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Hepatic Albumin and Urea Synthesis

THE MATHEMATICAL MODELLING OF THE DYNAMICS OF [^{14}C]CARBONATE-DERIVED GUANIDINE-LABELLED ARGININE IN THE ISOLATED PERFUSED RAT LIVER

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1. A mathematical model was constructed to define the dynamics of incorporation of radioactivity into urea carbon and the guanidine carbon of arginine in plasma albumin after the rapid intraportal-venous administration of $Na_2{}^{14}CO_3$ in the isolated perfused rat liver. 2. The model was formulated in terms of compartmental analysis and additional experiments were designed to provide further information on subsystem dynamics and to discriminate between alternative model structures. 3. Evidence for the rapid time-constants of labelling of intracellular arginine was provided by precursor–product analysis of precursor [^{14}C]carbonate and product [^{14}C]urea in the perfusate. 4. Compartmental analysis of the dynamics of newly synthesized urea was based on the fate of exogenous [^{13}C]urea, endogenous [^{14}C]urea and the accumulation of [^{12}C]urea in perfusate water, confirming the early completion of urea carbon labelling, the absence of continuing synthesis of labelled urea, and the presence of a small intrahepatic urea-delay pool. 5. Analysis of the perfusate dynamics of endogenously synthesized and exogenously administered [6-^{14}C]arginine indicated that although the capacity for extrahepatic formation of [^{14}C]-urea exists, little or no arginine formed within the intrahepatic urea cycle was transported out of the liver. However, the presence of a rapidly turning-over intrahepatic arginine pool was confirmed. 6. On the basis of these subsystem analyses it was possible to offer feasible estimations for the parameters of the mathematical model. However, it was not possible to simulate the form and magnitude of the dynamics of newly synthesized labelled urea and albumin which were simultaneously observed after administration of [^{14}C]carbonate on the basis of a preliminary model which postulated that both products were derived from a single hepatic pool of [6-^{14}C]arginine. On the other hand, these observed dynamics could be satisfied by a two-compartment arginine model, which also provided an explanation for discrepancies observed between albumin synthesis measured radioisotopically and immunologically. This was based on a relative overestimation of [^{14}C]urea specific radioactivity resulting from the rapid dynamics of [^{14}C]carbonate and the [^{14}C]urea subsystem relative to the labelled albumin subsystem. The effects of arginine compartmentalization could be minimized in the model by minor slowing of the rate of [^{14}C]carbonate turnover or by constant infusion of [^{14}C]carbonate, both of which permitted valid determination of albumin-synthesis rates.

The use of mathematical models in the analysis of metabolic systems has concentrated traditionally on attempting to fit experimental test data to some specified model not necessarily corresponding to physiological features, with a good statistical fit being considered adequate (Berman, 1963). Useful results have been obtained in this way even if these have been of somewhat restricted applicability. The attention that metabolic processes are now receiving from systems scientists means that the procedures for formulating and identifying models are being applied on the basis of accepted physiological phenomena as well as simple observational data. This approach leads to models which describe more accurately physiological structure and behaviour as well as being more useful for predictive purposes. These considerations separate the identification and estimation of parameters which are described here from much previous work where model parameters were required simply to yield a good fit between model and test responses for some class of inputs.

The principles of mathematical modelling have been applied to the [^{14}C]carbonate method for the

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measurement of the synthesis rate of liver-produced proteins (McFarlane, 1963; Reeve *et al.*, 1963). However, only partial validation of the postulated model was obtained *in vivo*. Failure to achieve true pulse-labelling of urea and protein after a single intravenous injection of [^{14}C]carbonate led to the concept of 'continuing synthesis' (Jones *et al.*, 1968). In contrast, measurements of synthesis made in the isolated perfused liver, in which the [^{14}C]carbonate can be administered directly into the portal vein, might avoid this shortcoming. The isolated perfused liver provides an ideal system in which to test this hypothesis, since it is possible to present to the liver relatively undiluted [^{14}C]carbonate (of high specific radioactivity) over a short time-interval, thereby achieving early maximal labelling of intrahepatic [6-^{14}C]arginine, plasma albumin and urea, whereas non-incorporated [^{14}C]-carbonate is rapidly diluted in a relatively large pool of plasma bicarbonate which is in equilibrium with rapidly exchangeable volatile CO_2.

In the course of investigation of these aspects of [^{14}C]carbonate dynamics, which was aimed at providing a model of intrahepatic [6-^{14}C]arginine metabolism, it became necessary to measure the specific radioactivity of ^{14}C-labelled albumin and ^{14}C-labelled urea at frequent and equally spaced intervals over a prolonged time-course after pulse administration of [^{14}C]carbonate into the portal vein of the perfused liver. By using a perfusate in which the plasma component was obtained from a different species it was possible simultaneously to quantify the net synthesis of rat albumin by a method independent of tracer dynamics (Mancini *et al.*, 1965). The discrepancies observed in the rates of synthesis determined by these two methods, when considered together with the dynamics of appearance of radioactively labelled albumin and urea after pulse-labelling with [^{14}C]carbonate, led us to suggest certain modifications to current concepts of hepatic arginine metabolism. A mathematical model was formulated in terms of compartmental analysis, and further experiments were designed to facilitate discrimination between alternative model structures. From the tests carried out, data were obtained which are incompatible with the concept of a single precursor pool of labelled guanidine carbon destined for both albumin and urea synthesis. The model proposed provides evidence that the influence of subcompartments of intrahepatic arginine is apparent only in the context of the very rapid radioisotope dynamics of the isolated perfused rat liver, after a pulse input of [^{14}C]-carbonate.

Materials and Methods

Isolated liver perfusions

For each experimental situation two perfusions were carried out. In addition two types of perfusion fluid were used. (1) Bovine erythrocytes, thrice washed in 0.9% (w/v) NaCl containing urea (AnalaR, 30mg/100ml), were suspended to a packed cell volume (v/v) of 28–32% in Krebs–Ringer bicarbonate solution (Krebs & Henseleit, 1932) containing bovine serum albumin (Armour, Eastbourne, Sussex, U.K.; 3g/100ml), urea (30mg/100ml) and L-amino acids in the following concentrations (mM: asparagine 0.027, threonine 0.252, serine 0.532, glutamine 0.368, proline 0.325, alanine 0.409, glycine 0.294, valine 0.188, methionine 0.045, isoleucine 0.131, leucine 0.152, tyrosine 0.089, phenylalanine 0.076, lysine monohydrochloride 0.346, histidine monohydrochloride 0.073, arginine monohydrochloride 0.175, tryptophan 0.069, cysteine monohydrochloride 0.061) which approximated to the normal plasma concentrations observed by Fisher & Kerly (1964) and Scharff & Wool (1964). This perfusate was supplemented with insulin and cortisol as described by John & Miller (1969) and heparin (heparin sodium salt; Evans Medical, Liverpool, U.K.; 100mg/100ml). (2) Rat erythrocytes obtained from fresh rat blood drawn by cardiac puncture were washed three times in heparinized 0.9% (w/v) NaCl to remove plasma and buffy coat and resuspended in freshly drawn heparinized rabbit plasma to provide a packed cell volume (v/v) of 28–32%. The plasma was obtained from normally fed rabbits.

Male Sprague–Dawley rats (300–400g body wt.) were lightly anaesthetized with ether. The bile duct was cannulated (P10 nylon cannula; Portex, Hythe, Kent, U.K.) and the portal vein exposed and cannulated. Intrahepatic rat blood was flushed out *in situ* with 20–30ml of warmed oxygenated perfusate, which was then discarded and the liver was transferred to a recycling system maintained under constant flow of humidified O_2+CO_2 (95:5). The initial volume of the perfusate was 250ml, and before the injection of radioisotopes a sample of 2.5ml was withdrawn from the reservoir for a baseline plasma urea determination. Thereafter the exact timing, the volumes of all samples removed and the packed cell volumes of all samples were recorded. Blood flow ranged from 7.5 to 10.0ml/min throughout perfusion.

