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Effect of L-Alanine Infusion on Gluconeogenesis and Ketogenesis in the Rat *in vivo*

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1. In 48h-starved 6-week-old rats the ^{14}C incorporation *in vivo* into blood glucose from a constant-specific-radioactivity pool of circulating [^{14}C]alanine or [^{14}C]lactate confirmed that lactate is the preferred gluconeogenic substrate. 2. Increasing the blood [alanine] to that occurring in the fed state increased ^{14}C incorporation into blood glucose 2.3-fold from [^{14}C]alanine and 1.7-fold from [^{14}C]lactate. 3. When the blood [alanine] was increased to that in the fed state, the ^{14}C incorporation into liver glycogen from circulating [^{14}C]alanine or [^{14}C]lactate increased 13.5- and 1.7-fold respectively. 4. The incorporation of ^{14}C into blood acetoacetate and 3-hydroxybutyrate from a constant-specific-radioactivity pool of circulating [^{14}C]oleate was virtually abolished by increasing the blood [alanine] to that existing in the fed state. However, the [acetoacetate] remained unchanged, whereas [3-hydroxybutyrate] decreased, although less rapidly than did its radiochemical concentration. 5. It is concluded that during starvation in 6-week-old rats, the blood [alanine] appears to influence ketogenesis from circulating unesterified fatty acids and inversely affects gluconeogenesis from either lactate or alanine. A different pattern of gluconeogenesis may exist for alanine and lactate as evidenced by comparative ^{14}C incorporation into liver glycogen and blood glucose.

Alanine, one of the primary gluconeogenic substrates (Ross *et al.*, 1967; Felig, 1975; Garber *et al.*, 1976), is released in substantial amounts from skeletal muscle (Blackshear *et al.*, 1975; Felig, 1975; Garber *et al.*, 1976). In addition, the elevation of this amino acid in blood above 0.6mM causes a marked decrease in the blood [3-hydroxybutyrate] and alters the overall production of ketone bodies (Ozand *et al.*, 1977). These findings suggest that this amino acid may have a regulatory role in both gluconeogenesis and ketogenesis. Therefore a series of experiments was initiated to investigate such a combined regulatory role of alanine. Among the questions posed is the possible influence of L-alanine on glucose formation from lactate as well as from alanine itself. In addition, one may ask if the formation of ketone bodies from circulating unesterified fatty acids is inhibited directly by alanine, as would occur if the liver is the site of action for the anti-ketogenic effect of alanine (Ozand *et al.*, 1977).

The present studies confirm the presumption that ketone-body formation from circulating unesterified fatty acid is decreased by alanine infusion, and show that glucose formation from circulating alanine and lactate is also influenced by the concentration of alanine in the blood. These results further suggest that the processes of gluconeogenesis and ketogenesis, although generally believed to be under coordinate control processes, at least during starvation, may be regulated by independent mechanisms.

Experimental

Animals and reagents

Wistar rats of both sexes were used: the age of the animals was 5–6 weeks. The animals were fed on regular laboratory chow *ad lib* (Ralston Purina Co., St. Louis, MO, U.S.A.) until they were placed in metabolic cages and starved for 48h immediately before experimentation. The animals had free access to water. All reagents and enzymes for the perfusion studies and biochemical analyses were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., except for alanine dehydrogenase (EC 1.4.1.1; from *Bacillus subtilis*), which was obtained from Boehringer Mannheim Corp., New York, NY, U.S.A. The ^{14}C-labelled compounds were purchased from New England Nuclear Corp., Boston, MA, U.S.A., or Amersham/Searle Corp., Arlington Heights, IL, U.S.A. In experiments where sodium oleate was used, this compound was dissolved with 16% (w/v) bovine serum albumin in 0.9% NaCl. The albumin used was essentially freed of unesterified fatty acids and citric

acid by the procedures of Chen (1967) and Lopes-Cardozo & VandenBergh (1972) respectively.

Perfusion procedure

The perfusion procedure has been described by Ozand *et al.* (1977). This procedure was modified to provide rapid labelling of the circulating intermediate pool by injecting a priming dose in 0.9% NaCl (2.0ml/kg body wt.) into the vena femoralis and followed within 1min by constant perfusion of the same compound in 0.9% NaCl (12.0ml/h per kg body wt.) by using a Sage pump. The Sage pump employed was model 341 from Orion Research, Cambridge, MA, U.S.A. The detailed compositions of the perfusion solutions are described for each experiment. The samples of blood (50–100μl) equal to the amount of perfused fluid were obtained from the contralateral arteria femoralis at 4–5min intervals. These samples were quickly mixed with 1ml of 1M-$HClO_4$ at 0°C, centrifuged at 1000*g* for 10min and the deproteinized supernatant fluids as well as the precipitated protein pellets were kept at −95°C until analysed.

