Gluconeogenesis from glycine and serine in fasted normal and diabetic rats

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1. Non-anaesthetized normal and diabetic rats were fasted for 1 day, and [U-¹⁴C]glycine, or [U-¹⁴C]serine, or [U-¹⁴C]- plus [3-³H]-glucose was injected intra-arterially. The rates of synthesis *de novo*/irreversible disposal for glycine, serine and glucose, as well as the contribution of carbon atoms by the amino acids to plasma glucose, were calculated from the integrals of the specific-radioactivity-versus-time curves in plasma. 2. The concentrations of both glycine and serine in blood plasma were lower in diabetic than in fasted normal animals. 3. The rates of synthesis *de novo*/irreversible disposal of both amino acids tended to be lower in diabetic animals, but the decrease was statistically significant only for serine (14.3 compared with 10.5 $\mu\text{mol}/\text{min per kg}$). 4. Of the carbon atoms of plasma glucose, 2.9% arose from glycine in both fasted normal and diabetic rats, whereas 4.46% of glucose carbon originated from serine in fasted normal and 6.77% in diabetic rats. 5. As judged by their specific radioactivities, plasma serine and glycine exchange carbon atoms rapidly and extensively. 6. It was concluded that the turnover of glycine remains essentially unchanged, whereas that of serine is decreased in diabetic as compared with fasted normal rats. The plasma concentration of both amino acids was lower in diabetic rats. Both glycine and serine are glucogenic. In diabetic rats the contribution of carbon atoms from glycine to glucose increases in direct proportion to the increased glucose turnover, whereas the contribution by serine becomes also proportionally higher.

INTRODUCTION

Glycine has been found to be a poor precursor of glucose in isolated hepatocytes from fed, but not from fasted, rats (Remesy *et al.*, 1983). Hepatocytes isolated from cat liver convert glycine into glucose more readily (Beliveau & Freedland, 1982). In the intact rat, especially if on a high-protein diet, glycine was readily taken up by the liver and converted into glucose (Remesy *et al.*, 1983). In the intact organism, glycine is catabolized by several pathways, of which at least two are potentially glucogenic: (i) the conversion of glycine into aminoacetone and via methylglyoxal (2-oxopropanal) into pyruvate and glucose; (ii) the coupled reactions of glycine dehydrogenase and serine hydroxymethyltransferase (Snell, 1984), having the overall balance of:

The extent of the contribution of glycine to the carbon atoms of circulating glucose *in vivo* has not been estimated.

Serine, which may be derived from glycine as above, is glucogenic, since it may be deaminated by serine dehydratase to pyruvate, or converted by transamination into hydroxypyruvate and ultimately glucose. On the basis of experiments with liver homogenates (Rowell *et al.*, 1979) or isolated liver cells (Sandoval & Sols, 1974), serine dehydratase is the preferred pathway of serine catabolism in the rat. Transamination, on the other hand, accounts for a large part, if not all, of serine catabolism in cat (Beliveau & Freedland, 1982) and human (Snell, 1986) liver. Again, a quantitative evaluation of the contribution of serine to glucose formation *in vivo* is lacking.

The primary aim of the present experiments was to calculate in normal and diabetic rats the rates of synthesis *de novo* and of irreversible disposal of glycine and serine, as well as their contribution to glucose formation. Since in fed animals the rate of gluconeogenesis is low, all experiments were carried out on rats fasted for 24 h. The experiments were extended to diabetic rats, in which gluconeogenesis is presumably near to, or at, its maximal rate. In particular, answers to the following questions were sought: (i) what is the steady-state rate of synthesis *de novo* and irreversible disposal of glycine and serine in fasted normal and diabetic rats? (ii) what is the extent of the interchange of ¹⁴C atoms between plasma glycine and serine? (iii) what is the transfer of ¹⁴C atoms from injected [U-¹⁴C]glycine or [U-¹⁴C]serine to glucose? This was taken as an index for gluconeogenesis from the respective amino acid.