Radioisotope administration

All radioisotopes were injected 30–45min after the start of perfusion via a three-way tap directly into the portal vein over a period of 20–30s. $Na_2{}^{14}CO_3$ (>50mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) was diluted with 0.9% NaCl to a concentration of 200μCi in 3ml and stored in sealed ampoules under N_2. [^{13}C]Urea was prepared in the Biophysics Department of the National Institute for Medical Research, London N.W.7, U.K., from $Ba^{13}CO_3$ (Nuclear Equipment Chemical Corp.,

Farmingdale, N.Y., U.S.A.). CO_2 was liberated by adding 4M-H_3PO_4 to the $Ba^{13}CO_3$ under vacuum and then converted into [^{13}C]urea by high-temperature reaction with NH_3 (Calvin *et al.*, 1949). The [^{13}C]-urea used in these experiments had an enrichment of 61.5 atoms % excess of ^{13}C, and a purity of 96% based on gasometric recovery of CO_2 after treatment with urease. About 18mg was dissolved in 2ml of 0.9% NaCl immediately before injection. [6-^{14}C]Arginine (25–40mCi/mmol; The Radiochemical Centre) was diluted in 0.9% NaCl and 5μCi in 2ml was injected into the portal vein.

Urea carbon determinations

Plasma urea concentration was measured by an automated diacetyl monoxime method on a Technicon AutoAnalyzer II (Skeggs, 1957). The remaining plasma (about 2.0ml) was deproteinized by addition of 6.5ml of water, 0.5ml of 10% (w/v) sodium tungstate and 0.5ml of 0.335M-H_2SO_4 and then centrifuged. The clear supernatant was decanted through cotton-wool into a round-bottom flask and evaporated to dryness. The sediment was redissolved in 0.5ml of 0.31M-sodium pyrophosphate–0.12M-orthophosphoric acid buffer (pH7.0) and transferred quantitatively with CO_2-free water into a reaction vessel. Urea carbon was liberated by reaction with urease (urea amidohydrolase, EC 3.5.1.5) at room temperature and quantified in a high-vacuum line at constant volume as described previously (Tavill *et al.*, 1968). $^{14}CO_2$ was then transferred to trapper (2-phenethylamine–methanol) and scintillator {0.5% 2,5 diphenyloxazole, 0.005% 1,4-bis-(5-phenyloxazol-2-yl)benzene [Nuclear Enterprises (G.B.) Ltd., Sighthill, Edinburgh, U.K.] in anhydrous toluene, sulphur-free (AnalaR)} for radioactive counting in a Philips liquid-scintillation spectrometer with correction for quenching by the external-standard channels-ratio method.

When urea carbon enrichments were required the CO_2 was transferred from the high-vacuum line to one limb of a Rittenberg tube which was cooled in liquid N_2 to −196°C. This was fed into the spectrometer inlet system of a Nier-type (60°C) mass spectrometer, which measured the ion intensities of the CO_2 at *m/e* 44 and 45. The atoms % excess of ^{13}C in the sample was calculated from the ratio of the ion intensities (*R*) at *m/e* 44:45 by using the formula:

$$\text{atoms \% of } ^{13}C = \frac{100}{R+1}$$

The atoms % excess of ^{13}C in the sample was obtained by subtracting the ^{13}C abundance of natural CO_2 (from a solid-CO_2 source) from this measurement. The specific radioactivity of the same sample of CO_2 was obtained by condensation on to 2-phenethylamine–methanol and counting as described above.

Albumin guanidine carbon determination

Stable albumin was measured after HCl–ethanol extraction and assayed by the biuret method against known albumin standards (Fernandez *et al.*, 1966). The remaining plasma (5ml) was treated with an equal volume of 10% (w/v) trichloroacetic acid, centrifuged at 1000*g* for 15min and the supernatant discarded. The precipitate was washed with approximately twice its volume of 5% (w/v) trichloroacetic acid, centrifuged and the supernatant again discarded. Ethanol (approximately three times the volume of the original plasma) was added, mixed thoroughly and the suspension centrifuged. The supernatant was dialysed overnight against running tap water, concentrated to approx. 5ml, and the protein concentration determined by biuret reaction (Korner & Debro, 1956). Protein was hydrolysed with 6M-HCl for 20h at 110°C. The acid hydrolysates were concentrated, neutralized and made to react sequentially with activated arginase (L-arginine amidinohydrolase, EC 3.5.3.1) and the urease as described previously (Tavill *et al.*, 1968). Liberated CO_2 was measured and counted for radioactivity as described for urea carbon.

Measurement of specific radioactivity of circulating precursor $Na_2{}^{14}CO_3$

Samples (20ml) of blood were withdrawn from the reservoir of the perfusion and 5ml was pipetted immediately into the centre bulb of a pre-cooled reaction vessel and frozen to −30°C in a mixture of solid CO_2–acetone. After evacuation the free and combined CO_2 was liberated by addition of 1.0ml of 4M-citric acid–tungstic acid [2ml of 10% (w/v) sodium tungstate+10ml of 0.038M-H_2SO_4+10ml of water] from the side arm of the reaction flask. The labelled CO_2 was measured and counted for radioactivity as described above.

Measurement of specific radioactivity of precursor [6-^{14}C]arginine and product [^{14}C]urea

Blood withdrawn from the perfusion was immediately centrifuged at 1000*g* and about 4.0ml of plasma was deproteinized as described above. One-half of the protein-free supernatant was used for determination of the [^{14}C]urea specific radioactivity as described above, and the other half was retained for measurement of [6-^{14}C]arginine specific radioactivity. This was carried out by incubation of the supernatant at pH9.0 overnight with 2–3mg of arginase. The specific radioactivity of [^{14}C]urea derived from this reaction was measured by further incubation of the supernatant with urease at pH7.0 as described above.

Quantitative radial immunodiffusion

Newly synthesized rat albumin was measured by single radial immunodiffusion (Mancini *et al.*, 1965) against a monospecific anti-(rat albumin) serum.

Duplicate standards of immunologically pure rat albumin were run on each plate together with the plasma samples, and albumin concentrations were read off the standard curve on which (diameter)^2 was plotted against mg of protein.

Calculations

(1) *Urea-synthesis rate.* The urea pool was calculated as the product of plasma urea concentration and total perfusate volume. Allowance was made for samples removed during the course of perfusion by adding the total urea in each sample to the subsequent urea pool. The total urea pool was plotted against time and synthesis rate calculated from the regression line thus obtained.

(2) *Albumin-synthesis rate by radial immunodiffusion.* The total albumin pool was calculated from the product of concentration and perfusate plasma volume. Albumin removed during sampling was calculated as for urea removals, and was included in the total pool. A graph of total albumin against time allowed the best-fitting straight line to be drawn, and from this line the synthesis rate was measured.

(3) *Total ^{14}C incorporated into urea.* Total radioactivity is the product of the urea carbon pool at each time-point and the urea carbon specific radioactivity of the sample removed at that time. Allowance was made for [^{14}C]urea radioactivity removed, in the same manner as for stable urea, by using specific radioactivity and stable urea concentration to calculate the [^{14}C]urea removed. Time of complete labelling and total ^{14}C incorporated into urea were obtained by plotting cumulative radioactivity against time.

(4) *Total [6-^{14}C]arginine incorporated into albumin.* The time-course of albumin specific radioactivity was plotted to ascertain the peak of specific radioactivity of the arginine guanidine carbon of albumin. Total [6-^{14}C]arginine radioactivity incorporated into albumin was calculated as the sum of the total radioactive pool at the point of maximal labelling and the cumulative radioactivity removed in early samples.

(5) *Synthesis rate of albumin by the [^{14}C]carbonate method.* The common precursor–product relation implies that the specific radioactivities of two products of a common radioactive precursor are equal. In other words the ratios of radioactivity to newly synthesized mass are equal, i.e.

