Short-lived radionuclides in nutritional physiology.
A model study with L-[*Me*-11C]methionine in the pig

BY C.-G. STÅLNACKE, B. JONES[1], B. LÅNGSTRÖM[2], H. LUNDQVIST, P. MALMBORG, S. SJÖBERG[2] AND B. LARSSON

Department of Physical Biology, Gustaf Werner Institute, University of Uppsala, Box 531, S-751 21 Uppsala, Sweden
[1]*Department of Clinical Chemistry, Clinical Centre, College of Veterinary Medicine, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden*
[2]*Department of Organic Chemistry, University of Uppsala, Box 531, S-751 21 Uppsala, Sweden*

1. A 'new' carbon radioisotope, 11C, for use in nutritional studies is presented. It has a 20 min half-life, and decays by positron emission giving annihilation photons of 511 keV energy (Wolf & Redvanly, 1977). Thus repeated studies can be made with short time intervals and the distribution of radioactivity in the experimental animal can be detected externally.

2. 11C was produced with a tandem Van de Graaff accelerator and L-[*Me*-11C]methionine was synthesized and used in model experiments in the pig. The tracer was administered intravenously through a catheter in the jugular vein of pigs weighing between 40 and 100 kg. In a series of experiments, one pig received a low-methionine diet supplemented with DL-methionine to give three different levels of methionine intake.

3. The radioactivity distribution between liver and muscle was measured as a function of time by external detectors for 2–3 h after administration. Blood and exhaled CO_2 were sampled and measured for radioactivity.

4. The results indicate that 11C is a useful radionuclide in nutritional studies in intact large domestic animals.

The amino acids are of great importance in the breeding of domestic animals. Our basic knowledge of the metabolism and distribution of amino acids in different organs is mainly based on experiments with small laboratory animals utilizing 3H-, 14C- or 35S-labelled compounds. Such techniques have been applied with success in nutritional studies. Thus, for example, the correlation found between total intake of a specific amino acid and oxidation of its 14C-labelled analogue has been used to estimate amino acid requirements in the rat (Brookes *et al.* 1972). For larger animals there are problems with contamination when these long-lived radionuclides are used. Experiments with pure β-emitters also often require the killing of series of animals. 11C-labelled amino acids may here represent a better alternative, since this short-lived positron-emitting radionuclide, yielding 511 keV photons, allows external detection. Its physical half-life of 20 min implies low radiation dose to the animal and eliminates contamination problems.

The aim of this investigation was not primarily to gain new knowledge of the pig's physiology but to show that information on amino acid behaviour in a large animal can be obtained by the use of 11C-labelled amino acids. Thus, this paper, in the main, deals with the technique itself. The radioactivity in blood, liver, muscle and exhaled air was followed as a function of time after intravenous administration of L-[*Me*-11C]methionine. The experimental design and measurements appear applicable to experiments on other large animals as well as to clinical studies on human subjects.

