to lactate, accounting for 50% of the lactate turnover rate. Lactate turnover and lactate conversion to glucose were 81.8 ±6.2 and 16.7 ±1.1 mg/kg per hr, respectively. Approximately 20% of the glucose turnover was derived from lactate under these conditions. During the administration of ethanol, the blood lactate concentration doubled and the lactate turnover rate declined slightly. Lactate conversion to glucose was markedly inhibited, decreasing from 16 to 5 mg/kg per hr, and the per cent of the glucose turnover derived from lactate decreased from 18 to 6. Despite the marked inhibition of lactate conversion to glucose, neither the blood glucose concentration nor the glucose turnover rate changed. Both glucose recycling and glucose conversion to lactate were decreased, indicating that ethanol inhibited peripheral glucose utilization. There was no difference in the degree of inhibition of lactate incorporation into glucose produced by ethanol when nonfasted subjects were compared with two subjects who had fasted for 48–72 hr despite the presence of hypoglycemia in the latter.

These results indicate that starvation is not a prerequisite for ethanol inhibition of gluconeogenesis from lactate in humans but is necessary for the development of hypoglycemia. Inhibition of lactate incorporation into glucose in nonfasted subjects is probably masked by a concomitant increase in glycogenolysis which prevents hypoglycemia. Ethanol decreases glucose conversion to lactate as well as lactate conversion to glucose, thus inhibiting the Cori cycle.

Received for publication 28 April 1970 and in revised form 11 August 1970.

INTRODUCTION

Although there have been numerous studies of ethanol's effect on blood glucose concentrations and glucose homeostasis in man (1–6), relatively little is known concerning its effect on glucose synthesis per se. Except for a few experiments, summarized recently (7) in which ethanol was demonstrated to inhibit glucose synthesis from alanine, glutamate, glycerol, and lactate by surviving slices and biopsy segments of human liver, there have been no in vitro nor in vivo studies of its effect on gluconeogenesis in man. In a single study in which ethanol decreased the hepatic uptake of glycerol (8), an important precursor of glucose, glucose production was not measured. The paucity of information concerning the effect of ethanol on human gluconeogenesis is in striking contrast to that which has been obtained from extensive in vitro studies in animals (9–13). It is primarily as a result of the latter, in which ethanol has been demonstrated to inhibit gluconeogenesis from amino acids and other glucose precursors, that the mechanisms by which it produces hypoglycemia have been defined.

An interesting paradox of ethanol's effect on glucose homeostasis has been that while it inhibits gluconeogenesis by liver tissue from nonfasted humans and rabbits and briefly fasted rats (4, 7), it does not produce hypoglycemia in man or other animals unless its administration is preceded by a 48–72 hr fast (2, 14). It has been suggested that the nutritional status of the organism determines whether ethanol induces hypoglycemia (9). In the presence of an adequate hepatic supply of 3-carbon precursors, such as occurs in the fed state, there is sufficient substrate for cytoplasmic reoxidation of the NADH that is formed during the metabolism of ethanol, and inhibition of gluconeogenesis does not occur. In contrast, in the absence of sufficient quantities of 3-carbon precursors, such as occurs with prolonged fasting, greater proportions of the NADH must be reoxidized in the mitochondrion with resulting impairment of gluconeogenesis. Although such statements imply that ethanol only inhibits gluconeogenesis when

substrate availability is limited, it is possible, as suggested recently (14), that inhibition of hepatic gluconeogenesis may occur in the nonfasted subject but be masked by the presence of adequate glycogen stores and concomitant glycogenolysis. Recent studies from this laboratory which demonstrated a prompt inhibitory effect of ethanol on lactate incorporation into glucose in the absence of hypoglycemia (15) support such a proposal.

With these thoughts in mind, we have utilized techniques which have recently been described for the quantification of glucose and lactate interconversion (16) to examine the effect of ethanol on gluconeogenesis in man.

METHODS

Lactate and glucose turnover studies were performed before and during the administration of ethanol in four male and four female volunteers aged 19–35 yr. Informed consent was obtained from all subjects. Their vital statistics are presented in Table I. Each study was begun between 7:30 and 8:00 a.m. after a fast of 10–12 hr. Two types of studies were performed, one in which only glucose turnover was measured and the other in which both lactate and glucose turnover were measured. When only glucose turnover was measured, glucose-1-^{14}C was infused at a constant rate for 8 hr. When both were measured, the techniques utilized were modified from those described previously (16) in the following way: lactate and glucose turnover was determined sequentially over an 8 hr period on 1 day rather than over 4-hr periods on consecutive days. We have assumed that the metabolism of lactate and glucose determined in two successive 4-hr periods on the same day represents the pattern of metabolism for both substrates during the total infusion period of 8 hr. 50 μCi of sodium L-(+) lactate-U-^{14}C (Nuclear-Chicago, Des Plaines, Ill.) was administered over the first 4 hr through an indwelling polyethylene venous cannula by a primed infusion technique in which the ratio of priming dose (μCi) to infusion rate (μCi/hr) was 1:1. 50 μCi of glucose-1-^{14}C was then administered from the 5th through the 8th hr by a primed infusion technique in which ratio of priming dose (μCi) to infusion rate (μCi/hr) was 2:1. When only one isotope was infused, the ratio of the priming dose to infusion rate was the same as used in the sequential study of that substrate. The total dose of radioactivity received in the single infusion was the same as that received in the sequential infusion study.