MATERIALS AND METHODS

Animals

Experiments were carried out on male Sprague-Dawley rats. The animals had free access to water and food pellets (26% protein, 9% fat, 62% carbohydrate, 3% fibre, on a dry-weight basis) and were housed at 20–21 °C with a 12 h-light (07:00–19:00 h)/12 h-dark cycle. For all groups of animals, food was withdrawn 1 day before the experiment. At 3–4 days before the experiment, a plastic cannula (Clay-Adams PE-50) was introduced into the carotid artery (Popovic & Popovic, 1960). Diabetes was produced by the injection of 35 mg of alloxan/kg body wt. dissolved in 0.1 M-acetate buffer, pH 4.4, into the arterial cannula. These rats were used 72 h after the injection, when the concentration of glucose in their plasma ranged between 20.8 and

46.0 $\mu\text{mol/ml}$. During the course of the experiments, the animals were in a dynamic steady state with respect to plasma glycine, serine and glucose.

Experimental design

[U- ^{14}C]Glycine (50–60 μCi) was injected into 13 normal rats via the cannula. After repeated rinsing with 0.9% NaCl and 0.9% NaCl/heparin (20 units/ml), from eight rats eight blood samples (each about 0.75 ml) were withdrawn, at 10, 20, 40, 80, 120, 160, 220 and 290 min after the injection of the tracer. From the other five rats the samples were withdrawn at 1, 2, 4, 7, 10, 20, 40, 80 and 120 min. The plasma concentrations of labelled and unlabelled glucose were determined in all samples, and the concentrations of labelled and unlabelled glycine and serine in the samples taken between 1 and 220 min.

A similar amount of [U- ^{14}C]glycine was injected into ten diabetic rats. From five rats samples were withdrawn between 10 and 290 min, and from the other five between 1 and 120 min with a sampling schedule as for the normal rats.

[U- ^{14}C]Serine was injected into eight normal and seven diabetic rats. Blood samples were taken between 10 and 290 min from four normal and four diabetic rats. In the other rats samples were taken between 1 and 120 min. The same sampling schedules were followed as in the experiments with labelled glycine.

Finally, to eight normal and seven diabetic rats, 25 μCi of D-[3- ^3H]glucose and 10 μCi of D-[U- ^{14}C]glucose were injected simultaneously. The plasma concentrations of unlabelled and labelled glucose (with respect to both labels) were determined in samples withdrawn at 2, 5, 8, 13, 25, 40, 80, 110 and 150 min after the injection of the tracers.

Blood samples were centrifuged within 10 min from withdrawal, at room temperature for 1 min in a high-speed Eppendorf 5412 centrifuge. The red blood cells were resuspended in 0.25 ml of 0.9% NaCl and were re-injected into the carotid artery within 5 min.

Chemical analysis

The concentration of glucose was determined in 0.01 ml of plasma with a Beckman II glucose analyser. Radioactive glucose was isolated as follows: a measured amount of plasma (0.1–0.25 ml), made up to 2 ml with glass-distilled water, was loaded on top of a column (0.6 cm diameter, 3 cm high) of Bio-Rad AG 1-X8 resin (acetate form). The column was rinsed with 4 ml of water. More than 99.8% of lactate and pyruvate, but no glucose, are retained on the column. The effluent was collected and deproteinized with $\text{BaOH}_2/\text{ZnSO}_4$. After centrifugation (500 g for 15 min) at room temperature, the supernatant was shaken (200 cycles/min) with equal amounts (50 mg/ml) of Dowex 50W-X8 (H^+ form) and Amberlite CG-4B (OH^- form) for 30 min in glass-stoppered tubes. The tubes were centrifuged (500 g for 15 min), and the supernatants were placed into similar tubes and shaken again with a new batch of the same resins for another 30 min (Chiasson *et al.*, 1974). By this procedure about 4% of glucose and 99.8% of glycine and serine are removed.