$$\frac{R_u}{M_u} = \frac{R_g}{M_g} \qquad (1)$$

where M_u = rate of urea carbon synthesis (mg/h),
R_u = total radioactivity as [^{14}C]urea (d.p.m.),
M_g = rate of synthesis of arginine guanidine carbon in albumin (mg/h),
R_g = total radioactivity as [6-^{14}C]arginine in albumin (d.p.m.),
z_g = mg of arginine guanidine carbon/mg of albumin,
z_u = mg of carbon/mg of urea,
$r_g(n)$ = specific radioactivity of albumin guanidine carbon in the nth sample (d.p.m./mg),
r'_g = peak specific radioactivity of albumin guanidine carbon (d.p.m./mg),
$r_u(n)$ = specific radioactivity of urea carbon in the nth sample (d.p.m./mg),
r'_u = peak specific radioactivity of urea carbon (d.p.m./mg),
$A_s(n)$ = mass of albumin removed in the nth sample (mg),
A_p = mass of albumin in perfusate at peak specific radioactivity (mg),
$U_s(n)$ = mass of urea removed in the nth sample (mg),
U_p = mass of urea in perfusate at peak specific radioactivity (mg),
U_t = rate of urea synthesis (mg/h),
A_t = rate of albumin synthesis (mg/h),
m = the number of samples removed before peak specific radioactivity is reached,

$$R_g = z_g\left[\sum_{n=1}^{m} A_s(n)r_g(n) + A_p r'_g\right]$$

$$R_u = z_u\left[\sum_{n=1}^{m} U_s(n)r_u(n) + U_p r'_u\right]$$

$$M_u = z_u U_t$$

$$M_g = R_g\frac{M_u}{R_u}. \qquad \text{from (1)}$$

Now

$$A_t = \frac{M_g}{z_g} \qquad (2)$$

$$= \frac{z_g\left[\sum_{n=1}^{m} A_s(n)r_g(n) + A_p r'_g\right]}{z_g} \times \frac{z_u U_t}{z_u\left[\sum_{n=1}^{m} U_s(n)r_u(n) + U_p r'_u\right]}$$

$$= \left[\sum_{n=1}^{m} A_s(n)r_g(n) + A_p r'_g\right] \times \frac{U_t}{\left[\sum_{n=1}^{m} U_s(n)r_u(n) + U_p r'_u\right]} \qquad (3)$$

Experimental and Results

I. *Dynamics of incorporation of $Na_2{}^{14}CO_3$ into [^{14}C]urea (Fig. 1)*

Two perfusions were performed by using the bovine erythrocyte–bovine albumin-supplemented perfusate. A period of 45 min was allowed for stabilization

of the system before the injection of 200μCi of $Na_2{}^{14}CO_3$ into the portal vein. Thereafter samples of 20ml were withdrawn from the portal vein inflow cannula at approx. 10min intervals for the first 1h and at 30min intervals for the subsequent 3–4h. The specific radioactivity of the total $H^{14}CO_3$, $^{14}CO_3$ and $^{14}CO_2$ was compared with that of [^{14}C]urea in the same sample over the time-course of the two experiments. The carbonate specific radioactivity was maximal at 10min and fell to less than 5% of this value by 60min. The die-away curve of carbonate specific radioactivity passed through the peak of [^{14}C]urea specific radioactivity, which was reached between 30 and 40min and thereafter declined at a linear rate with time.

II. *Comparison of* [^{12}C]-, [^{13}C]- *and* [^{14}C]-*urea dynamics* (*Fig.* 2)

Two perfusions using the heterologous bovine system were carried out. The amino acid supplementation was increased tenfold on the concentrations quoted in the Materials and Methods section in order to generate higher rates of urea synthesis. The $Na_2{}^{14}CO_3$ (200μCi) and [^{13}C]urea (18.2mg and 17.7mg in the two experiments) were injected simultaneously into the portal vein, and blood samples (20ml) withdrawn from the mixing reservoir at short intervals over a period of 150–180min. Plasma was analysed for total urea concentration, [^{14}C]urea specific radioactivity and [^{13}C]urea enrichment as described in the Materials and Methods section. In both experiments the pool of unlabelled urea increased linearly with time over 120min. The specific radioactivity of endogenous [^{14}C]urea reached its maximum at 30min, whereas the exogenous [^{13}C]urea enrichment increased over the period 10–20min and then declined together with the [^{14}C]urea.

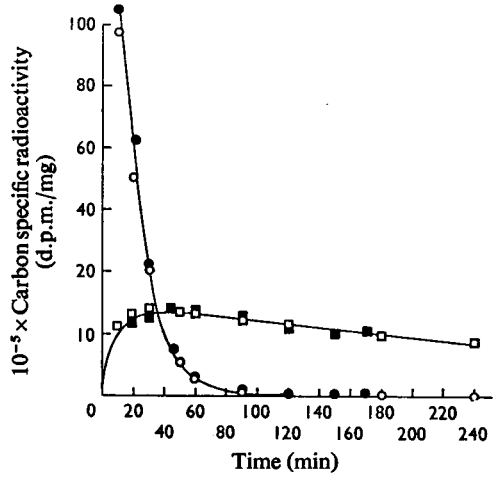

Fig. 1. *Time-course of specific radioactivity of dissolved and combined CO_2 and urea carbon after the administration of 200μCi of $Na_2{}^{14}CO_3$ into the portal vein of the isolated perfused rat liver*

Results of two experiments are given: ○, ●, acid-releasable CO_2 specific radioactivity (d.p.m./mg of carbon); □, ■, urea specific radioactivity (d.p.m./mg of carbon). Time-course is given in minutes after the addition of radioisotope.

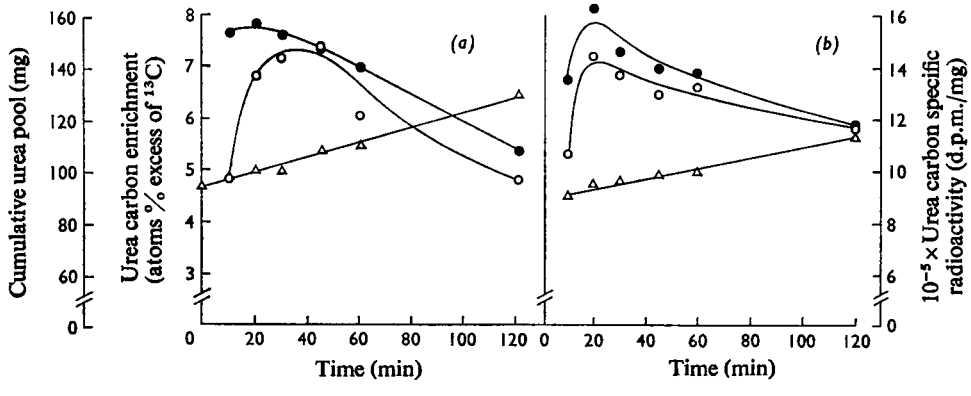

Fig. 2. *Two experiments (a and b) to compare the dynamics of exogenous* [^{13}C]*urea* (●), *endogenous* [^{14}C]*urea* (○) *and endogenous* [^{12}C]*urea* (△)

The stable [^{13}C]urea (18.2mg and 17.7mg) and radioactive $Na_2{}^{14}CO_3$ (200μCi) were administered at zero time into the portal vein of the isolated perfused liver.

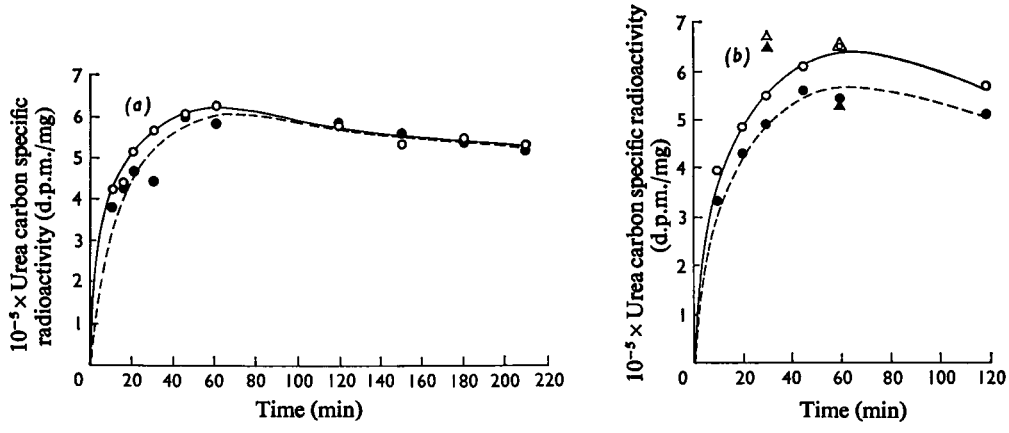

Fig. 3. *Two experiments to detect the possible release of endogenously synthesized [6-^{14}C]arginine by the isolated perfused rat liver*

(*a*) Comparison of the specific radioactivities (d.p.m./mg) of urea carbon after incubation of mixed perfusate with urease (○) or with arginase followed by urease (●). (*b*) Comparison of specific radioactivities (d.p.m./mg) of urea carbon after incubation of perfusate with urease or with arginase followed by urease. ○, Mixed perfusate, urease alone; △, hepatic-venous outflow perfusate, urease alone; ●, mixed perfusate, arginase plus urease; ▲, hepatic-venous outflow, arginase plus urease. Precursor $Na_2{}^{14}CO_3$ (200μCi) was administered at zero time into the portal vein of the liver.