The short half-life of 11C implies the necessity for production of the labelled substance in close proximity to the experiment. In our experience, however, 11C has routinely been transported, for use in other investigations, 70 km from the accelerator. The 11C technique depends on efficient interdisciplinary collaboration. Initially the costs involved may be quite
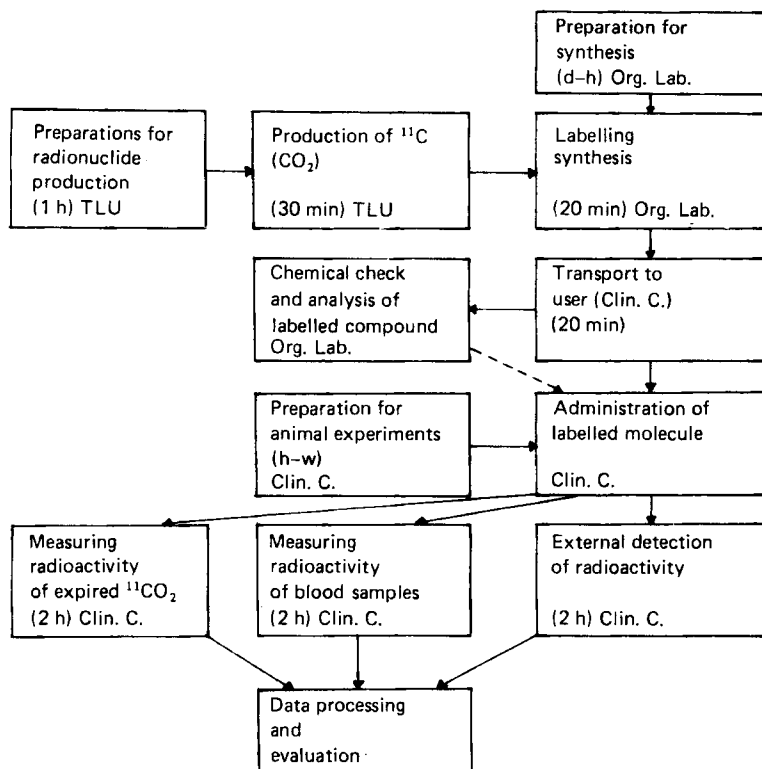
Fig. 1. Experimental design, showing the successive stages and their time duration (in parentheses) when pigs were administered L-[Me-^{11}C]methionine. The different locations are given as: TLU, Tandem accelerator Laboratory in Uppsala; Org. Lab., laboratories at Gustaf Werner Institute and Department of Organic Chemistry at Uppsala University; Clin. C., Department of Clinical Chemistry, College of Veterinary Medicine, Swedish University of Agricultural Sciences, Ultuna, Uppsala.

high but with the establishment of production techniques for biomedical applications the experiment economy tends to be comparable with more conventional methods.

MATERIAL AND METHODS

The design of the experiments is shown in Fig. 1.

Production of ^{11}C-labelled methionine

The radionuclide ^{11}C was produced at the Tandem accelerator Laboratory in Uppsala (TLU) by bombarding a nitrogen gas target system with a 10 MeV, 10 μA proton beam. The nascent ^{11}C atoms produced in the nuclear reaction ^{14}N(p,α)^{11}C reacted with trace amounts of oxygen present in the target gas. The ^{11}CO$_2$ formed was trapped in a molecular sieve (5 A) and transported to a nearby chemical laboratory.

[^{11}C]methyl iodide was synthesized and used for alkylation of the sulphide anion of L-homocysteine in liquid ammonia as described previously (Långström & Lundqvist, 1976; Långström, 1980). The L-[Me-^{11}C]methionine was obtained in a 65–90% chemical yield 30–40 min after the end of bombardment. The specific radioactivity of labelled amino acid was estimated to be approximately 400 MBq/μmol and the radiochemical purity determined by liquid chromatography (LC) to be 99·5%. Typically 100 MBq (3 mCi)-labelled amino acid was obtained in the physiological saline (9 g sodium chloride/l) used for injection.

Detectors and data handling

Cylindrical NaI(Tl)-crystals (diameter 51 mm, height 76 mm) were used externally to detect disintegrating ^{11}C in chosen structures. Samples of blood, exhaled CO_2, urine and faeces were measured in an NaI(Tl)-crystal of well type. All detectors were connected to single-channel analysers set at 511 keV. Counts accumulated for 1 min were transferred to a data log (Texas Silent 700 Terminal, Texas Instrument Inc., Houston, Texas, USA, with cassette deck) for off-line computer evaluation.

For normalization, $Na_2^{11}CO_3$ dissolved in a water phantom viewed by the external detectors was used. A sample from the phantom fluid was measured in the well detector and the relative sensitivity of all detectors was thus obtained. A portion of the injected radioactivity used in the experiment was also measured in the well detector. The radioactivity measured by the external detectors could then be normalized to permit calculation of the relative amount of administered radioactivity in the observed tissue volume and its deviation from conditions of homogeneous dilution in the body. The observed radioactivity was corrected for background, radioactive decay, detector efficiency and dead time losses. The standard deviations of the normalized values were calculated.