To determine the specific radioactivities (SA) of amino acids in the plasma, 250 μl samples of plasma were used. After addition of 0.125 ml of 0.01 M-HCl and 0.125 ml of 1 μM -norleucine in 0.01 M-HCl as internal standard, 0.5 ml of 20% (w/v) trichloroacetic acid was added to

each sample. The precipitated protein was removed by centrifugation for 5 min in an Eppendorf 5412 centrifuge. Amino acids were separated from the trichloroacetic acid by mixing the solutions with an equal volume of Dowex 50W-8X (20–50 mesh) ion-exchange resin equilibrated with 0.01 M-HCl. The resin fractions were washed with 3×5 ml of water. After this, the amino acids were eluted with 6 ml of 7% (v/v) ammonia. The eluates were dried on a Savant Speed-Vac Concentrator. To remove amides and acetylated derivatives from the amino acid samples, the samples were treated with 6 M-HCl for 45 min at 106 $^\circ\text{C}$ *in vacuo*. After removal of the HCl, again with the Savant Speed-Vac Concentrator, derivatives of the dried samples were prepared with phenyl isothiocyanate, and the amino acids were separated and quantified by reverse-phase h.p.l.c. (Bidlingmeyer *et al.*, 1984). The separations were carried out on an Altex-ODS Ultrasphere C-18 column, by using an acetonitrile gradient of 0–100% eluent A to 100% eluent B in 30 min at a flow rate of 1 ml/min. Eluent A consisted of a 6% (v/v) solution of acetonitrile in 140 mM-sodium acetate/3.6 mM-triethylamine adjusted to pH 6.4 with acetic acid. Eluent B was 60% (v/v) acetonitrile in water. Peaks were detected by monitoring the A_{260} with a Bio-Rad detector. The quantities of amino acids in the eluates were calculated by using an Apple II Plus microcomputer and the Chromatochart software from Interactive Microware Inc., obtained from Mandel Scientific Co. (Richmond, Ont., Canada), to analyse data from the Bio-Rad absorbance detector. Absorbance values obtained from the analysis of known amounts of amino acids were used to convert absorbances into nanomoles. The absorbance peaks corresponding in elution time to serine and glycine were collected by hand, and the radioactivity in them was determined by scintillation counting. Specific radioactivities of amino acids were first calculated as d.p.m./nmol and then converted into d.p.m./ μg of C atoms. From the amount of the internal standard, norleucine, detected in the processed samples relative to the amount of glycine and serine, the concentrations of glycine and serine in plasma were calculated.

Specific radioactivity (SA) of all metabolites was expressed in units of d.p.m./ μg of C atoms. In the Figures SA(t) is shown as normalized with respect to the injected amount of tracer (M^* , expressed in units of d.p.m. $\times 10^6$).

Calculations

The time course of the changes of specific radioactivity in time SA(t)/ M^* , was described as a sum of two exponential terms for the injected labelled substrate. To describe SA(t)/ M^* for glucose after the injection of labelled glycine or serine, the difference of two exponential terms forced to SA(0) = 0 was fitted. The SA(t) curve for plasma serine after the injection of labelled glycine, and the SA(t) curve for plasma glycine after the injection of labelled serine, were adequately described by a sum of two exponentials for all points at $t > 1, 2$ or 4 min. To calculate the integral from 0 to infinity of these curves, the correction for the area between $t = 0$ and $t = 1, 2$ or 4 min was applied as described by Corney & Heath (1970).

The rate of synthesis *de novo*, which in a dynamic steady state is equal to the irreversible disposal rate (R_i) of glycine, serine or glucose was calculated as the ratio of the injected radioactivity (M^* , d.p.m.) divided by the

Table 1. Rates of synthesis *de novo*/irreversible disposal (R_i , $\mu\text{mol}/\text{min}$ per kg), and plasma concentrations (μM) and plasma clearance rates (ml/kg per min) of glycine, serine and glucose in normal 24 h-fasted and diabetic rats

The numbers of rats in each group are given in parentheses. R_i was calculated from the SA/M^* curves shown in the Figures. The range of the best estimate was calculated from the area under the curve $\pm$ its s.d. Plasma clearance is calculated as $R_i/\text{plasma concentration}$. For the plasma concentration and clearance, means $\pm$ s.e.m. are shown. The plasma concentration is shown as the mean value of the average concentrations for each animal. The difference between normal fasted and diabetic rats is significant at $*P < 0.05$ or $**P < 0.01$.