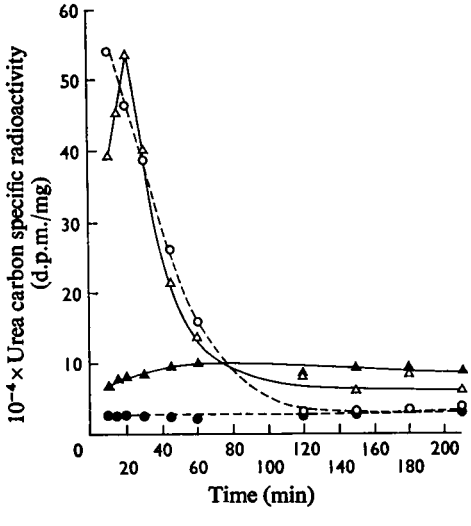

Fig. 4. *Dynamics of urea carbon specific radioactivity (d.p.m./mg) after the administration of [6-^{14}C]arginine (5μCi) into the portal-vein inlet at zero time in two experiments*

Expt. 1, in the presence of liver: △, incubation of perfusate with arginase and urease; ▲, incubation with urease alone. Expt. 2, in the absence of liver: ○, incubation of perfusate with arginase and urease; ●, incubation with urease alone.

III. *Detection of extrahepatic formation of labelled urea (Fig. 3)*

Two perfusions were carried out by using the heterologous bovine perfusate. Sampling was continued for 120–210min and the protein-free plasma supernatants were incubated either sequentially with arginase and urease or with urease alone. In addition, in the second perfusion, samples were obtained from the hepatic venous outflow directly from the liver at 30 and 60min after administration of the $Na_2{}^{14}CO_3$. Until about 50min the specific radioactivity of the urea carbon exceeded the specific radioactivity of the mixed arginine guanidine carbon and urea carbon. Thereafter the differences were minimal. The specific radioactivity of hepatic-venous outflow urea was greater than that of mixed perfusate urea at 30min but the difference had disappeared by 60min after administration of precursor [^{14}C]carbonate. As with the mixed perfusate the specific radioactivity of urea was higher after the single incubation than that after the sequential incubation with arginase and urease.

IV. *Metabolism of administered [6-^{14}C]arginine (Fig. 4)*

Two perfusions were carried out in which the precursor was *guanidine*-^{14}C-labelled arginine. Both experiments were supplemented with amino acids in a tenfold higher concentration than that given in the

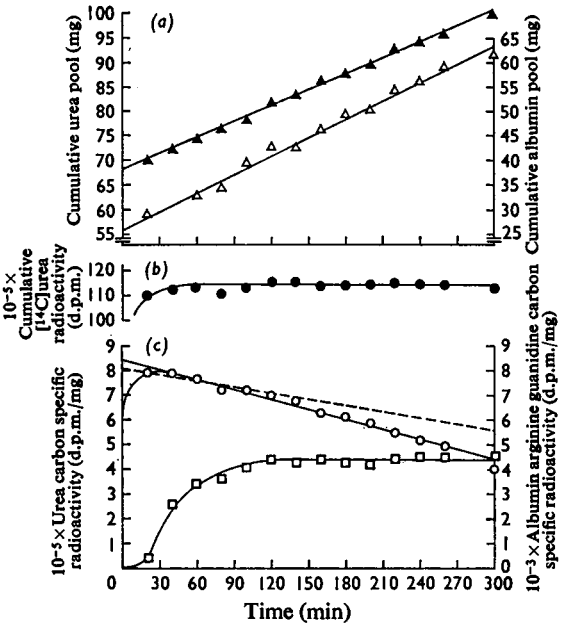

Fig. 5. *Simultaneously observed dynamics of ^{14}C-labelled arginine guanidine carbon in albumin and [^{14}C]urea after the rapid injection of $Na_2{}^{14}CO_3$ (200 μCi) into the portal vein of the isolated perfused liver (Expt. 1)*

The liver was perfused with rabbit plasma and washed rat erythrocytes, and samples were withdrawn from the mixing reservoir at 20min intervals for measurement of specific radioactivity, urea carbon concentration and rat albumin concentration by radial immunodiffusion. ▲, Cumulative urea pool (mg) (*a*); △, cumulative rat albumin pool (mg) (*a*); ●, cumulative [^{14}C]urea radioactivity (d.p.m.) (*b*); ○, [^{14}C]urea specific radioactivity (d.p.m./mg of carbon) (*c*); ----, corrected [^{14}C]urea specific radioactivity (d.p.m./mg of carbon) (*c*); □, specific radioactivity of arginine guanidine carbon in albumin (d.p.m./mg of carbon) (*c*). Time-course is shown in minutes after the administration of $Na_2{}^{14}CO_3$.

Materials and Methods section. The labelled arginine (5 μCi) in 0.5ml of 0.9% NaCl was administered over 20s into the portal-vein inlet tube, and blood samples (20ml) were withdrawn at frequent intervals over the first 1h, with a final sample at 210min. Incubation of deproteinized plasma supernatant was carried out either with arginase followed by urease or with urease alone in order to measure the specific radioactivity of urea derived from circulating arginine and that of endogenously synthesized urea respectively. Both perfusions were carried out on the same day by using the bovine heterologous perfusate which was constituted from a common pool of erythrocytes. The liver was omitted from the circuit of the second experiment, thereby providing a control system for the detection of circulating arginase activity. With the liver included, the [^{14}C]urea specific radioactivity after the sequential incubation rose during the first 20min and then fell rapidly over the next 120min. The specific radioactivity of endogenously synthesized urea rose to a plateau at 60–80min and remained almost constant thereafter. In the absence of the liver the maximum specific radioactivity after the sequential incubation was observed 10min earlier, at the first sampling time. The rate of fall was similar, but the final value was lower than with the liver in the system. Endogenously synthesized urea was detected very early, at 10min, and thereafter its specific radioactivity rose very gradually. Between 120 and 210min the values were just below those observed with the sequential incubation.

V. *Dynamics of urea and albumin labelling after administration of [^{14}C]carbonate and comparison of synthetic rates measured by radioisotopic and immunological methods (Fig. 5)*

Two perfusions were carried out by using 250ml of the fresh rat erythrocyte–rabbit plasma perfusate. The donor liver was flushed out *in situ*, transferred in isolation to the perfusion cabinet, and the blood volume restored to 250ml with 0.9% NaCl. In the second experiment a greater volume of blood was used in the flushing procedure and in consequence the packed cell volume, plasma albumin and urea concentrations were somewhat lower than in the first experiment. Samples (15ml) were withdrawn at zero time and at 20min intervals thereafter for 300min, and centrifuged immediately. Samples (3.0ml) were used for [^{12}C]urea and [^{14}C]urea measurements, the remainder being used for the determination of albumin concentration, assay of rat albumin by the radial-immunodiffusion method and the specific radioactivity of guanidine carbon of arginine in purified albumin.

(i) *Dynamics of appearance of [^{14}C]urea.* Maximal [^{14}C]urea specific radioactivity was reached very rapidly. In Expt. (1) the first sample taken at 20min yielded the highest specific radioactivity. In Expt. (2) the peak was between 20 and 40min after injection of radioactive isotope. Thereafter [^{14}C]urea specific radioactivity fell linearly throughout the course of the experiment. However, when correction was made for the continuing synthesis of unlabelled urea into a pool of [^{14}C]urea which was falling as a result of sampling, the linear rate of fall of specific radioactivity was decreased (Fig. 5). When allowance was made for radioactively labelled urea removed in sampling, a cumulative plot of [^{14}C]urea radioactivity could be drawn. This confirmed the completion of urea labelling between 30 and 40min in Expt. (1), whereas in Expt. (2) a small but continuing rise in total [^{14}C]urea was noted.

Table 1. *Data used for the calculation of the albumin-synthesis rate in the isolated perfused rat liver*

In both experiments $Na_2{}^{14}CO_3$ (200 μCi) was administered as a single pulse into the portal vein 45 min after the start of perfusion with a heterologous perfusate of washed rat erythrocytes and fresh heparinized rabbit plasma.