Analyses of intermediates

Glucose, lactate, alanine, acetoacetate and 3-hydroxybutyrate were analysed by a micro-auto-analytic procedure previously described (Ozand *et al.*, 1975). L-Alanine was assayed by using alanine dehydrogenase (Yoshida & Freese, 1970; Ozand *et al.*, 1975). Results were expressed as the average of all values at indicated time intervals±S.E.M.; the Student's single *t* test was used to calculate the statistical significance (White, 1951). Analysis of ^{14}C-labelled acetoacetate and acetoacetate plus 3-hydroxybutyrate was performed with the precipitated Denigés salt before and after treatment with 3-hydroxybutyrate dehydrogenase respectively essentially as described by McGarry & Foster (1969). The determination of [^{14}C]oleate in blood was performed by liquid-scintillation counting after extraction of the $HClO_4$-precipitated pellet by the procedure of Lauwreys (1969).

Isolation and identification of radioactive compounds (produced in gluconeogenesis)

This procedure is a combination of the methods outlined by Windmeuller & Spaeth (1974) and Chiasson *et al.* (1977). The acidified supernatant fluid of blood centrifuged at 1000*g* for 10min received a mixture of L-[3-^3H]alanine (10–30nCi), DL-[2-^3H]lactate (3–6nCi) and D-[6-^3H]glucose (3–6nCi). The samples were neutralized by the addition of 1M-KOH to pH 6–6.5 and the $KClO_4$ pellets discarded after centrifugation at 1000*g* for 10min. The sample was then passed through 0.5ml of Dowex-50 (H^+ form), and the resin washed with water. The combined eluates were neutralized to pH7–8 and passed through a 1.5ml column of Dowex-1 (formate form). The resin was again washed with water. The eluates were then combined and freeze-dried. The residue was dissolved in 3ml of water and labelled glucose was determined by mixing 1ml of this sample with 10ml of Triton X-100 counting solution and counting for radioactivity in a liquid-scintillation counter. The composition of the scintillator was 5g of 2,5-diphenyloxazole and 0.3g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of toluene and 500ml of Triton X-100. The Dowex-50 (H^+ form) columns were eluted repeatedly with 2ml of 4M-NH_3 and the combined eluates were freeze-dried and then dissolved in 0.5ml of 40mM-L-alanine solution. Two samples (50μl) were applied to a strip [1.5in (3.8cm)×23in (58.4cm)] of Whatman 3MM paper and subjected to high-voltage electrophoresis in 1.64M-formic acid, pH1.55, at 4000V, 400A, for 20min. The alanine spot was located by ninhydrin with a standard of non-radioactive alanine run simultaneously on a parallel marker strip. The Dowex-1 (formate form) column was eluted with five successive washes each of 2ml of 12M-formic acid and the eluates were freeze-dried as above, except that 40mM-L-lactate was used to dissolve the residue. Two samples (50μl) were subjected to high-voltage electrophoresis exactly as above. This procedure quantitatively separates pyruvate from lactate. The lactate spot was located by staining a parallel marker strip with lactate dehydrogenase/NAD^+/Nitro Blue Tetrazolium/phenazine methosulphate isoenzyme dye (Brewer, 1970). The alanine or lactate spots from experimental strips were cut into small pieces and placed into 1ml of water and 10ml of the previously described toluene/Triton X-100-based fluor. The samples were left at 4°C overnight, and the paper strips were removed next morning and the solutions counted for radioactivity. The standard procedures (Okita *et al.*, 1957) for double radioisotope counting were used in an LKB Ultrobeta liquid-scintillation counter.

At the end of each experiment the animals were killed and the liver glycogen was purified essentially by the procedure of Somogyi (1957). This procedure was modified as follows: the initial 33% (v/v) ethanol precipitate was dissolved in 5% (w/v) trichloroacetic acid at 4°C and the denatured protein was discarded by centrifuging at 2000*g* for 10min. The glycogen was precipitated with 70% ethanol. The final precipitate was hydrolysed with 1M-HCl in boiling water for 30min. The hydrolysed solution was neutralized to pH7.0 with the addition of KOH, a portion of which was analysed for glucose. To another portion 3nCi of [6-^3H]glucose was added and was further purified by passage through Dowex-50 (H^+ form) and Dowex-1 (formate form) columns as outlined for the isolation of labelled intermediates from blood. A portion of the eluate from the Dowex-1 column was counted for radioactivity with the Triton X-100-based solution as scintillant as described previously.

Calculations

The average d.p.m., concentration and specific radioactivities of alanine, lactate and glucose were determined at 5 min intervals for 50–70 min. A linear regression fitting a set of data points to a straight line was used involving the method of least squares where r^2 is the proportion of total variation about the mean ($r^2 = 1$ indicates perfect fit). The rate of glucose formation was determined from the steady-state specific radioactivities of intermediates when either [^{14}C]alanine or [^{14}C]lactate was perfused. The rates of radioisotope appearance in glucose were expressed as nmol of alanine or lactate incorporated into 1 μmol of blood glucose/min. The results will be discussed in terms of [alanine] = (>0.8 mM) being characteristic of fed animals and [alanine] = (<0.3 mM) being characteristic of starved animals.