Amino acid	Rats	Irreversible disposal rate ($\mu\text{mol}/\text{min}$ per kg)	Plasma	
			Concn. (μM)	Clearance (ml/kg per min)
Glycine	Normal fasted (13)	14.8 (12.8–17.5)	586 ± 52	25.4 ± 4.5
	Diabetic (10)	12.2 (10.9–13.8)	$394 \pm 35^{**}$	29.7 ± 4.3
Serine	Normal fasted (8)	14.3 (12.6–15.7)	265 ± 20	54.1 ± 7.2
	Diabetic (7)	10.5 (9.4–12.0)	$187 \pm 12^*$	56.1 ± 7.8
Glucose	Normal fasted (8)	32.7 (31.2–34.0)*	7390 ± 216	4.43 ± 0.26
	Diabetic (7)	38.2 (36.8–39.7)	$34033 \pm 5166^{**}$	$1.12 \pm 0.25^{**}$

area under the $\text{SA}(t)$ curve of the respective metabolite in the plasma after the injection of the tracer labelled with ^{14}C (Shipley & Clark, 1972). The standard error (s.d.) of the estimated area under the curve was calculated from the standard errors of the four parameters of the fitted double-exponential curve by the delta process (Rao, 1973). A BMDP routine on an Amdahl computer was used for these calculations. The plasma clearance of a metabolite was taken as $R_i/\text{plasma concentration}$. The turnover rate of glucose was calculated as M^*/area under the $\text{SA}(t)$ curve for D-[3- ^3H]glucose. For the definition of the parameters of turnover see below.

The ratio of areas under the $\text{SA}(t)$ curves from zero to infinity was taken as the estimate of the fraction of the turnover of the product (such as glucose) which arises from the primarily labelled precursor (Shipley & Clark, 1972).

The standard error of quantities calculated as the ratio or the product of two other calculated quantities [ratio of areas under the $\text{SA}(t)$ curves, the rate of transfer of C atoms from precursor to product or plasma clearance rates] was calculated as described by Topping (1958). The significance of difference between such compound quantities was estimated from the ratio (z) of the difference of their mean values and the square root of their common variance. The level of P was defined in terms of standard deviations around the mean: see Table 1 of Fisher, 1967).

Terminology used in the interpretation of parameters of turnover

The term $M^*/\text{SA} \cdot dt$ is a form of the Stewart Hamilton principle used to calculate the rate of flux across a system. The calculation assumes that the system is in a dynamic steady state, i.e. the inflow and outflow are equal and do not change with time during the period of observation. In the study presented the system is the mass of metabolite (glycine, serine or glucose) which intermixes 'rapidly' with the injected tracer. The metabolite in the plasma forms part of this mass, which may also include molecules in some part of, or the entire, extracellular volume, and possibly also some intracellular molecules in rapid equilibrium with the latter. When

referring to the flux rate of a metabolite, it is possible to distinguish between the rate of irreversible disposal (equal to the rate of synthesis *de novo*) and the rate of turnover. The former (R_i) is the rate of net loss from the system. A molecule which, after having left the system to be metabolized, is resynthesized and returned will not be counted as net loss. By the same reasoning, R_i also equals the rate of synthesis *de novo*. The turnover rate (R_T) measures unidirectional loss and the rate of entry from any source. Evidently R_T is higher than R_i . Since the ^3H atom attached to the C_3 of glucose is removed when glucose is metabolized to pyruvate and is not returned to circulating glucose, $M^*/\int \text{SA}(^3\text{H}, t) \cdot dt$ is a measure of R_T . Some ^{14}C atoms of metabolized glucose are re-incorporated into glucose released by the liver, so $M^*/\int \text{SA}(^{14}\text{C}, t) \cdot dt$ is a measure of R_i . In the present study the contribution of a precursor to the synthesis *de novo* of a product was examined. Accordingly R_i was used in the calculation of the parameters shown in Tables 2 and 3.

Materials

[U- ^{14}C]Glycine (100 mCi/mmol), [U- ^{14}C]serine (> 150 mCi/mmol), D-[3- ^3H]glucose (10–20 Ci/mol) and D-[U- ^{14}C]glucose (15 Ci/mol) were purchased from Amersham Canada Ltd., Oakville, Ontario, Canada. All other chemicals were obtained from Fisher Scientific Co. (Toronto, Ontario, Canada) and were of the highest purity available.