		Expt. 1	Expt. 2
Plasma albumin concentration	(mg/ml)	24.7	18.0
$10^{-3}\times$ Cumulative arginine guanidine carbon radioactivity in albumin	(d.p.m.)	72.06	46.46
$10^{-5}\times$ Cumulative radioactivity in urea carbon	(d.p.m.)	110.4	86.61
Urea-synthesis rate from cumulative [^{12}C]urea pool	(mg/h)	6.53	5.45
Albumin-synthesis rate from [^{14}C]carbonate	(mg/h)	2.01	1.40
Albumin-synthesis rate by immunodiffusion	(mg/h)	7.53	10.06

(ii) *Dynamics of appearance of [6-^{14}C]arginine-labelled albumin.* Labelled albumin at very low specific radioactivity was detectable at 20 min. Thereafter specific radioactivity rose, reaching a peak between 120 and 160 min in Expt. (1). In Expt. (2) the relative delay in urea and albumin labelling was similar. Thereafter little or no decline was observed in albumin specific radioactivity up to 5h.

(iii) *Synthesis of stable urea.* Since all samples withdrawn from the perfusion were left at room temperature before centrifuging it was assumed that complete distribution of labelled and unlabelled urea in erythrocyte and plasma water had occurred. Serial measurements of plasma urea concentration showed a linear increase for 4–5 h. Rates of synthesis determined from the cumulative [^{12}C]urea pool were similar in the two experiments (Table 1).

(iv) *Synthesis of stable albumin.* After a short delay rat albumin appeared in the perfusate circulation and accumulated at a linear rate for 4–5 h (Table 1). The rates of synthesis determined from the cumulative pool of rat albumin were 7.53 mg/h (Expt. 1) and 10.06 mg/h (Expt. 2). The concentration of total perfusate albumin was 24.7 mg/ml and 18.0 mg/ml in the two experiments.

(v) *Synthesis rate of albumin determined by the [^{14}C]carbonate method.* Substitution of the urea-synthesis rate, cumulative [^{14}C]urea radioactivity and cumulative [6-^{14}C]arginine radioactivity in albumin into the final synthesis eqn. (2) yielded synthesis rates for albumin of 2.01 mg/h and 1.40 mg/h respectively (Table 1). As Fig. 5 and Table 1 show, the maximum specific radioactivity of urea carbon was 70-fold higher than that reached by [6-^{14}C]-arginine in albumin in both experiments.

VI. *Mathematical modelling of experimental data*

Model construction (*Fig.* 6). The postulated model of CO_2 fixation has at its centre the separation of intracellular arginine into two principal compartments (2 and 3). At the outset this was based on the subcellular separation of the two processes of ureogenesis and protein synthesis; the former proceeds by a series of enzymic reactions in mitochondria and cytosol, arginine itself being synthesized one step before urea formation, whereas exported protein is synthesized on the rough endoplasmic reticulum from pre-formed arginyl-tRNA.

Since in the experimental tests the tracer material was injected directly into the liver via the portal vein, a liver CO_2 compartment (0) is included. During the first minute after injection [^{14}C]carbonate (of high specific radioactivity) is available to the liver for incorporation into arginine. That which is not incorporated during this time passes into the circulation of the perfusing medium [represented by the perfusate CO_2 compartment (1), from which subsequent transfer into the liver occurs, although much (about 99%) is discharged into the non-recycling atmosphere of CO_2+O_2 (5:95) and is lost to the system]. It is assumed that the pathway into arginine from the liver CO_2 compartment exists for not more than 1 min after injection, since this is the approximate time for complete injection of the [^{14}C]carbonate solution and replacement of the liver blood volume. For comparison with previous studies in which a constant infusion of [^{14}C]carbonate was used, this is delivered into the perfusate (compartment 1) and so compartment (0) does not exist (Fig. 6*b*).

The pathway of urea synthesis from arginine (compartment 2) contains a delay compartment (4), which is postulated to account for the finite time required for attainment of maximum enrichment in the perfusate urea compartment (6) in the experiments in which [^{13}C]urea was injected into the portal vein. In contrast, the albumin pathway from arginine (compartment 3) is influenced by a pure delay, τ, which represents the translation time of an albumin molecule. This is then subjected to a compartmental delay (5) caused by intrahepatic albumin on the secretory pathway to the circulating plasma albumin (compartment 7). Finally a catabolic compartment (8) is included to take into account the role of the liver in albumin degradation (Cohen & Gordon, 1958). However, it cannot be assumed that this compartment is totally discriminatory in its catabolic role. Other liver-produced proteins which have become labelled, or unlabelled tissue or plasma protein, may be degraded and may release arginine for reutilization via compartments (2) and (3). Although no information is available it is assumed that arginine can be recycled to an equal extent by these two pathways.

Model analysis. Except for the urea compartment,

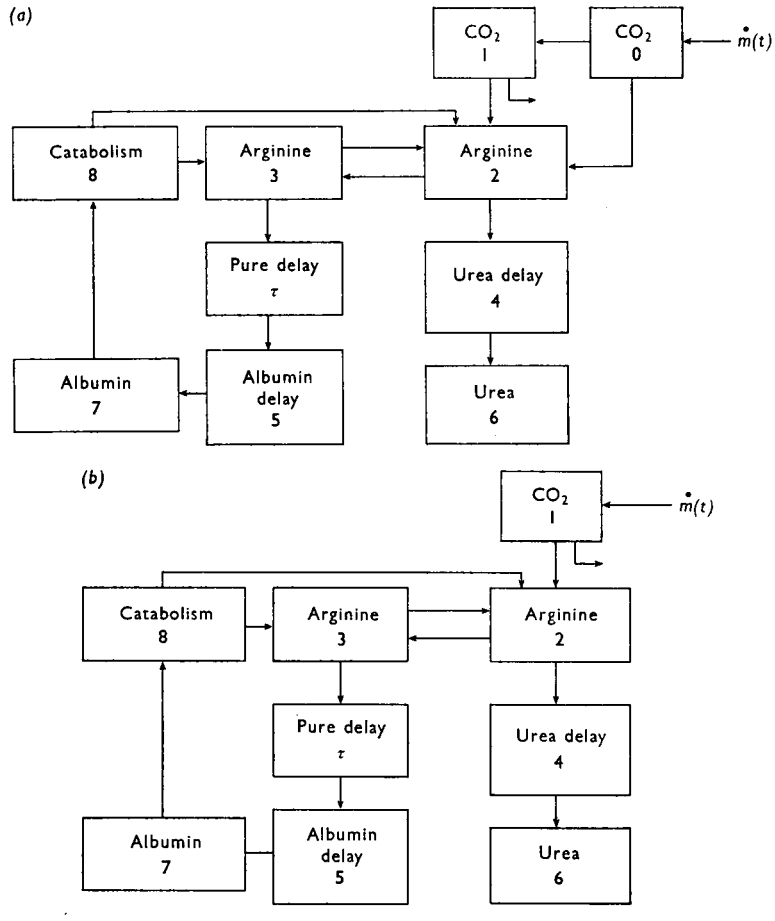

Fig. 6. *Multicompartment model for the dynamics of albumin and urea labelling (a) after pulse injection of $Na_2{}^{14}CO_3$ into the portal vein of the isolated perfused rat liver, and (b) during a constant infusion of $Na_2{}^{14}CO_3$ into the mixing reservoir of the isolated perfused rat liver*

it is assumed that the masses of unlabelled guanidine arginine carbon in each compartment remain constant during the experimental tests. There is, for example, a very small change in the unlabelled carbon in the albumin compartment (represented by net synthesis), but any error introduced is less than 1%. The increase in mass of unlabelled carbon in the urea compartment (6) is measured during the tests and the information used in the appropriate equation.

Since in all other respects the tests provided a true tracer situation, radioactivity balance equations can be written for the labelled material in each compartment.