Results

Steady-state incorporation of label from [^{14}C]alanine into glucose and lactate at [alanine] occurring in starved animals

The incorporation of ^{14}C into glucose and lactate from concentrations of [1-^{14}C]alanine occurring in starved animals is shown in Fig. 1. In this experiment the circulating pool of alanine was labelled with a priming infusion of [1-^{14}C]alanine followed by a constant perfusion (see the legend of Fig. 1). Fig. 1(*a*) shows that this priming-perfusion sequence did not significantly alter the chemical concentrations of lactate, 3-hydroxybutyrate, glucose or alanine observed in starved animals at zero time. The results in Fig. 1(*b*) demonstrate that this procedure resulted in the labelling of circulating alanine, glucose and lactate. The specific radioactivities of the circulating alanine and lactate remained essentially constant after the priming dose. In contrast, the specific radioactivity of the glucose continually increased.

Steady-state incorporation of label from [^{14}C]alanine into glucose and lactate at [alanine] observed in fed animals

The data presented in Fig. 2 show the effect of a priming dose of [1-^{14}C]alanine, followed by infusion of alanine at a lower specific radioactivity. This was designed to label the circulating pool of alanine immediately, and maintain the circulating alanine concentration at a value equal to that observed in fed animals. There was no significant alteration in chemically assayed lactate and glucose during this period. In contrast, the chemical concentration of alanine rose from approx. 300 μM to about 1 mM and remained essentially unchanged for the duration of the infusion. This increase in blood [alanine] was accompanied by an abrupt decline in the concentration of circulating 3-hydroxybutyrate, which persisted throughout the course of the experiment.

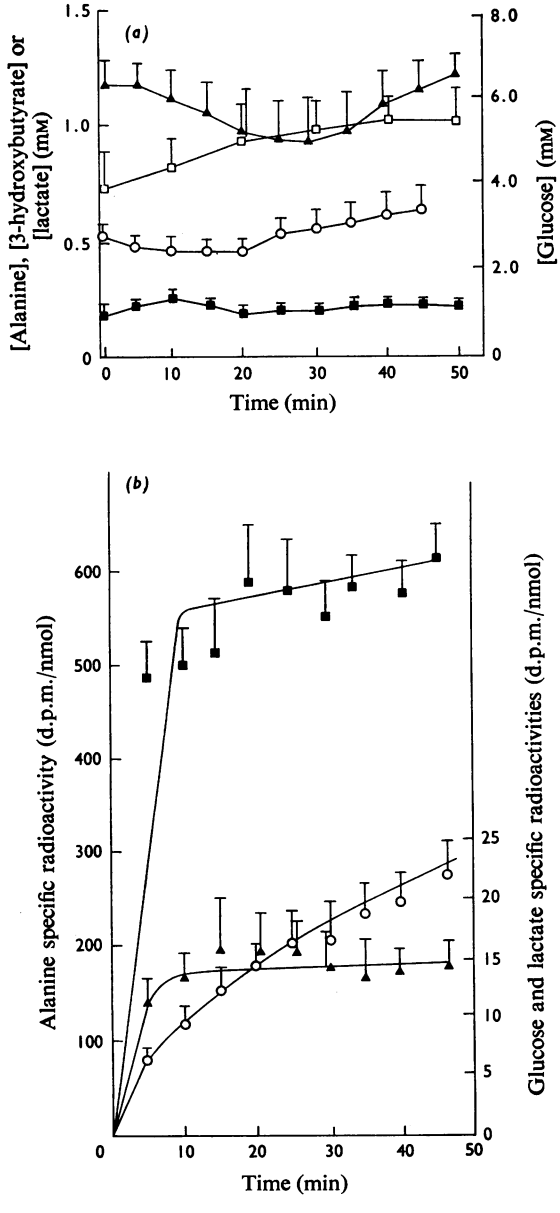

Fig. 1. *Label incorporation into glucose from [^{14}C]alanine at concentrations of blood alanine occurring in starved animals*

The priming dose was 30 μCi of L-[1-^{14}C]alanine (0.05 mg/kg body wt. in 0.9% NaCl) and the perfusion dose was 90 μCi of L-[1-^{14}C]alanine (0.15 mg/h per kg body wt. in 0.9% NaCl). The average concentrations (±S.E.M.) of L-alanine (■), 3-hydroxybutyrate (□), lactate (▲) and glucose (○) in six animals are shown in Fig. 1(*a*). The average specific radioactivities (±S.E.M.) of L-alanine (■), lactate (▲) and glucose (○) are shown in Fig. 1(*b*).