Blood was withdrawn at 30-min intervals through an indwelling venous catheter placed in the opposite arm that was kept patent with a slow saline infusion of isotonic saline or of a dilute heparin solution. 10 ml of whole blood was deproteinized with perchloric acid and neutralized with potassium hydroxide. The neutralized deproteinized extract was chromatographed on Dowex 1-X8 resin (formate form) (Dow Chemical Co., Midland, Mich.) and the eluates were counted and analyzed for glucose and lactate by methods described recently (16). While these procedures are essentially those that we have previously used for lactate turnover, they depart in the following ways from those used for glucose turnover: (*a*) blood samples were previously deproteinized and neutralized with barium hydroxide and zinc sulfate rather than with perchloric acid and potassium hydroxide, and (b) the neutralized extracts were previously chromatographed on Amberlite MB-3 resin (bicarbonate form) (Rohm and Haas Co., Philadelphia, Pa.) rather than Dowex 1-X8 resin. We used Dowex 1-X8 resin for separation of glucose and lactate during the infusion of glucose-1-^{14}C because the lactate fraction isolated from the Amberlite MB-3 resin in previous studies was contaminated with nonlactate radioactive intermediates of glucose metabolism, which spuriously elevated the lactate specific activity values and produced excessive estimates of "lactate derived from glucose." To determine the extent to which chromatography of plasma extracts on Dowex 1-X8 resin reduced the contamination of the lactate-containing eluate, we injected a rat with lactate-^{14}C, fractionated the deproteinized neutralized plasma extract by the above techniques, and subjected the lactate fraction to ascending one and two dimensional paper chromatography.[1] In addition, lactate in four individual patient samples was isolated as the acetaldehyde-dimedone derivative.[2] Both techniques revealed that over 94% of the radioactivity in the lactate fraction was actually in lactate. This modification was employed to circumvent the necessity for formation of the acetaldehyde-dimedone derivative of lactate. There were no significant differences in glucose specific activity values when the Somogyi precipitation and Amberlite MB-3 chromatography technique was compared with perchloric acid precipitation and Dowex 1-X8 chromatography. However, lactate specific activity values and estimates of lactate derived from glucose during the infusion of glucose-1-^{14}C were approximately 50% of those obtained when Amberlite fractionation was used. Glucose recycling was determined from the blood glucose specific activity and the radioactivity in carbon-6 of glucose as determined from the formaldimedone derivative (17). Minute ventilatory volume, CO_2 and $^{14}CO_2$ content of expired air were determined as described previously (16). Glucose inflow and outflow rates were calculated by the method of Steele (18). Lactate and glucose kinetics and interconversion

TABLE I

Vital Statistics of the Eight Subjects Studied

Subject	Age	Sex	Height	Weight*	Per cent‡ ideal body weight	Diabetes§ mellitus
H. B.	31	M	173	95.5	+27	0
S. O.	30	M	175	80.9	+14	0
P. T.	29	F	178	63.2	−9	0
T. A.	30	F	173	55.0	−14	0
C.D.	31	M	185	90.0	+10	0
V. W.	28	F	170	61.8	−3	0
G. S.	39	M	163	65.0	+2	0
P. O.	26	F	154	65.9	+16	0

* With clothes.

‡ Metropolitan Life Insurance Tables, 1959.

§ On the basis of an oral glucose tolerance test (40 g/m^2) or a 2 hr postprandial blood sugar.

[1] Whatman No. 1 filter paper and isopropanol: pyridine: acetic acid: water (8:8:1:4) and phenol: NH_3 (phenol saturated with 0.1 M NH_3 v/v) solvent systems.

[2] Analyses kindly performed by Dr. Gilbert Searle, San Francisco, Calif.

rates were calculated from the relationships shown in the Appendix. All values were changed from millimoles to milligrams to facilitate comparison with pre-existing data, and the results were expressed as milligrams per kilogram per hour.

Each subject was admitted to the Clinical Research Center of the University of Alabama Medical Center the night before the ethanol study. In those studies in which lactate and glucose turnover were determined sequentially, ethanol (19.6 g) as bourbon, blended whiskey, or Scotch was administered orally in water or a dietary, carbohydrate-free beverage at 2-hr intervals from 2:00 a.m. until completion of the study. Blood alcohol levels, measured at 2-hr intervals just before the administration of the next dose of ethanol, ranged from 35 to 75 mg/100 ml. In those studies in which only lactate or glucose turnover was determined, ethanol was administered only during the 5th–8th hr at a rate of 9.8 g/hr.

RESULTS

Results of a representative study in which glucose-1-^{14}C was infused for 8 hr is shown in Fig. 1. As shown in panel A of Fig. 1, the blood glucose concentration declined slightly during the infusion in this subject from 78 to 72 mg/100 ml. This degree of fall in the blood glucose concentration was observed in most of the studies. The glucose specific activity ranged from 5.56 to 6.35 $\times$ 10^5 dpm/mmole but with the exception of the 90- and 120-min values varied only 5% during the infusion period. The 30- and 60-min specific activity values are not plotted since equilibration and distribution of the isotope occurs during this period and the values are not representative of the isotopic steady state. The blood lactate specific activity values increased during the early phases of the infusion, reaching constant values during the period from 150 to 240 min (panel B of Fig. 1). After reaching this plateau, there was often a tendency for the lactate specific activity to increase slightly during the remainder of the infusion. The specific activity of glucose due to recycling (panel C of Fig. 1) increased progressively throughout the infusion. Glucose inflow and outflow rates were always closely balanced (panel D of Fig. 1).