Liver CO_2 compartment (0):

$$\frac{da_0}{dt}\cdot Q_0 = \dot{m}(t) - k_{01}a_0Q_0 - \begin{cases} k_{02}a_0Q_0 & (t \leqslant 1.0\text{min}) \\ 0 & (t > 1.0\text{min}) \end{cases}$$

Perfusate CO_2 compartment (1):

$$\frac{da_1}{dt}\cdot Q_1 = k_{01}a_0Q_0 - k_{12}a_1Q_1$$

Arginine compartment (2):

$$\frac{da_2}{dt}\cdot Q_2 = 0.01k_{12}a_1Q_1 + k_{82}a_8Q_8 + k_{32}a_3Q_3 - k_{23}a_2Q_2 - k_{24}a_2Q_2 + \begin{cases} k_{02}a_0Q_0 & (t \leqslant 1.0\text{min}) \\ 0 & (t > 1.0\text{min}) \end{cases}$$

Arginine compartment (3):

$$\frac{da_3}{dt}\cdot Q_3 = k_{23}a_2Q_2 - k_{32}a_3Q_3 + k_{83}a_8Q_8 - k_{35}a_3Q_3$$

Urea-delay compartment (4):

$$\frac{da_4}{dt}\cdot Q_4 = k_{24}a_2Q_2 - k_{46}a_4Q_4$$

Albumin-delay compartment (5):

$$\frac{da_5}{dt}\cdot Q_5 = k_{35}a_3(t-\tau)Q_3 - k_{57}a_5Q_5$$

Urea compartment (6):

$$\frac{d[a_6\cdot Q_6]}{dt} = k_{46}a_4Q_4$$

Plasma-albumin compartment (7):

$$\frac{da_7}{dt}\cdot Q_7 = k_{57}a_5Q_5 - k_{78}a_7Q_7$$

Albumin-catabolism compartment (8):

$$\frac{da_8}{dt}\cdot Q_8 = k_{78}a_7Q_7 - k_{83}a_8Q_8 - k_{82}a_8Q_8$$

Where a = specific radioactivity of material in the relevant compartment (d.p.m./mg of C),
Q = mass of unlabelled guanidine carbon in relevant compartment (mg),
k = rate constant (min^{-1}),
$\dot{m}(t)$ = rate of administration of injected material (d.p.m./min),
t = time (min).

Subscripts refer to the compartments as defined in Fig. 6. Double subscripts indicate radioactive-material transfer from the first specified compartment to the second.

Experimental test simulation. Digital computer simulation was carried out by using the simulation language MIMIC. The parameter estimates can be classified into three categories: (1) those based on experimental measurements or evaluated by subsystems analysis, (2) those approximated to acceptable physiological data, and (3) those postulated which on testing proved themselves compatible with the dynamics and magnitudes of the observed specific radioactivities.

Estimates of rate constants. Information about many of the rate constants is not well defined, but since a number of experimental tests are available which effectively decompose the system into a number of small subsystems most of the rate constants can be reliably estimated.

(i) Carbonate subsystem (section I, Fig. 1). This was tested by injection of [^{14}C]carbonate into compartment (0) followed by measurement of the appearance and disappearance of the labelled material in the perfusate CO_2 compartment (1). k_{02} exists only for the first minute. By experimental design almost all the labelled material is transferred from the liver CO_2 compartment (0) to the perfusate within 1.0–1.5 min, so that a suitable value of k_{01} could be chosen. This then enabled an estimate to be made for k_{12}, assuming a 1% efficiency of the incorporation of labelled carbon into arginine (McFarlane, 1963).

(ii) Urea subsystem (sections I, II, V; Figs. 1, 2 and 5). The overall urea pathway was simulated from the point of injection of [^{14}C]carbonate in compartment (0) to the appearance of [^{14}C]urea in compartment (6). It was also tested by injection of a known mass of [^{13}C]urea into the urea-delay compartment (4) and measurement of the atoms % excess of ^{13}C in the perfusate compartment (6). By utilizing the data on the synthesis of unlabelled urea the value of k_{46} could be estimated and inserted in the overall pathway.

Fig. 6 shows that in addition to the direct pathway, other pathways exist via arginine (3). However, it was argued that these would have comparatively little effect on the observed perfusate urea specific radioactivity. For example, the process of albumin catabolism in the isolated liver is so slow that it would not have any appreciable effect on urea synthesis over the test period. Therefore the arginine-recycling pathways were decoupled, and simulation of the direct pathway was carried out, enabling k_{24} to be estimated. Subsequent estimation of the parameters of the arginine-return pathway led to errors in urea specific radioactivity as a result of decoupling which did not exceed 1%.

(iii) Albumin subsystem (section V, Fig. 5). The specific radioactivity of the guanidine carbon of arginine in albumin (compartment 7) was measured during the time-course after the injection of [^{14}C]-carbonate. The delays in the albumin pathway were adjusted so that the peak albumin specific radioactivity occurred at between 120 and 160 min after injection. To obtain the experimentally observed values it was necessary to assume that $k_{23} > k_{32}$ by a factor of between 1.2 and 1.5:1 to account for net transfer of arginine from its site of synthesis (compartment 2) to a form (activated and tRNA-bound) at its site of utilization (compartment 3). k_{35} and k_{57} were chosen from the observed 20 min time-lag before the first significant appearance of labelled albumin in the perfusate, which agrees with the data of Morgan & Peters (1971). About one-half this time is assumed to be the pure delay effect owing to the finite translation time of the protein. This enabled the synthetic pathway to be simulated. On the catabolic pathway the assumption of a time-constant of several days for k_{78} is compatible with the minimal fall-off of albumin specific radioactivity during the test period once the peak had been attained. A time-constant was adopted for k_{82} and k_{83} on the basis of an intracellular catabolic compartment (8) in close proximity to the precursor arginine pools.

(iv) Arginine precursor subsystem. It was necessary to substitute parameter estimates of Q_2 and Q_3 and k_{23}, k_{32} which would both satisfy the requirement for

maintaining equalization of specific radioactivities in compartments (2) and (3) within the time-course of a constant-infusion experiment and also be compatible with the magnitudes of the observed specific radioactivities in the product compartments (6) and (7) in the single-pulse experiments described here.

Estimates of compartment magnitudes. Apart from the omission of compartment (0) and the substitution of $\dot{m}(t)$ of 8.8×10^8 d.p.m./min over a 0.5 min period for a value of 1.1×10^6 d.p.m./min throughout the whole time-course, the parameter estimates for the pulse injection and those for a constant infusion were identical.

Q_0 and Q_1 are based on CO_2 extractable with strong acid from blood equilibrated at a partial pressure of 40 mmHg (≡5% concentration, v/v), namely 25 mmol/litre in 4.0 and 250 ml respectively; Q_2 plus Q_3 approximates to the hepatic intracellular arginine concentration observed by Mayer & Shafrir (1972); Q_4 is obtained from the urea in total liver water assuming that it exists at the same concentration as in plasma; Q_5 is based on a feasible value of 5 mg for total hepatic intracellular albumin (A. G. Morton & A. S. Tavill, unpublished work); Q_6 is based on the increase in the circulating urea pool from 68 mg to 101 mg of urea over a 300 min period (see section V, iii); Q_7 represents the mass of circulating albumin, 5.0 g. This changes by about 1% over 300 min and is therefore assumed to be constant for the purposes of simulation.

Q_8 and k_{83}, k_{82} were considered together. The catabolism compartment (8) is derived both from albumin and other liver and plasma proteins. In theory arginine derived from degradation will be available for reutilization by both precursor compartments, 2 and 3. The values of Q_8 and k_{83}, k_{82} were chosen by reciprocal adjustments of the fractional rate of turnover and the pool size to satisfy the final simulation of the dynamics and magnitude of the urea and albumin specific radioactivities (category iii above).

Overall simulation. The simulation was finally carried out by using the following parameter estimates:

$Q_0 = 1.2\pm0.1$ mg
$Q_1 = 60.0\pm3.0$ mg
$Q_2 = 0.0108$ mg
$Q_3 = 0.007$ mg
$Q_4 = 0.36\pm0.07$ mg
$Q_5 = 0.02\pm0.001$ mg
$Q_6 = 13.6+0.0213\,t$ mg
$Q_7 = 20.0$ mg
$Q_8 = 0.40\pm0.01$ mg
$Q_2/Q_3 = 1.54\pm0.04$
$\tau = 10.0\pm0.8$ min
$k_{01} = 10.0\pm1.0\,\text{min}^{-1}$
$k_{02} = 0.40\pm0.03\,\text{min}^{-1}$
$k_{12} = 0.035\pm0.005\,\text{min}^{-1}$
$k_{23} = 0.08\pm0.003\,\text{min}^{-1}$
$k_{32} = 0.058\pm0.012\,\text{min}^{-1}$
$k_{24} = 2.0\pm0.4\,\text{min}^{-1}$
$k_{35} = 0.10\pm0.01\,\text{min}^{-1}$
$k_{46} = 0.06\pm0.01\,\text{min}^{-1}$
$k_{57} = 0.035\pm0.003\,\text{min}^{-1}$
$k_{78} = 0.000035\pm0.00003\,\text{min}^{-1}$
$k_{82} = 0.001\pm0.0004\,\text{min}^{-1}$
$k_{83} = 0.001\pm0.0004\,\text{min}^{-1}$

The limits indicated in the parameter estimates represent variations which would lead to a 5% change in the peak specific radioactivity in the response curves.