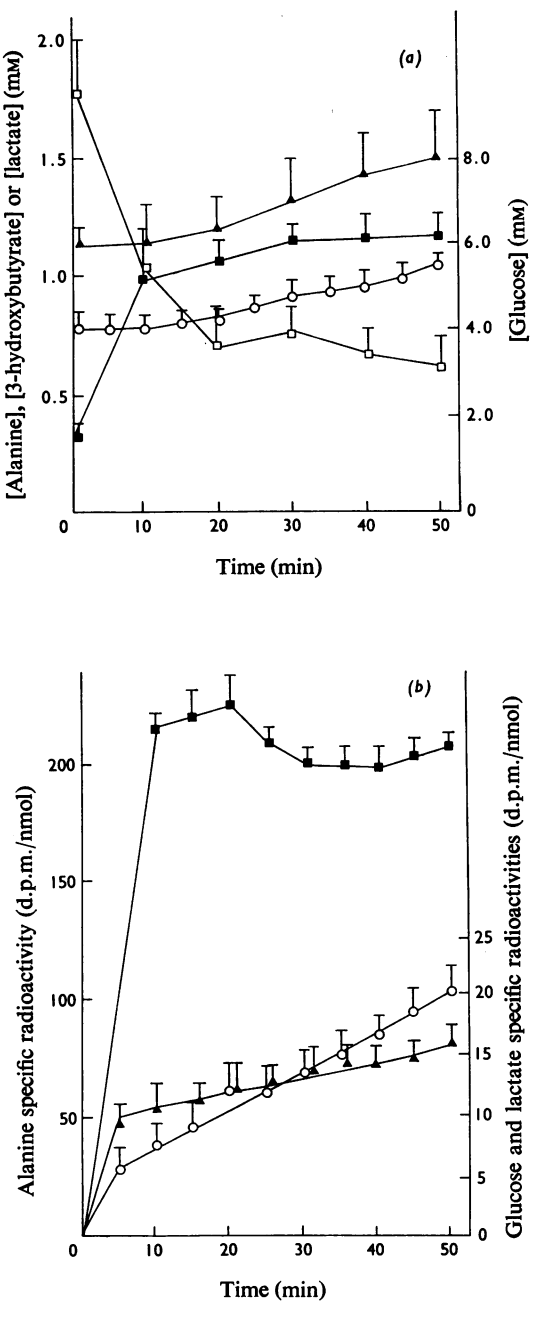

Fig. 2. *Label incorporation into glucose from [^{14}C]alanine at concentrations of blood alanine occurring in fed animals*

The priming dose was 40μCi of L-[1-^{14}C]alanine (100mg/kg body wt.) and the perfusion dose was 90μCi of L-[1-^{14}C]alanine (204mg/h per kg body wt.). The symbols for Figs. 2(*a*) and 2(*b*) are the same as those in Fig. 1. The results were the average±S.E.M. in six animals.

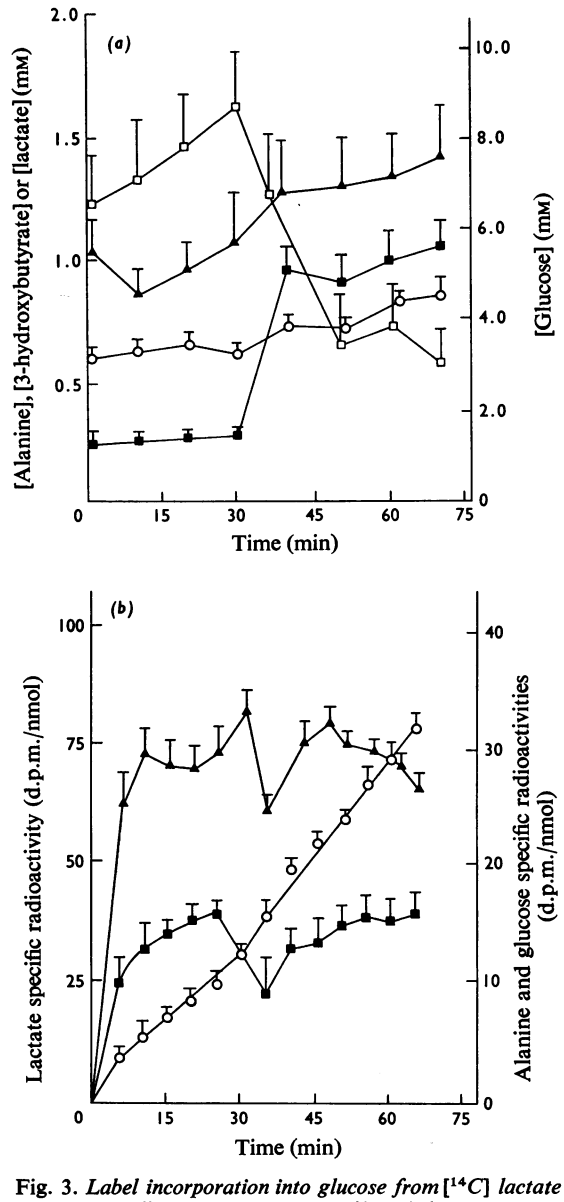

Fig. 3. *Label incorporation into glucose from [^{14}C] lactate at different concentrations of blood alanine*

The animals were injected with a priming dose of 33μCi of L-[^{14}C]lactate (0.5mg/kg body wt. in 0.9% NaCl) at zero time. The perfusion fluid contained 72μCi of L-[1-^{14}C]lactate (1.1mg/h per kg body wt.) from zero time to 30min. The animals were given a second priming dose after 30min that contained 9.5μCi of L-[1-^{14}C]lactate (0.15mg) and L-alanine (130mg/kg body wt.) in 0.9% NaCl after which they received a perfusion solution that contained 96μCi of L-[1-^{14}C]lactic acid (1.4mg) and L-alanine (204mg/h per kg body wt.) until 70min. The symbols for Figs 3(*a*) and 3(*b*) were the same as those in Fig. 1. The results were the average±S.E.M. in seven animals.