The effects of ethanol on the same parameters during an 8 hr glucose-1-^{14}C infusion are shown for a representative study in Fig. 2. In contrast to the specific activity time course curve for glucose, lactate, and recycled glucose shown in Fig. 1, ethanol produced significant changes which were identifiable within 30 min. The glucose specific activity (panel A of Fig. 2) increased during the period of ethanol administration and was approximately 10% greater than that observed in the preceding control period (0–240 min). In contrast to the steady increase in the lactate specific activity that occurred during the period from 270 to 480 min in the absence of ethanol, the specific activity remained the same or declined in its presence (panel B of Fig. 2). As indicated by the failure of the recycled glucose specific activity to rise throughout the infusion period (panel C of Fig. 2), ethanol inhibited glucose recycling. Despite the changes in glucose recycling and glucose

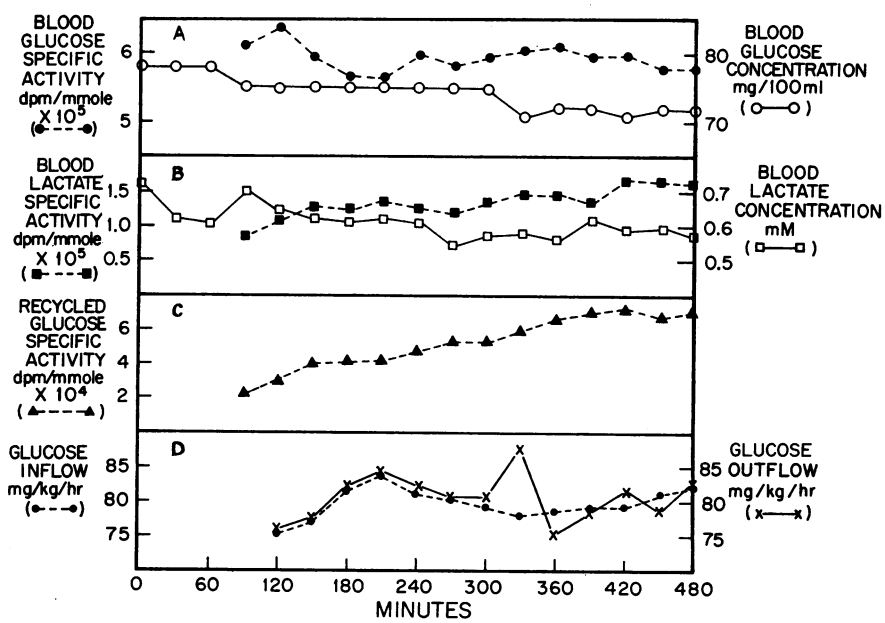

FIGURE 1 Blood glucose and lactate concentrations; specific activity-time course curves for glucose, lactate, and recycled glucose; and glucose inflow-outflow rates during the infusion of glucose-1-^{14}C.

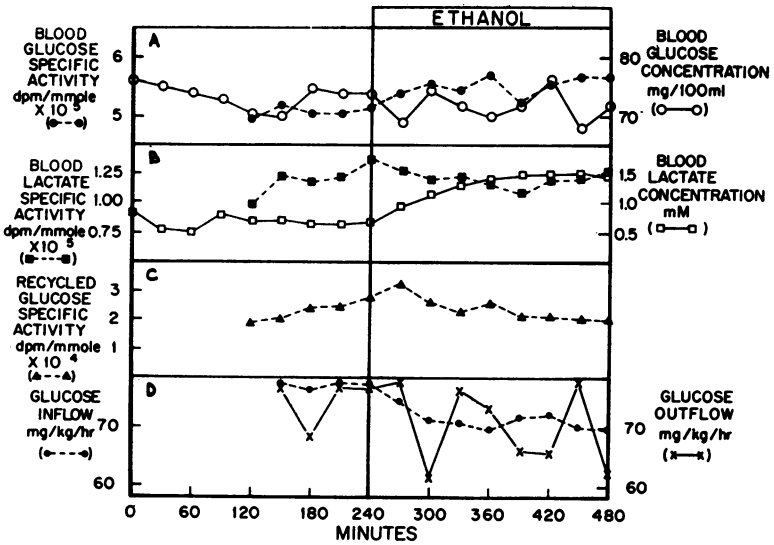

FIGURE 2 Same as Fig. 1. Effect of ethanol.

conversion to lactate, glucose inflow and outflow rates remained closely balanced (panel D of Fig. 2).