The listed set of parameter values enables simulated results to be obtained which agree reasonably well with experimental test responses and are compatible with known mass-transfer rates in the steady state. The simulated specific radioactivities of the plasma combined-CO_2 in compartment (1) are given in Fig. 7 and show a peak which occurs about 5 min before the first experimental sample (Fig. 1). The chosen parameters, although not reproducing the observed decay curve exactly, suggest that by 60 min the precursor $^{14}CO_2$ specific radioactivity has fallen to 5% of its maximum value. In these circumstances the simulated urea specific-radioactivity curve closely mirrors both the dynamics and magnitude of the experimental test response (Fig. 1).

The simulated albumin dynamics observed in association with these parameters show the required

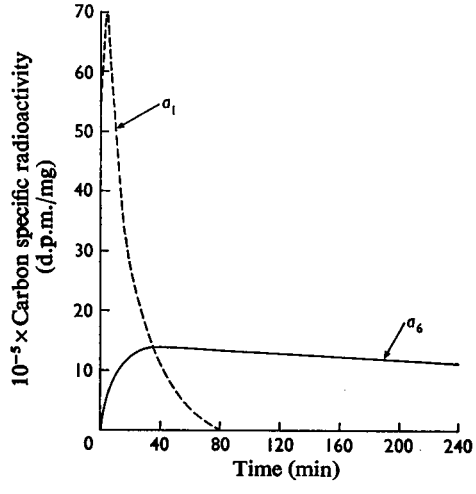

Fig. 7. *Simulated dynamics of the specific radioactivities of dissolved and combined CO_2 (a_1) and urea carbon (a_6) after the rapid injection of $Na_2{}^{14}CO_3$ (200 μCi) into the portal vein of the isolated perfused liver*

Time-course is shown in min after the injection of radioactive isotope. The theoretical curves are based on the model shown in Fig. 6(*a*).

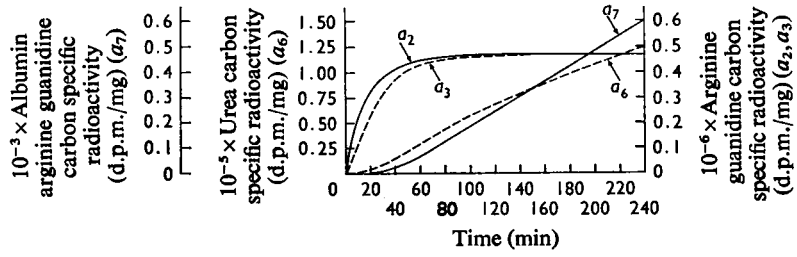

Fig. 8. *Simulated dynamics of the specific radioactivities (d.p.m./mg) of guanidine carbon of precursor arginine (a_2, a_3), urea carbon (a_6) and arginine guanidine carbon of albumin (a_7) during a constant infusion of $Na_2{}^{14}CO_3$ (30μCi/h) into the mixing reservoir of the isolated rat liver preparation*

Time-course is shown in min after the start of the radioactive isotope infusion. The theoretical curves are based on the model shown in Fig. 6(*b*).

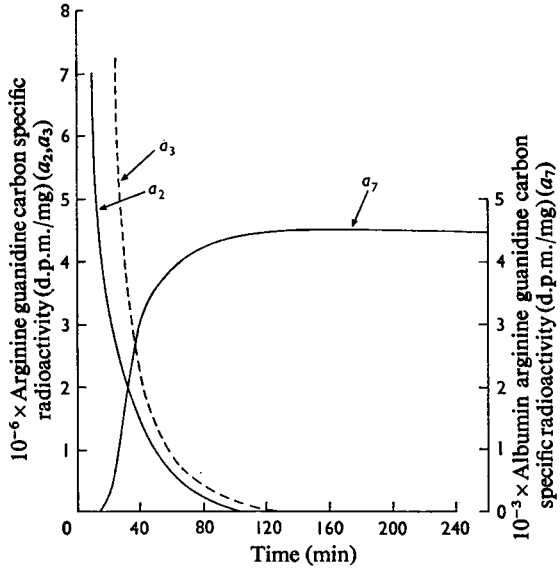

Fig. 9. *Simulated dynamics of the specific radioactivities (d.p.m./mg) of guanidine carbon of precursor arginine (a_2, a_3) and arginine guanidine carbon of albumin (a_7) after a single rapid injection of $Na_2{}^{14}CO_3$ (200μCi) into the portal vein of the isolated perfused rat liver*

Time is shown in min after the administration of radioactive isotope. The theoretical curves are based on the model shown in Fig. 6(*a*).

lag period of 20 min and the delay of 120 min before maximum specific radioactivity is attained (Fig. 9). No way could be found to satisfy both the relative delay in the dynamics of urea and albumin and the difference in the magnitude of their peak specific radioactivities other than by the insertion of the second precursor arginine compartment (3). In the presence of the observed CO_2 dynamics the simulated arginine precursor specific radioactivities in compartments (2) and (3) fail to equilibrate throughout the period of synthesis and release of labelled urea and albumin. However, if the same parameters are retained the administration of precursor [^{14}C]carbonate as a constant infusion permits complete equalization of the specific radioactivities of a_2 and a_3 at 200 min (Fig. 8). For practical purposes the ratio between a_6 and a_7 becomes constant after about 180 min, so that the choice of 240 min for the time of sampling in a constant-infusion experiment would provide valid relative specific radioactivities of urea and albumin for substitution in the albumin-synthesis equation.

Discussion

Exploitation of the functional relationship between the guanidine carbon of arginine and urea carbon for the calculation of albumin-synthesis rates *in vivo*, has yielded results which have been validated in overall terms against catabolic rates under steady-state conditions in both animals and man (McFarlane, 1963; Tavill *et al.*, 1968; Wochner *et al.*, 1968; Kirsch *et al.*, 1968). However, the calculations required the additional identification of complex metabolic pathways by means of isotopically labelled urea and albumin and parameter estimations which were obtained by deconvolution analysis (Jones *et al.*, 1968, 1970).

In the process of development of the isolated rat liver perfusion system for the study of albumin synthesis it seemed logical to administer the [^{14}C] carbonate directly into the portal vein. Since the extravascular fluid pool and the gut and renal compartments have been eliminated one has only to measure the accumulation of labelled urea and albumin in the perfusate and allow for quantities removed in sampling. These simplifications have enabled us to examine the [^{14}C]carbonate method in terms of metabolic

subsystems and unit processes of biochemistry. Having postulated a model on the basis of feasible parameters and structure it was possible to test the individual components of the system by designing specific input–output situations and to examine the overall system by comparison with an independent non-isotopic method for measurement of albumin synthesis.

By using separate or combined inputs of [^{14}C]-carbonate, [^{13}C]urea or [6-^{14}C]arginine it was possible to demonstrate pulse-labelling and a true precursor–product relationship between $^{14}CO_2$ and [^{14}C]urea. The presence of a urea-delay pool was postulated and confirmed by comparing the dynamics of [^{13}C]urea and endogenous [^{14}C]urea. Although the capacity of extrahepatic arginase to hydrolyse substrate quantities of arginine was observed no evidence was found for substantial leakage of [6-^{14}C]arginine which had been synthesized within the liver. Although there were clear indications for continued synthesis of unlabelled arginine throughout perfusion the hepatic synthesis of [6-^{14}C]arginine was completed between 30 and 60min after administration of [^{14}C]-carbonate. In this way a partial description of the parameters of the arginine, urea and albumin subsystem was achieved.