The specific radioactivities of circulating alanine and lactate remained unchanged (cf. Figs. 1*b* and 2*b*). In contrast, during this perfusion the specific radioactivity of glucose increased progressively. The specific radioactivities of lactate in experiments of both Figs. 1(*b*) and 2(*b*) were essentially the same, and in each case these values were considerably lower than the steady-state specific radioactivity of alanine.

Steady-state incorporation of label from [^{14}C]lactate into glucose at [alanine] occurring in fed and starved animals

Fig. 3 shows the result of an initial perfusion of [1-^{14}C]lactate followed by a double infusion of labelled lactate and unlabelled alanine to increase the circulating [alanine] to the [alanine] occurring in fed animals. During the latter part of the experiment [alanine] was maintained at approx. 1 mM and the specific radioactivity of lactate was sustained at the value established before the alanine administration by adjustment of the perfusion concentrations. To accomplish this, the [1-^{14}C]lactate infusion had to be increased from 1.1 to 1.4 mg/h per kg body wt. The results presented in Fig. 3(*a*) show that there was only a slight increase in lactate after a transient and marginal decline at 15 min. On administration of alanine at 30 min, the expected increase of alanine to the concentration observed in fed animals occurred. Concomitant with this effect was a decrement in [3-hydroxybutyrate]. Throughout the course of the perfusion a marginal increase in circulating blood [glucose] was observed. Although there was some fluctuation, the specific radioactivities of the circulating lactate and alanine were maintained relatively constant except for the brief periods immediately after the injection of priming doses. The rate of radioisotope appearance in glucose increased significantly during the second period when the alanine concentration was maintained at approx. 1 mM.

Rates of ^{14}C incorporation into blood glucose and liver glycogen from [^{14}C]alanine and [^{14}C]lactate

The rates of radioisotope incorporation into blood glucose from ^{14}C-labelled precursors are summarized in Table 1. The incorporation of label from [1-^{14}C]alanine into glucose was more than doubled ($P<0.001$) when the blood alanine concentration was increased from approx. 0.3 mM to 1 mM. Similarly, the rate of ^{14}C appearance in blood glucose from the [1-^{14}C]lactate was increased 1.7-fold ($P<0.05$) when the blood [alanine] was increased.

The amount of ^{14}C in purified liver glycogen of these experiments is also shown in Table 1. The increase of blood alanine (i.e. approx. 3-fold) from Expt. 1 to Expt. 2 (cf. Figs. 1 and 2) was accompanied by an increased incorporation of ^{14}C into liver glycogen of some 13.5-fold by the end of the experiment. When the blood alanine was maintained at approx. 0.3 mM for 30 min and then increased to approx. 1 mM (cf. Fig. 3*a* and Expt. 3, Table 1), the incorporation of ^{14}C into glycogen from [^{14}C]lactate was 183 nmol/μmol of glucose. If, however, the [alanine] was not increased after 30 min, the incorporation of ^{14}C into liver glycogen was 110 nmol/μmol of glucose at the end of the experiments. This represents a 1.7-fold

Table 1. *Rate of label incorporation into blood glucose and liver glycogen from [^{14}C]lactate and [^{14}C]alanine in starved rats*
The rates of gluconeogenesis were calculated as outlined in the Experimental section. The results as outlined in Figs. 1–3 were used. *n* indicates the number of animals; $r^2 \pm$S.E.M. is the average of the proportion of total variation and its standard error. The animals were killed at times indicated, when the radioisotope incorporation into glycogen was measured.

Experiment	Incorporation into blood glucose (nmol of precursor into 1 μmol of glucose per min)	Incorporation into liver glycogen at the end of the experiment (nmol of precursor in 1 μmol of glucose)	Time of killing (min)
(1) From [^{14}C]alanine at a concentration of approx. 0.3 mM of blood alanine	0.61 ± 0.11 ($n=6$) ($r^2=0.91 \pm 0.01$)	3.1 ± 1.8 ($n=6$)	50
(2) From [^{14}C]alanine at a concentration of 1 mM of blood alanine	1.38 ± 0.10 ($n=6$) ($r^2=0.97 \pm 0.01$) $P<0.001$ compared with (1)	41.5 ± 9.9 ($n=6$)	50
(3) From [^{14}C]lactate at a concentration of approx. 0.3 mM of blood alanine*	9.27 ± 1.78 ($n=10$) ($r^2=0.98 \pm 0.01$)	110.3 ± 6.5 ($n=4$)	70
(4) From [^{14}C]lactate at a concentration of 1 mM of blood alanine	15.49 ± 2.24 ($n=7$) ($r^2=0.98 \pm 0.01$) $0.05>P>0.02$ compared with (3)	183.1 ± 23.1 ($n=7$)	70

* These animals received no alanine in the second priming dose and the label incorporation into blood glucose is not shown in Fig. 3. Initial priming solution and perfusion solutions were the same as described for Expt. 3 (cf. Fig. 3).