The combination of these results with previous observations that ethanol produced a prompt and sustained inhibition of lactate conversion to glucose (15) led us to design experiments in which glucose and lactate interconversion could be determined on the same day. Results of a representative study in which L-(+) lactate-U-^{14}C and glucose-1-^{14}C were administered sequentially are shown in Fig. 3. Studies of this type were performed on two separate occasions, before and during the administration of ethanol. Blood lactate specific activity values (panel A of Fig. 3) increased progressively during the infusion of lactate-U-^{14}C. This pattern was observed in four subjects while in the other two the lactate specific activity values achieved a plateau between 150 and 240 min. In previous studies in which lactate-U-^{14}C was infused for 8 hr, lactate specific activity plateaued by 240 min but often demonstrated a small secondary rise (15). We believe that this finding is due to conversion of endogenously labeled glucose to lactate which raises the activity of the lactate pool in the face of a constant infusion of lactate-U-^{14}C. Lactate turnover for each subject is the mean of four individual values calculated from the lactate specific activities at 150, 180, 210, and 240 min. The individual coefficient of variation for lactate turnover determined from the average of these four values ranged from 1.8 to 10.0% with a mean of 5.75% ±0.95 (SEM, n = 8). We therefore believe that, while the patients were not in an absolute radioisotopic steady state, the lactate turnover calculated from these data is a reasonable approximation of the steady-state value. The blood glucose specific activity values (panel B of Fig. 3) rose progressively during the infusion of lactate-U-^{14}C. The glucose specific activity values at 180, 210, and 240 min were used to calculate the per cent of the glucose turnover which was derived from lactate. After discontinuation of the lactate-^{14}C infusion and during the administration of glucose-1-^{14}C, the blood lactate specific activity fell (panel C of Fig. 3), reaching constant levels during the period from 390 through 480 min. The specific activity of glucose (panel D of Fig. 3) became constant within 60 min of the beginning of the primed-constant infusion of glucose-1-^{14}C. Glucose turnover for each subject was the mean of six individual values obtained at 30-min intervals from 330 through 480 min. The lactate specific activity values at 390, 420, 450, and 480 min during the infusion of glucose-1-^{14}C were used to calculate the per cent of lactate that was derived from glucose.

The glucose kinetics of six subjects determined by the above described sequential techniques before the administration of ethanol are presented in Table II. The mean glucose turnover rate was 94.0 mg/kg per hr ±3.8 (SEM). This value is approximately 80% of the glucose turnover rate determined when glucose-1-^{14}C was infused for 4 hr on the day after the infusion of lactate-U-^{14}C and when samples were chromatographed on Amberlite rather than Dowex resin. This discrepancy is most likely due to the ^{14}C remaining in glucose from the preceding infusion of lactate-^{14}C and a higher glucose specific activity than would have been obtained had the glucose turnover not been performed sequentially. To estimate the extent to which this carry-over influenced the glu-

cose specific activity, we performed experiments in which the infusion of lactate-^{14}C was not followed by glucose-^{14}C and measured the rate of disappearance of ^{14}C from glucose. Blood glucose specific activity decayed at a rate of 20% per hr and it can therefore be estimated that residual ^{14}C in glucose could account for 10–20% of the glucose specific activity during the period of glucose-^{14}C infusion. Glucose recycling constituted 14.5% of the glucose turnover rates versus the value of 10.4% for the previous studies. However, the absolute rates of recycling were not appreciably different. Minor differences were also observed when glucose oxidation rates for both procedures were compared. However, the most striking changes were observed in the calculated rates of glucose conversion to lactate. With the more refined techniques used in this study, the mean glucose to lactate conversion rate was 40.7 mg/kg per hr ±2.1. Residual ^{14}C in lactate after cessation of the lactate-^{14}C infusion could result in the overestimation of glucose conversion to lactate during the infusion of glucose-^{14}C. In studies in which the lactate-^{14}C infusion was not followed by glucose-^{14}C, lactate specific activity fell precipitously and by 360 min it had decreased to a value that was less than 20% of that at 240 min. Considering the lactate specific activities that were generated during the infusion of glucose-^{14}C, this carry-over could account for only 10–15% of the values that were actually observed. However, the glucose to lactate conversion rate observed in the current study is less by approximately 50% than the value obtained previously in which Amberlite chromatography was utilized. As a result, the per cent of the glucose turnover converted to lactate represented 50 rather than 66% of the turnover rate. We believe that these figures are more accurate than those that we reported previously.

Lactate kinetics for these same subjects are shown in Table III. Lactate turnover was 81.8 mg/kg per hr ±6.2, a value that is essentially identical to that determined previously. Similarly, there was essentially no difference in lactate oxidation rates nor in the conversion of lactate to glucose when both techniques were compared. This would be predicted since Dowex resin was also used for separating lactate from glucose in the lactate turnover studies in that series. Lactate conversion to glucose was 16.7 mg/kg per hr ±1.1 and accounted for approximately 21% of the lactate turnover rate.