RESULTS

The mean body weight of the 21 fasted normal rats injected with either tracer glycine or serine was 448 ± 9 g, and that of the 17 diabetic animals was 405 ± 13 g. The fasted normal rats injected with tracer glucose weighed 440 ± 13 g and the diabetics 383 ± 16 g. The mean plasma concentrations of glycine, serine and glucose in fasted normal and diabetic rats are shown in Table 1. The mean plasma concentrations of glycine and serine in the fasted normal rats were significantly higher than in diabetics ($t = 2.95$, $P < 0.05$ for glycine; $t = 2.98$, $P < 0.1$ for serine).

Fig. 1. Specific radioactivity (SA, as d.p.m./ μ g of C) of plasma glycine, serine and glucose after the intra-arterial injection of $[U-^{14}C]$ glycine at zero time

The SA has been normalized with respect to the injected amount of labelled glycine ($M^* = \text{d.p.m.} \times 10^6$). Abscissa shows time after the injection of labelled glycine (min).

In Fig. 1(a), the curves for specific radioactivity versus time of plasma glycine, serine and glucose after the injection of $[U-^{14}C]$ glycine are shown for fasted normal rats. In Fig. 1(b) curves for diabetic animals are shown. In Fig. 2 similar data observed after the injection of $[U-^{14}C]$ serine are shown in fasted normal and diabetic rats.

The rates of synthesis *de novo*/irreversible disposal (R_i) of glycine, serine and glucose calculated in fasted normal and diabetic rats are shown in Table 1. There was a significant decrease in R_i of serine in diabetic as compared with fasted normal rats ($z = 2.08$, $P < 0.05$). The small decrease in the R_i of glycine was not statistically significant. There was no difference between the plasma clearance of either glycine or serine in diabetic and fasted normal animals.

The ratio of the integrals under the specific-radioactivity-versus-time curves are shown in Table 2. Estimated from the transfer of ^{14}C atoms, 30–31% of the C atoms in circulating serine arises from glycine in either fasted normal or diabetic rats. About 21–22% of plasma glycine originates from serine. The same fraction (about 2.9%) of the C atoms in plasma glucose arise from glycine in fasted normal and diabetic animals. In fasted

Fig. 2. Specific radioactivity (SA, as d.p.m./ μ g of C) of plasma serine, glycine and glucose after the intra-arterial injection of $[U-^{14}C]$ serine at zero time

The SA has been normalized with respect to the injected amount of labelled serine ($M^* = \text{d.p.m.} \times 10^6$). Abscissa shows time after the injection of labelled serine.

Table 2. Ratio of integrals of the fitted $SA(t)/M^*$ curves of plasma glycine, serine and glucose after the injection of labelled glycine or serine in normal fasted and diabetic rats

The integral of the $SA(t)/M^*$ of the injected tracer is the denominator of the ratio (= 100%). Values are shown as the means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. The difference between normal fasted and diabetic rats is significant at $*P < 0.05$.

Rats...	Ratio (%)	
	Normal fasted	Diabetic
Serine/glycine	30.3 $\pm$ 6.0 (13)	31.4 $\pm$ 5.41 (10)
Glycine/serine	21.5 $\pm$ 3.4 (8)	22.0 $\pm$ 3.9 (7)
Glucose/glycine	2.95 $\pm$ 0.46 (13)	2.92 $\pm$ 0.35 (10)
Glucose/serine	4.46 $\pm$ 0.52 (8)	6.77 $\pm$ 0.87* (7)

normal rats 4.46%, and in diabetics 6.77%, of the C atoms of plasma glucose arise from serine. The difference is significant ($z = 2.29$, $P < 0.05$).

The rate of transfer of C atoms from glycine to serine,

Table 3. Calculated rates of transfer of C atoms, in μg of C/min per kg and as the percentage of the rate of synthesis *de novo* (μg of C/min per kg) of the precursor in normal fasted and diabetic rats

Values are shown as the means $\pm$ S.E.M. for the numbers of experiments in parentheses. The difference between normal fasted and diabetic rats is significant at * $P < 0.01$ or ** $P < 0.001$.