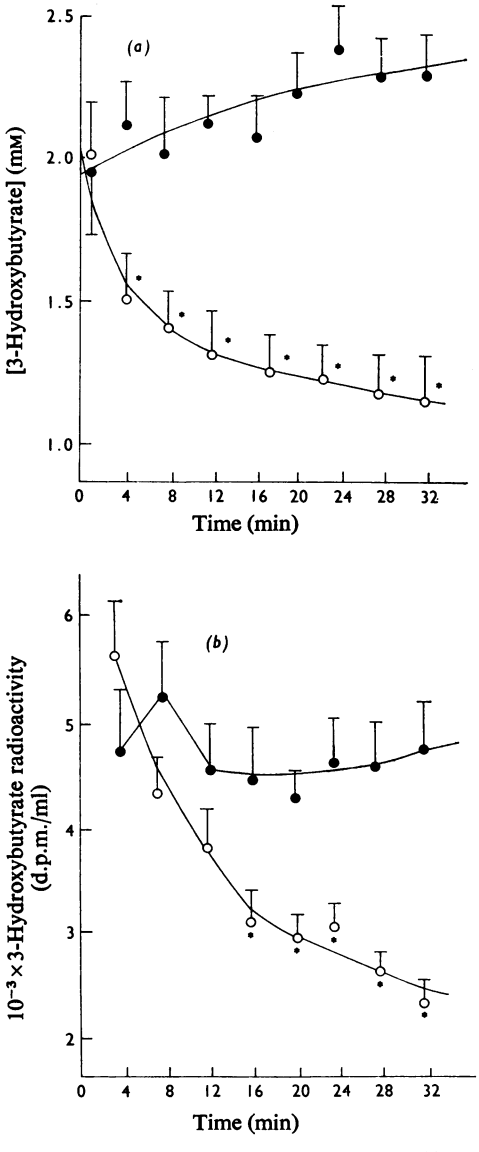

Fig. 4. *Formation of 3-hydroxybutyrate from* [1-^{14}*C*]*oleate*
The priming dose contained 20.3 μCi of sodium [1-^{14}C]oleate (15.2mg), bovine serum albumin (800mg) and either (●) D- or (○) L-alanine (105mg/kg body wt.) in 0.9% NaCl. The results were the average of eight animals (±S.E.M.). * indicates statistically significant results at $P<0.02$ confidence level. (*a*) shows the changes of [3-hydroxybutyrate] and (*b*) the changes in the total radioactivity of 3-hydroxybutyrate.

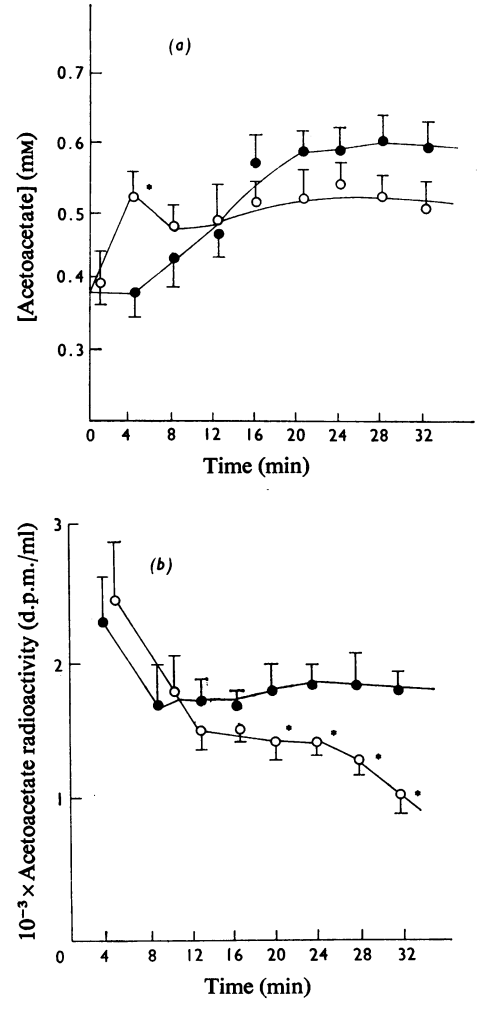

Fig. 5. *Formation of acetoacetate from* [1-^{14}*C*]*oleate*
Experimental conditions were the same as in Fig. 4.

increase in ^{14}C incorporation into glycogen from lactate when the [alanine] is below 0.3 mM rather than when it is approx. 1 mM.