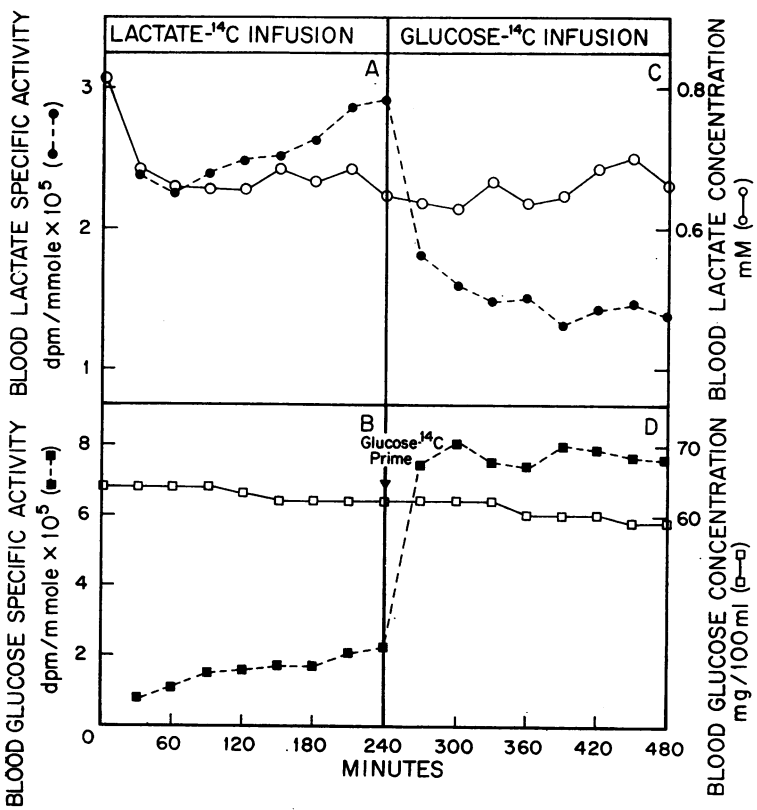

FIGURE 3 Sequential infusions of L-(+) lactate-U-^{14}C and glucose-1-^{14}C. Blood glucose and lactate concentrations and specific activity-time course curves.

TABLE II
Glucose Kinetics

Subject	Turnover	Recycle		Oxidation		Conversion to lactate	
	mg/kg per hr	*mg/kg per hr*	%	*mg/kg per hr*	%	*mg/kg per hr*	%
T. A.	90.7	13.3	14.7	8.9	9.8	46.8	51.6
C. D.	88.7	11.0	12.3	8.6	9.7	34.0	38.3
G. S.	110.0	17.6	16.0	13.3	12.1	40.7	45.1
P. T.	93.7	16.9	18.0	9.5	10.2	35.0	37.3
S. O.	100.3	12.8	12.8	25.4	25.3	47.3	47.1
H. B.	80.8	10.8	13.4	13.2	16.4	40.4	49.9
Mean	94.0	13.7	14.5	13.2	13.9	40.7	44.9
±SEM	3.8	1.1	0.8	2.4	2.3	2.1	2.2
Mean	123.2	12.9	10.4	17.2	13.9	81.3	66.0
±SEM	7.3	1.0	0.8	2.5	2.0	7.0	4.4
(previous study) (16)							

Due to the improved separation of lactate from other radioactive intermediates of glucose metabolism, the specific activity of lactate and therefore the per cent of lactate derived from glucose was lower, as indicated above. As a result, the per cent of lactate turnover derived from glucose was 50.9 ±1.9, a value that is approximately 60% of our previously reported values.

The effect of ethanol on blood glucose concentration and glucose turnover and disposal are shown in Fig. 4. The values demonstrated in the figure are those obtained before and during ethanol. There was no significant difference in blood glucose concentrations before or during ethanol. The mean glucose turnover rate decreased in five of the six subjects from 94.0 ±3.8 to 87.3 ±3.1 mg/kg per hr; however, the change was not significant. Glucose recycling was markedly inhibited in all subjects and decreased by approximately 70% from 14.3 ±1.1 pre-ethanol to 4.6 ±0.3 mg/kg per hr during ethanol. Glucose conversion to lactate was reduced in five and unchanged in one subject. The mean conversion rate decreased from 40.7 ±1.1 to 32.5 ±1.1 mg/kg per hr. When the values for glucose recycling and glucose conversion to lactate were combined, ethanol produced a 35% reduction from 56.3 ±1.6 to 37.0 ±1.0 mg/kg per hr.

The effect of ethanol on blood lactate concentration and lactate turnover and disposal is shown in Fig. 5. The blood lactate concentration during the administra-

TABLE III
Lactate Kinetics

Subject	Turnover	Turnover from glucose	Conversion to glucose		Glucose turnover from lactate	Oxidation	
	mg/kg per hr	%	*mg/kg per hr*	%	%	*mg/kg per hr*	%
T. A.	111.6	42.0	20.8	18.6	22.9	7.8	7.0
C. D.	63.6	53.4	12.5	19.7	14.1	4.4	6.9
G. S.	79.0	51.5	16.7	21.2	16.7	8.2	10.4
P. T.	69.4	50.4	17.9	25.8	19.1	6.1	8.8
S. O.	82.2	57.4	14.7	17.9	14.7	13.7	16.6
H. B.	80.7	50.5	17.4	21.6	21.6	7.9	9.8
Mean	81.8	50.9	16.7	20.8	18.2	8.0	9.9
±SEM	6.2	1.9	1.1	1.1	1.4	1.2	1.3
Mean	81.4	88.0	18.2	22.4	15.1	9.4	11.5
±SEM	5.0	4.0	0.7	2.4	0.8	1.5	1.4
(previous study) (16)							