Rats...	Rate of transfer of C atoms			
	Normal fasted		Diabetic	
	(μg of C/min per kg)	(as % of R_i of precursor)	(μg of C/min per kg)	(as % of R_i of precursor)
Glycine from serine	77 $\pm$ 13 (8)	15 $\pm$ 2.5	64 $\pm$ 8 (7)	22 $\pm$ 2.8
Serine from glycine	153 $\pm$ 19 (13)	45 $\pm$ 5.3	118 $\pm$ 16 (10)	42 $\pm$ 5.7
Glucose from glycine	69 $\pm$ 3 (13)	19 $\pm$ 0.8	80 $\pm$ 3* (10)	27 $\pm$ 1.0**
Glucose from serine	104 $\pm$ 5 (8)	20 $\pm$ 1.0	185 $\pm$ 7** (7)	49 $\pm$ 7.1**

and vice versa, as well as their contribution to circulating glucose, were calculated as the irreversible disposal rate multiplied by the fractional contribution to the turnover of the product compound (Table 3). Any difference in the rate of flow of C atoms (μg of C/min per kg) between fasted normal and diabetic rats reflects the change both in R_i of the product and in the fraction of the product arising from the given precursor. When the transfer of C atoms is expressed as a fraction of the rate of irreversible disposal of the precursor (by definition equal to the rate of its synthesis *de novo*), only the fraction of the metabolized amino acids converted into glucose is different between fasted normal and diabetic rats. This difference is much larger with serine than with glycine as the precursor.

The turnover rate of glucose from the specific-radioactivity-versus-time curves of D-[3- ^3H]glucose was $42.5 \pm 1.9 \mu\text{mol/min per kg}$ in fasted normal and $47.8 \pm 7.4 \mu\text{mol/min per kg}$ in diabetic rats. The difference is not significant.

DISCUSSION

Turnover of glycine and serine

In normal rats fasted for 1 day, the rates of synthesis *de novo*/irreversible disposal (R_i) of glycine and serine are of about the same order of magnitude as that of alanine, 13–21 $\mu\text{mol/min per kg}$ (Freminet & Leclerc, 1980; Golden *et al.*, 1981). As compared with fasted normal rats, in diabetic rats the plasma concentrations of both glycine and serine were lower by about the same degree. Since, however, the R_i of both amino acids was lower (albeit statistically significant only for serine), their plasma clearance remained unchanged.

In view of the presumed increase in protein breakdown in severely diabetic rats, the essentially unchanged R_i may appear to be paradoxical. However, in man 81 % of the turnover of glycine in plasma was estimated to be by synthesis *de novo* and not by protein breakdown. Neither does the rate of glucose utilization have a significant effect on the turnover of glycine (Robert *et al.*, 1982). Using [^{15}N]glycine as the tracer, Nissim & Lapidot (1986) found an increased turnover of glycine in diabetic rabbits. Their finding, however, may be a reflection of the label, ^{15}N , being diluted by pools not shared by ^{14}C atoms.

The first step in serine synthesis from a substrate other than glycine, the enzyme D-3-phosphoglycerate dehydrogenase, has been reported to be decreased in activity in diabetes, or indeed in any condition in which gluconeogenesis is increased (Salach *et al.*, 1972). The activities of the other two enzymes involved in the synthesis of serine, 3-phosphoserine:2-oxoglutarate aminotransferase and 3-phosphoserine phosphatase, are decreased to a lesser extent (Hayashi *et al.*, 1975). The decreased rate of synthesis *de novo* in diabetic rats indicates that lower enzyme activity leads to a lower flux of substrate. The marginal decrease in glycine turnover is, at least in part, due to the smaller flux of carbon atoms from serine to glycine. As judged by the rapid and sizable incorporation of ^{14}C atoms from injected labelled glycine into plasma serine, and from injected labelled serine into plasma glycine, there is a considerable interchange of C atoms between the two amino acids. This may be accounted for by the coupled reactions catalysed by mitochondrial serine hydroxymethyltransferase and glycine dehydrogenase, a 'glycine cycle' as proposed by Snell (1984). This cycle may be present in both the liver and the kidney (Lowry *et al.*, 1987). In spite of the rapid exchange of ^{14}C atoms between the two amino acids in the plasma, their specific radioactivities remained distinctly different and their C atoms cannot be considered to be in a common plasma pool.