Perfusion of the isolated rat liver with heterologous perfusate permitted the simultaneous measurement of net synthesis of plasma albumin by two independent methods. The first non-radioisotopic method depends on the accurate sequential quantification of small amounts of newly synthesized and released homologous albumin within the large pool of pre-existing heterologous plasma. The second method necessitates the measurement of both radioisotopic and non-radioisotopic parameters of urea and albumin synthesis. The 3–4-fold difference in albumin-synthesis rate as determined by the two methods necessitates further analysis in the light of the data provided in these experiments as well as those offered by other workers who have used the [^{14}C]carbonate method. Although there is considerable evidence that albumin-synthesis rates determined *in vivo* in man and animals by the [^{14}C]carbonate method under steady-state metabolic conditions closely mirror degradation rates measured independently, relatively few data are available from the perfused rat liver. Nevertheless, it appears that administration of $Na_2{}^{14}CO_3$ by constant infusion into a homologous perfusion system yields albumin-synthesis rates that are comparable with rates separately determined in a heterologous system by the radial-immunodiffusion technique (Hoffenberg *et al.*, 1971). These workers' choice of a 4h time-point for comparison of the specific radioactivities of [^{14}C]urea and [6-^{14}C]arginine in labelled albumin was arbitrary but was opportune in ensuring good agreement. Other workers (Kirsch *et al.*, 1969, 1973; Kelman *et al.*, 1972) have chosen to administer the [^{14}C]carbonate as a single pulse into the reservoir of the perfusion and to compare the specific radioactivities of [^{14}C]urea and [6-^{14}C]-arginine-labelled albumin 2–3h later. Albumin-synthesis rates under these circumstances were about 35% of those obtained by the constant-infusion technique. Rothschild and co-workers have for some time used a single portal-vein injection of [^{14}C]-carbonate to measure the synthesis of albumin by the perfused rabbit liver (Rothschild *et al.*, 1968, 1969, 1971). They have reported that synthetic rates determined from 2½h perfusion data corresponded reasonably well to simultaneous measurements based on quantitative radial immunodiffusion (Oratz *et al.*, 1973). An explanation must be provided for the disparity observed in the present studies, the agreement when [^{14}C]carbonate is constantly infused (Hoffenberg *et al.*, 1971) and the delay in achieving maximum labelling of albumin which cannot be explained solely by delay in release (Morgan & Peters, 1971). The explanation for all three phenomena may lie in the methodological approach or in current physiological concepts.

Analysis of eqn. (3) indicates that a falsely low albumin-synthesis rate could result from underestimation of the numerator (albumin specific radioactivity or urea-synthetic rate) or overestimation of the denominator (urea specific radioactivity). Since the method of albumin extraction yields a protein of at least 95% purity, it would be difficult to conceive an unlabelled impurity which could lead to at least a 3-fold underestimation of albumin specific radioactivity. Further, multiple sampling provides a means of ascertaining the point of achievement of maximum specific radioactivity. A systematic overestimation of urea specific radioactivity would also be difficult to explain on methodological grounds alone. Urea-synthetic rates are based on stable urea measurements at multiple time-points, and the results compare well with those published by Kirsch *et al.* (1969, 1973) and Kelman *et al.* (1972). In contrast, radial immunodiffusion demonstrated linear continued synthesis of albumin throughout perfusion at rates similar to or slightly lower than those observed *in vivo* (Jeejeebhoy *et al.*, 1972).

The disparity between the albumin-synthesis rates measured by the two techniques is therefore more likely to be based on precursor dynamics than on systematic errors in measurement. The [^{14}C]carbonate method can be accepted as valid only if arginine in newly synthesized plasma albumin and urea is derived exclusively from the same labelled intracellular arginine pool. If there are separate precursor pools for urea and albumin which are of similar specific radioactivity, no discrepancy will result even though the assumption is invalid. If the separate precursor pools are of different specific radioactivities, errors will result, the magnitude of which will directly

reflect the difference between the specific radioactivities of the two pools. Also inherent in this assumption is the now justified concept that guanidine-labelled arginine formed within the liver is not transported to an extracellular site at which labelled urea but not albumin can be synthesized.

It was therefore necessary to look for an explanation in terms of a physiologically feasible model which should be able to predict the magnitudes and dynamics of the test responses after the administration of a pulse of [^{14}C]carbonate into the portal vein, and in addition it should be compatible with the constant-infusion data produced in other studies. The mathematical model of the isolated perfused liver which has been produced is capable of simulating such experimental test data, and is useful in predictive studies for a wide range of stimuli. Nevertheless, one of the problems that arises even with a comparatively simplified system such as this is the inaccessibility of a number of system variables. For example, no separate measurement of the specific radioactivities in arginine compartments (2) and (3) can be made. This means that in terms of achieving the observed urea and albumin specific-radioactivity responses, the model is insensitive to variation in the mass of unlabelled carbon in most of the compartments. Those to which it is sensitive, Q_6 and Q_7, can be measured with an error of less than 5%.

In terms of achieving the desired peak magnitude of albumin specific radioactivity, the values chosen for k_{23}, k_{32}, k_{35}, k_{57} and τ are critical. For example, an increase in τ from 10 to 15min results in a 30% decrease in the peak value. In contrast, removal of compartment (3) or an increase in k_{23} and k_{32} considerably decreases the time delay in achieving maximum values for a_7 and produces an increase in the maximum albumin specific radioactivity relative to that of urea such that a higher rate of albumin synthesis would be calculated by substituting for a_6 and a_7 in eqn. (3).

An example of the structural insight which this isolated perfused liver study provides can be seen by the postulated compartmentalization of arginine. It is not possible to simulate the observed albumin specific radioactivities after a pulse input of [^{14}C]carbonate into the portal vein if a single operational arginine compartment is assumed. It appears that the dynamics of the precursor carbonate and urea systems are so rapid in comparison with the dynamics of the albumin system that the peak urea specific radioactivity is achieved without equilibration of the specific radioactivities of the arginine pools. In the test responses obtained experimentally in which the [^{14}C]carbonate specific radioactivity in compartment (1) falls to 5% of its peak value within 60min, the arginine dynamics are sufficiently significant to prevent equilibration of a_2 and a_3 even after 240min. However, if a simulation is carried out in which carbonate dynamics are delayed by decreasing the value of k_{12} to 0.015min^{-1} there is retention of 10% of the maximum carbonate specific radioactivity in compartment (1) after 120min, so that arginine specific radioactivities in compartments (2) and (3) become similar and constitute a single operational precursor pool.

It may be that the failure to achieve a true pulse input of [^{14}C]carbonate in human studies *in vivo* (Jones *et al.*, 1968) lessened the effects of the arginine dynamics and enabled valid rates of synthesis to be achieved (Tavill *et al.*, 1968; Jeejeebhoy *et al.*, 1972). The model which was simulated in response to a constant infusion of [^{14}C]carbonate demonstrates effective equilibration of arginine specific radioactivities within 180min. If calculations of albumin-synthesis rates in the perfused rat liver are based on infusion studies with data confined to times between 3 and 5h then substantially higher rates of albumin synthesis may be measured than when the radioisotope is delivered as a single pulse either into the portal vein or into the mixing reservoir. Clearly, the extent to which arginine compartmentalization is of practical importance depends on the method of radioisotope administration, but is also a function of the relative dynamics of albumin and urea, which may vary with the experimental system and test conditions and the species under investigation.

Evidence is accumulating based on kinetic data which supports the concept of intracellular compartmentalization of a variety of amino acids. Valine appears to exist in an expandable and non-expandable form in the isolated perfused rat liver (Mortimore *et al.*, 1972). The former was in rapid equilibrium with valine used for protein synthesis whereas the latter was more closely identified with amino acid released by intracellular proteolysis. Alternatively, amino acids such as glutamic acid which may be generated within the cell may undergo metabolic transformation at their site of synthesis before mixing with exogenous amino acid transported into the cell from the extracellular fluid (Berl *et al.*, 1962; Davis, 1972).

Although evidence for hepatic arginine compartmentalization has previously been offered (Palacios *et al.*, 1970) its anatomical nature is speculative. Re-utilization of arginine for protein synthesis is minimal because of its rapid hydrolysis to urea (Swick & Handa, 1956; Swick & Ip, 1974). These findings are compatible with the relatively rapid urea dynamics seen in the present studies which may be based on preferential hydrolysis of endogenous arginine by arginase rather than its activation for protein synthesis. Finally, it can be argued for albumin that its initial synthesis in precursor form (Judah & Nicholls, 1971; Geller *et al.*, 1972; Russell & Geller, 1973) could create a recycling pool of amino acids rich in arginine (Judah *et al.*, 1973) which is derived from

the peptide fragment of pro-albumin in close proximity to the rough endoplasmic reticulum.

Whichever explanation is correct for the postulated physical separation of the arginine precursor, it appears that the dynamics of the carbonate system and the urea system are so rapid in comparison with the dynamics of the albumin system that any sustained equilibrium of arginine specific radioactivities after a true pulse input of [^{14}C]carbonate is precluded. Any mechanism which delays the disappearance of [^{14}C]carbonate and decreases its overall dynamics, or which achieves a relative slowing of the urea dynamics or an acceleration in the movement of newly synthesized arginine to the sites of protein synthesis will have the effect of minimizing the role of compartmentalization and permitting the assumption of a common precursor arginine pool for urea and albumin. The combined mathematical and biochemical approach to the subsystem analysis of a relatively simplified experimental model such as the isolated perfused rat liver can provide insight with which to identify more complex systems *in vivo* and offers guidance as to the mode of administration of labelled precursors.

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