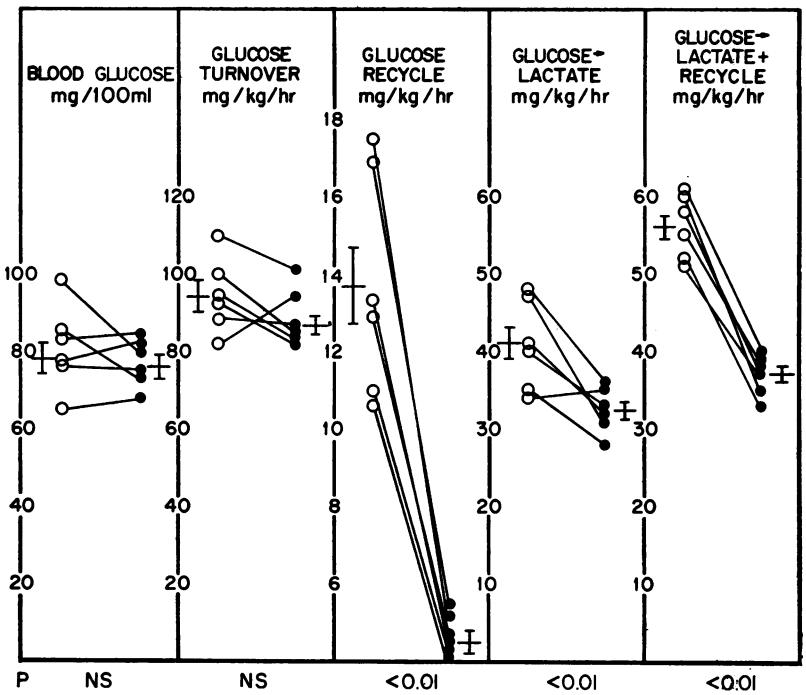

FIGURE 4 Effect of ethanol on glucose metabolism. The blood glucose concentration for each subject is the mean of 17 individual values obtained during the infusion periods.

tion of ethanol increased approximately 200% from 0.63 mmole/liter ±0.04 to 1.17 mmoles/liter ±0.06. The lactate turnover rate was lower in five subjects during ethanol but the differences were not significant. The decrease in lactate conversion to glucose during ethanol was striking. During the pre-ethanol period, 16.7 mg/kg per hr ±1.1 was converted to glucose while during ethanol this value decreased to 5.5 mg/kg per hr ±0.2. The per cent of glucose derived from lactate was reduced by 70% from 18 to 6%.

The effects in two subjects of a 72 hr fast alone and with ethanol on glucose turnover, glucose recycling, and glucose derived from lactate is shown in Table IV. With fasting alone, 35 and 60% of the glucose turnover was derived from lactate, findings consistent with our previous observations concerning lactate conversion to glucose during starvation (16). Ethanol produced significant hypoglycemia in both subjects, lowered glucose turnover and recycling, and inhibited lactate conversion to glucose. The inhibition of lactate conversion to glucose by ethanol in these subjects was no greater than that which occurred in fed individuals.

DISCUSSION

The metabolism of glucose and of lactate are intimately related. Glucose serves both as a precursor of lactate as well as a product of its metabolism. Glucose is converted to lactate by nonhepatic tissues, and the lactate is subsequently resynthesized into glucose by the liver, a process commonly known as glucose recycling or as the

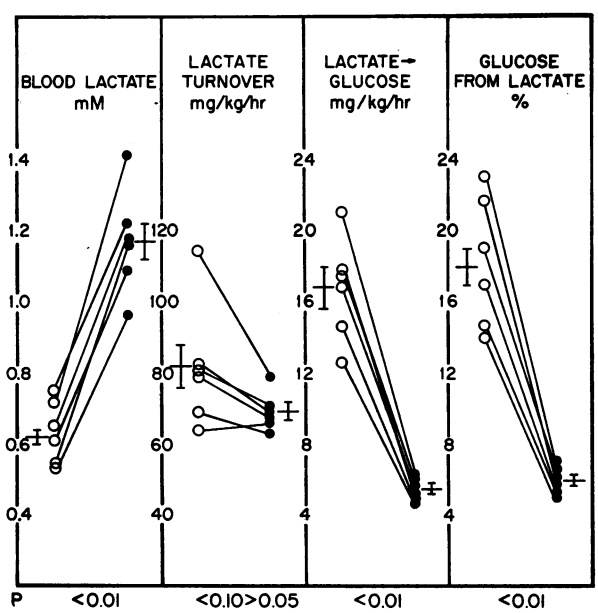

FIGURE 5 Effect of ethanol on lactate metabolism. The blood lactate concentrations for each subject is the mean of 17 individual values obtained during the infusion periods.

TABLE IV

Effect of Starvation and Ethanol

Subject	Fast*	Ethanol	Blood‡ glucose	Glucose turnover	Glucose recycling	Glucose from lactate
			mg/100 ml	*mg/kg per hr*	*mg/kg per hr*	*mg/kg per hr*
V. W.	+	0	53.5	55.9	11.3	19.6
	+	+	31.8	36.2	5.0	11.9
P. H.	+	0	41.2	58.9	13.3	35.0
	+	+	27.9	40.4	5.1	12.8

* 72 hr.

‡ Mean of 17 individual values obtained at 30-min intervals during the infusion period.