Ketogenesis from [1-^{14}*C*]*oleate in the presence of* [*alanine*] *occurring in fed and starved animals*

The results shown in Figs. 4 and 5 demonstrate the effect of alanine on the blood [3-hydroxybutyrate] and [acetoacetate]. Included in Figs. 4 and 5 are the specific radioactivities of these ketone bodies that have been derived from [^{14}C]oleate in the presence of D- or L-alanine. No differential effect of either isomer of alanine on the circulating concentration of [^{14}C]-oleate was observed (Fig. 6): in both cases there was a transient decrement in [^{14}C]oleate after the priming dose and a marginal increase during the remainder of the experiment. Furthermore, any effect ascribed to the infusion of D- or L-alanine should be assessed after the initial decline, since the immediate decrease

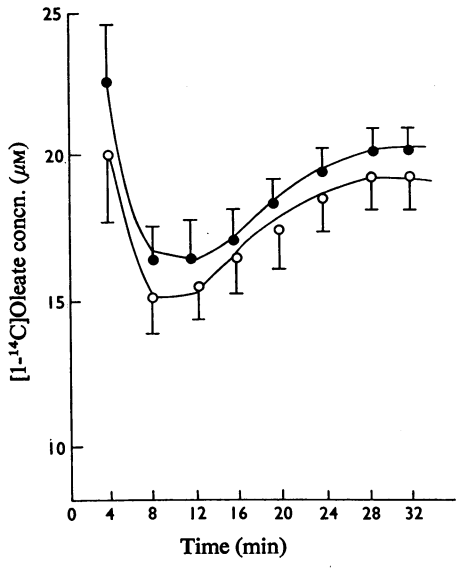

Fig. 6. *Concentration of* [1-^{14}C]*oleate in blood*
Experimental conditions were the same as in Fig. 4.

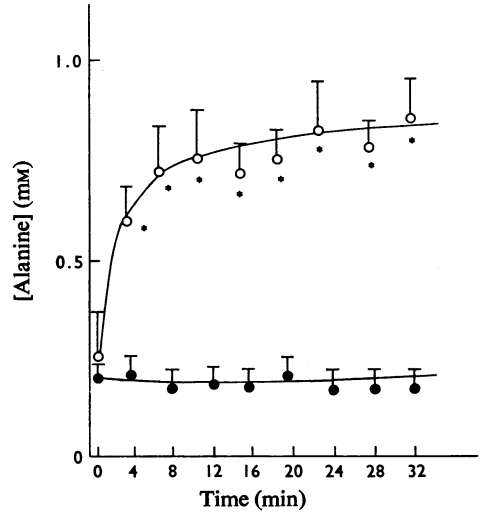

Fig. 7. *Concentration of alanine in blood*
Experimental conditions were the same as in Fig. 4.

in [^{14}C]oleate that occurred during the 4–8min interval would be expected to be reflected in decreased specific radioactivities of ketone bodies. The infusion of D-alanine had no effect on the blood [alanine], but the priming and perfusion with L-alanine did establish and maintain an [L-alanine] that was in the range of the fed state (i.e. nearly 1mM, Fig. 7). The data presented in Fig. 5(*a*) show that the chemical concentration of acetoacetate was not significantly altered by D- or L-alanine perfusion. In Fig. 5(*b*), however, it is clear that the concentration of [^{14}C]-acetoacetate, which was formed from the [^{14}C]oleate, was decreased by the infusion of L-alanine but not by D-alanine.

The results presented in Fig. 4(*a*) demonstrate that the chemical [3-hydroxybutyrate] in blood was rapidly lowered by L-alanine infusion but not by D-alanine. The corresponding concentration of 3-hydroxy-[^{14}C]butyrate in blood was also significantly decreased by the administration of L- but not of D-alanine (cf. Fig. 4*b*). The decrease in the label in 3-hydroxybutyrate was faster than the decline in its chemical concentration.

Discussion

The assessment of gluconeogenesis by the combined use of arterial–venous catheterization and radioactively labelled substrates has been cited recently as an appropriate method for the direct measurement of gluconeogenic-precursor utilization as well as the production of radioactively labelled glucose by the splanchnic bed (Chiasson *et al.*, 1977). It was also noted in their work that a tracer-determined precursor-conversion rate may provide a reliable index for fluctuations in gluconeogenesis when this process is not complicated by hormonal changes. In our previous studies with the present perfusion model, we demonstrated that significant changes in relevant hormones do not occur during the course of the procedure (Ozand *et al.*, 1977). In summary, the use of physiological concentrations of L-alanine and the present perfusion model appears to be an entirely appropriate model for the study of the influence of this amino acid on the regulation of gluconeogenesis and its influence on ketone-body production.