Formation of glucose from serine and glycine

Glycine is potentially glucogenic, since one route of its metabolism is its conversion into serine. It may also be catabolized to aminoacetone and yield pyruvate, although flux along this pathway is believed to be small in the mammalian liver (Bender, 1975). Isolated hepatocytes taken from fed rats form no glucose from glycine (Beliveau & Freedland, 1982; Remesy *et al.*, 1983). In hepatocytes from rats fasted for 20–44 h, however, glycine was found to be glucogenic (Remesy *et al.*, 1983).

Serine was shown to be glucogenic in isolated rat hepatocytes, but whereas more alanine is taken up than serine, a larger fraction of utilized serine is converted into glucose (Klim *et al.*, 1986). Two pathways of serine catabolism are glucogenic: conversion by deamination into pyruvate, catalysed by serine dehydratase, and into hydroxypyruvate by transamination, catalysed by serine:pyruvate aminotransferase. The former enzyme is

present only, and the second mainly, in the liver (Snell, 1984). The relative importance of the two pathways varies with species and the diet consumed. A rough inverse correlation between serine dehydratase activity and the size of the animal has been demonstrated (Rowell *et al.*, 1979). Although transamination is the major pathway in carnivorous animals (Rowell *et al.*, 1979; Beliveau & Freedland, 1982) and man (Snell, 1986), in rats the preferred gluconeogenic pathway is by deamination (Sandoval & Sols, 1974; Bhatia *et al.*, 1975; Rowell *et al.*, 1979). In cultured hepatocytes from normal and diabetic rats a relationship was found between glucose production and the activity of serine dehydratase (Mak & Pitot, 1981). Klim *et al.* (1986) reported that the activity of serine dehydratase increases with an increase in the plasma glucagon/insulin ratio *in vivo*. There is evidence, however, that the activities of both serine dehydratase and serine transaminase increase in diabetes (Rowell *et al.*, 1973; Snell, 1984). Our experiments on intact non-anaesthetized rats confirm the finding of an increased formation of glucose from serine in diabetes, previously investigated in isolated liver cells and perfused livers, but yields no direct evidence about the relative contribution of the alternative pathways to plasma glucose production. Indirect evidence, however, favours an increased flux along the serine dehydratase pathway, since, similarly to lactate and alanine, a higher fraction of glucose carbon originates from serine in the diabetic state. This suggests an increased flux along a pathway in which the formation of pyruvate is the common initial step.

Does the glycine → serine → glucose pathway account for all of the glucose formation from glycine? Calculated from the fractions of the R_1 of glycine converted into serine and the fraction of glucose arising from glycine, the glycine → serine → glucose pathway was found to account almost completely for glucose formation in diabetic, but less so in fasted normal rats.

Conceivably glycine may give rise to pyruvate (and thus to glucose) without the formation of serine by the aminoacetone pathway (Bender, 1975). However, the formation of glucose from glycine by pathways not including the formation of serine may be overestimated by the tracer method, because measurements of glucose formation from injected serine and from serine arising from glycine in the liver cell may not be strictly comparable. As pointed out by Beliveau & Freedland (1982), the second (labelled) C atom of glycine could be transferred by serine hydroxymethyltransferase to form labelled serine from labelled glycine. Labelled serine is thus formed in the mitochondria and conceivably metabolized preferentially by serine aminotransferase (see Snell, 1984). Beliveau & Freedland (1982) conclude that, in cats at least, 'even if serine and glycine are taken up by the mitochondria at the same rate, serine formed from glycine has the possibility to contain up to twice the radioactivity of added ^{14}C -serine. This would be reflected in a higher d.p.m. in ^{14}C -glucose from glycine versus serine'. Even if in rats the formation of glucose from serine by transamination may be a less significant pathway than in cats, their reasoning applies to the extent to which serine hydroxymethyltransferase may contribute to the formation of glucose. Nevertheless, it is possible that the increased serine dehydratase activity in diabetes accounts for a larger fraction of glucose formation from serine in diabetic than in normal rats.

Should the pathway of gluconeogenesis from glycine and serine pass through pyruvate, the rate of the transfer of ^{14}C atoms to glucose underestimates the true rate of gluconeogenesis, because of the 'metabolic exchange' of labelled and unlabelled carbon atoms in the hepatocellular oxaloacetate pool. The degree of underestimation requires a correction of about 1.5-fold in fasted rats (Hetenyi, 1978).

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