Cori cycle (19). As indicated in the current studies, approximately 45% of the glucose turnover is converted to lactate, accounting for 50% of the lactate turnover. These values are significantly lower than previous estimates from this laboratory (16), no doubt reflecting the refinement of our methodology and are consistent with values obtained in sheep in which lactate was isolated as the acetaldehyde-dimedone derivative (20). Lactate serves as a significant glucose precursor in the human, accounting for approximately 21% of the glucose turnover in the nonfasted state and 35% during prolonged starvation (16). Approximately 14% of the glucose pool is derived from lactate in sheep (20). As indicated in this and previous studies (16) of lactate conversion to glucose in man, an infusion period of 4 hr may not always be sufficient to allow achievement of constant glucose specific activity. In that regard, our calculations of the glucose turnover which is derived from lactate may underestimate the actual value. Inspection of the contour of the glucose specific activity curve during an 8 hr infusion of lactate-^{14}C (15) suggests that a plateau is reached at approximately 4–5 hr but that there is a secondary rise over the remainder of the infusion period. The latter may be due to increased specific activity of the glucose pool and greater participation of labeled glucose in the recycling process without any increase in recycling and does not necessarily reflect increased lactate incorporation into glucose. This interpretation is also supported by the observation that the lactate specific activity also rises during this interval but that the lactate concentration remains constant. A recent critical review of glucose recycling has emphasized the variability of this process in otherwise normal subjects and the probable importance of anxiety as a modifying factor (21). Certainly the wide range of lactate turnover rates observed in the current group of normal subjects supports such a proposal. The values for glucose recycling in the current study are similar to those of previous studies from this laboratory (16, 22). The data presented in this paper indicates that ethanol significantly influences the metabolism and interconversion of glucose and lactate. Ethanol not only decreased lactate incorporation into glucose but also inhibited glucose conversion to lactate, thus modifying both limbs of the Cori cycle.

As demonstrated in this and a previous study, the incorporation of lactate into glucose is inhibited during both the acute (15) and chronic phases of ethanol administration. The incomplete inhibition of lactate conversion to glucose by ethanol could be a result of either only partial blockade of hepatic gluconeogenesis or of continued incorporation of lactate into glucose by kidney since this tissue is not sensitive to the inhibitory effects of ethanol on gluconeogenesis (13). However, despite the significant reduction of gluconeogenesis from lactate, glucose turnover was only slightly decreased and blood glucose concentration was unchanged. Since only 20% of glucose production in humans who have been fasted for 10–12 hr is derived from lactate (16), failure to develop hypoglycemia could simply indicate the relative unimportance of lactate as a glucose precursor under these conditions. This seems unlikely since ethanol also decreases the uptake of glycerol (8), a major gluconeogenic precursor, by the human liver. Several more reasonable explanations may be possible. As demonstrated in dogs (14), the net effect of ethanol on the blood glucose concentration is determined by opposing actions on glucose production and utilization. Ethanol decreases peripheral glucose disposal, which tends to raise the blood glucose, as well as inhibiting hepatic glucose release, which tends to lower it. If maintenance of the blood glucose concentration in these subjects was due to concomitant and balanced changes in glucose production and utilization, then a greater decrease in the glucose turnover rate than was observed would have been expected. Since the glucose turnover rate was not significantly reduced by ethanol, we propose that the inhibition of gluconeogenesis in our subjects was masked by a concomitant increase in glycogenolysis.

Because hepatic glycogen stores are preserved in normal subjects fasted for short periods of time, it has not been possible to determine from measurements of peripheral or hepatic venous glucose whether ethanol has inhibited gluconeogenesis. Ethanol decreases glucose production by the perfused rat liver from animals fasted for only 18 hr (1, 11) and inhibits alanine incorporation into glucose by liver slices from nonfasted rabbits and man (4, 7). Our results indicate that starvation is not a prerequisite for ethanol inhibition of gluconeogenesis but is necessary for the development of hypoglycemia. This is supported by our additional observation that there was no difference in the degree of inhibition of gluconeogenesis produced by ethanol when our nonfasted subjects were compared with the two subjects who had fasted for 48–72 hr, despite the presence of hy-

poglycemia in the latter. This would suggest that ethanol increases NADH in the nonstarved as well as the starved state and diverts substrate away from gluconeogenesis. Krebs has indicated that the rate of the pyruvate carboxylase reaction in vivo is likely to be controlled by the concentration of pyruvate in the liver and that a shift in the $NAD^+/NADH$ ratio as a result of ethanol would reduce hepatic pyruvate concentration and decrease gluconeogenesis (23). Freinkel and his coworkers (4) also recognized that the hypoglycemic effect of ethanol would be buffered by the availability of preformed glycogen at the time of exposure but did not believe that inhibition of gluconeogenesis occurred until glycogen stores were exhausted.

In addition to decreased lactate conversion to glucose, these studies indicate that glucose conversion of lactate is also inhibited by ethanol. The decrease in glucose recycling observed in these studies was therefore not due entirely to inhibition of lactate conversion to glucose but also to decreased glucose conversion to lactate. The recovery of glucose in lactate and in recycled glucose, which partially reflects peripheral metabolism of glucose, was inhibited 35%, thus confirming the observation that ethanol inhibits peripheral glucose utilization in dogs (14). Whether this effect is due to ethanol directly or to acetate, which has been demonstrated to mediate many of its effects (24), cannot be stated. It is also possible that the increased availability of lactate that occurs with the administration of ethanol may be partially responsible for the inhibition of glucose utilization since lactate has been shown to block glucose oxidation and turnover in dogs (25).