The data of Figs. 1 and 2 demonstrate that perfused [^{14}C]alanine and [^{14}C]lactate reached an equilibrium value within 10min and remained essentially unchanged for the duration of the experimental period. The rate of radioisotope appearance in blood glucose was linear with time, and had not reached equilibrium at the end of the perfusion. This is not unexpected, since the rates of gluconeogenesis were relatively low (Table 1) and in this starved animal a variety of other unlabelled substrates are capable of supporting gluconeogenesis in addition to [^{14}C]-alanine and [^{14}C]lactate (e.g. serine, glycerol etc.) (Roobol & Alleyne, 1973; Scrutton & Utter, 1968). The injection of [^{14}C]alanine led to the appearance of label in lactate, and vice versa (Figs. 1–3). These equilibrations were also rapid and reached essentially constant values after 10min of perfusion. The

continual rate of radioactive isotope appearance in glucose indicated glucose formation from the injected precursors, i.e. [^{14}C]lactate and [^{14}C]alanine. The rate of label incorporation into glucose from [^{14}C]lactate was always higher than that observed with [^{14}C]alanine from either starved or fed animals (Table 1). This finding is in accordance with the previously published reports indicating that alanine is not preferred to lactate for gluconeogenesis in rats (Scrutton & Utter, 1968). The experiment in Fig. 3 clearly indicates that [alanine] $\geqq 1$ mM increases the rate of gluconeogenesis from lactate. These latter results are supported by the reports of Friedrichs & Schöner (1974), who demonstrated a stimulatory effect of alanine on gluconeogenesis from lactate in isolated kidney tubules and liver. Thus in starved rats the concentration of blood alanine may be more relevant to the regulation of gluconeogenesis from lactate rather than from alanine itself. It has been shown in this regard that during starvation the Cori cycle retains substantial activity and thus supports conversion of lactate into glucose (Freminet *et al.*, 1976). Furthermore, the comparison of data in Fig. 3 with those of Figs. 1 and 2, as outlined in Table 1, indicates a different pattern of hepatic glucose formation from alanine from that from lactate. The rate of label incorporation into blood glucose increased 2.3 times when the blood [alanine] was increased to approx. 1 mM, whereas the increase in ^{14}C concentration in the liver glycogen was 13.5-fold. When the ^{14}C-labelled precursor was [1-^{14}C]lactate and gluconeogenesis was stimulated by increasing the blood [alanine], the rate of ^{14}C incorporation into both blood glucose and liver glycogen were of equal magnitude (i.e. 1.7-fold in both cases). An explanation that is consistent with these results is that hepatic gluconeogenesis from alanine proceeds directly via liver glucose to liver glycogen. This glycogen is in turn in partial equilibrium with the glucose of the blood. Alternatively, when gluconeogenesis results from lactate there appears to be a formation of liver glucose that is in virtual equilibrium with blood glucose but not with liver glycogen. This formulation is consistent with the observations of Vernon & Walker (1972) in the developing rat.

The results presented in Figs. 4–7 provide the best evidence at present to suggest that the formation of ketone bodies is altered by the elevation of the blood [alanine] to that existing in the fed animals. Although the administration of L-alanine did not affect the chemical concentration of blood acetoacetate, it produced a substantial and immediate decline in the blood [3-hydroxybutyrate], which is consistent with previous reports (Genuth & Castro, 1974; Ozand *et al.*, 1977). As shown in Figs. 6 and 7, fed L-alanine concentrations do not affect the concentration of [^{14}C]oleate in the blood and hence the decrement in blood 3-hydroxybutyrate was not due to a decrease in the circulating [unesterified fatty acid]. It was noted in Figs. 4(*b*) and 5(*b*) that the incorporation of ^{14}C from [1-^{14}C]oleate into both ketone bodies was significantly less with L-alanine than with D-alanine as the experiment proceeded. The most interesting observation described above is the disproportionate decline in the chemical compared with the radiochemical concentration of 3-hydroxybutyrate. The ^{14}C incorporation into acetoacetate also declined, but the [acetoacetate] did not change. The decline in the specific radioactivities of ketone bodies was apparently not due to a complete inhibition of the ketogenic process, because if this were the explanation then one would expect their specific radioactivities to remain essentially constant. The data of Figs. 4(*b*) and 5(*b*) indicate that such was not the case. Taken together, these data support our previous findings and indicate that alanine stimulates the ketone-body production from a non-labelled source of acetyl-CoA in addition to promoting a lower rate of ketogenesis from unesterified fatty acids and retards the attainment of equilibrium between acetoacetate and 3-hydroxybutyrate (Ozand *et al.* 1977).

In conclusion, it is tempting to suggest that the source of the non-labelled acetyl-CoA units needed for ketogenesis in experiments of Figs. 4–7 may be alanine, pyruvate or lactate. The generation of ketones from pyruvate has been shown (e.g. Lopes-Cardozo & VandenBergh, 1972).

The present results are of interest in view of the observation that, in general, physiological conditions that lead to increased blood concentrations of ketone body also favour increases in gluconeogenesis (e.g. prolonged starvation). The observation that fluctuations of gluconeogenesis and of ketogenesis do not necessarily parallel each other has been reported (Mapes & Harris, 1976). The more common occurrence, however, is that these two processes occur in concert, such as during starvation (Exton, 1972). The results are of particular relevance to the divergent regulation of ketogenesis and gluconeogenesis in view of the fact that the present study was performed under conditions *in vivo*. The present observations provide a support for the concept that, during starvation, the regulation of ketone-body generation and glucose synthesis are under integrated, but independent, control.

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