As indicated by the pre-ethanol studies, approximately 50% of the normal lactate turnover is derived from glucose, the remainder presumably coming from muscle glycogen and other sources, perhaps amino acids. Despite the reduction in glucose conversion to lactate by ethanol, there was no change in the rate of production of lactate in the acute studies (15) nor lactate turnover in the chronic studies. This strongly suggests that the contribution of other sources to the lactate pool must have increased. While the deficit in the lactate pool which arises from the decreased glucose contribution may be made up by peripheral tissues, it is also possible that the liver makes a contribution. Ethanol increases the recovery of alanine-^{14}C in lactate in studies with rat liver slices (9) and the conversion of serine and alanine to lactate by the isolated perfused rat liver (13). Similar alterations in humans may account for the observation that ethanol not only blocks lactate by the liver but also increases hepatic vein lactate concentrations (26, 27). Since alanine is an important glucose precursor arising primarily from peripheral protein stores, its conversion to lactate, either in muscle or liver in the presence of ethanol, would decrease its availability for glucose synthesis and might be an important contributory factor in the development of hypoglycemia. It would be of some interest to known whether ethanol alters the concentrations of blood amino acids and alanine in particular. To our knowledge, such studies have not been performed. Recent studies indicate that reciprocal changes may be observed in blood amino acid concentrations in obesity and during nutritional manipulation, and unless specific measurements of individual acids is undertaken, important changes may be obscured (28, 29).

It is important to emphasize that metabolic data obtained with isotopic dilution techniques must be interpreted with some reservation. Estimates of lactate turnover from lactate-^{14}C disappearance may not reflect net lactate balance. Because of the rapid exchange of ^{14}C in lactate wtih pyruvate and its subsequent incorporation into other amino acids and tricarboxylic acid cycle intermediates without net disappearance of substrate, it is likely that the calculated turnover is greater than true lactate flux. Furthermore, since the rate of isotopic exchange is more rapid than true lactate metabolism, less ^{14}C is available for oxidation and conversion to glucose with the result that the disposal of lactate by these routes will be underestimated. The extent to which these exchange reactions contribute to the disappearance of the ^{14}C label and thereby overestimate true chemical flow of substrate and underestimate metabolism cannot be determined from these studies. However, in view of the fact that the turnover of lactate determined isotopically (80 mg/kg per hr) is approximately twice the estimated rate of lactate production (46 mg/kg per hr) by the three major tissues producing lactate (brain, erythrocytes, and muscle) (16) and because the incorporation of lactate into glucose and CO_2 can account for only 30% of the turnover, it is likely that this exchange is significant.

APPENDIX

$$\text{Lactate turnover (mmoles/hr)} = \frac{\text{lactate-}^{14}C \text{ infusion rate (dpm/hr)}}{\text{blood lactate specific activity (dpm/mmole)}} \quad (1)$$

$$CO_2 \text{ from lactate (\%)} = \frac{CO_2 \text{ specific activity (dpm/mmole)} \times 3 \times 100}{\text{blood lactate specific activity}} \quad (2)$$

$$\text{Lactate oxidation (mmoles/hr)} = \frac{\%\ CO_2 \text{ from lactate} \times \text{minute } CO_2 \text{ production (mmoles)} \times 60}{3} \quad (3)$$

$$\text{Glucose from lactate (\%)} = \frac{\text{blood glucose specific activity (dpm/mmole)} \times 100}{\text{blood lactate specific activity (dpm/mmole)} \times 2} \quad (4)$$

$$\text{Glucose from lactate (mmoles/hr)} = \text{\% glucose from lactate} \times \text{glucose turnover (mmoles/hr)} \quad (5)$$

$$\text{Lactate conversion to glucose (mmoles/hr)} = \text{glucose from lactate (mmoles/hr)} \times 2 \quad (6)$$

$$\text{Glucose turnover (mmoles/hr)} = \frac{\text{glucose-1-}^{14}\text{C infusion rate (dpm/hr)}}{\text{blood glucose-1-}^{14}\text{C specific activity (dpm/mmole)}} \quad (7)$$

$$\text{CO}_2\text{ from glucose (\%)} = \frac{\text{CO}_2\text{ specific activity (dpm/mmole)} \times 6 \times 100}{\text{blood glucose specific activity (dpm/mmole)}} \quad (8)$$

$$\text{Glucose oxidation (mmoles/hr)} = \frac{\text{\% CO}_2\text{ from glucose} \times \text{minute CO}_2\text{ production (mmoles)} \times 60}{6} \quad (9)$$

$$\text{Lactate from glucose (\%)} = \frac{\text{blood lactate specific activity (dpm/mmole)} \times 2 \times 100}{\text{blood glucose specific activity (dpm/mmole)}} \quad (10)$$

$$\text{Lactate from glucose (mmoles/hr)} = \text{\% lactate from glucose} \times \text{lactate turnover (mmoles/hr)} \quad (11)$$

$$\text{Glucose conversion to lactate (mmoles/hr)} = \frac{\text{lactate from glucose (mmoles/hr)}}{2} \quad (12)$$

ACKNOWLEDGMENTS

We wish to express our appreciation to Gail Yerby, Anne Stefanu, Sharon O'Grady, Marilyn DeSteno, Jack Friday, and Dan Martin for their technical assistance and to the nurses and staff of the Clinical Research Center of the University of Alabama Medical Center.

This work was supported by the Veterans Administration Hospital, Birmingham, Ala., and by U. S. Public Health Service Grants AM-09722-04, 5MO1-RR32-09, and TO1-AM05053-15.

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