Animal experiments and sample preparation

Twenty-two experiments were performed on three growing female pigs (nos. 1–3) raised as for slaughter (Yorkshire × Swedish landrace). The frequency of the experiments (approximately one per week) was determined mainly by the access to the accelerator.

All experiments were performed during daylight hours but not exactly at the same hour of the day. Effects of the diurnal rhythm were not evaluated. The pigs were fed approximately 1 h before administration of the labelled compound in most experiments. The first eleven experiments were performed in pigs nos. 1 and 2 to optimize the experimental design. These pigs were given a commercial standard diet throughout the experimental period. The amounts given were 1·95 kg/d at 50 kg body-weight given in two equal parts, with a gradual increase to 2·80 kg/d at 90 kg body-weight, i.e. in accord with Swedish standards for fattening pigs (see Table 3). Each animal was used until it reached a weight of 90–100 kg. During the first experiments pig no. 3 was given the same diet (control diet), but during the last seven experiments it was given a specially prepared low-methionine diet (Table 1), supplemented with DL-methionine (p.a. grade; Merck, Darmstad, W. Germany) 1·2, 0·6 and 0 g/kg, to give three different methionine levels in the diet. The different levels were given from at least 1 week before the first of two experiments at each level (Fig. 2).

During measurements the animal was held in a special cage which prevented it from moving. The anterior part of the animal was placed in a wooden box. From the box a tube led to an air-pump used for collection of $^{11}CO_2$ samples. The animal was tranquilized during the experiment by intravenous injection of Pentobarbital (Mebumal; ACO, Solna, Sweden) approximately 10 mg/kg body-weight.

To facilitate repeated blood sampling during the experiments, a silicon-rubber tube (Silastic* o.d. 2·16 mm, i.d. 1·02 mm; Dow Corning, Brussels, Belgium) was permanently placed in the jugular vein, led subcutaneously to the back and exteriorized over the posterior rib approximately 50 mm lateral to the spine. The end of the tube was fitted to an infusion cannula with a valve. The cannula and valve were covered with a piece of cloth for protection between the experiments. The operation to insert this tube was performed with the pig in dorso-lateral recumbency using an intravenous thiopenthal narcosis (Pentothal* Sodium; Abbot, North Chicago, Ill., USA).

At the start of each experiment approximately 40 MBq (1 mCi) L-[*Me*-¹¹C]methionine

Table 1. *The composition (g/kg) of low-methionine diet fed to pigs, and its analysed content of protein and some amino acids*

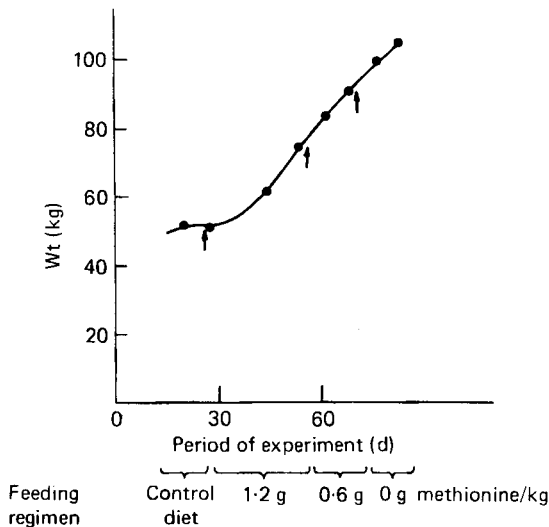| Ingredients		Analysed content	
V 65, whey powder	80	Crude protein*	157
Peas	150	Methionine	2·6
Barley	250	Cysteine	3·2
Maize starch	250	Lysine	9·4
Wheat	180	Threonine	7·7
Lucerne meal	50	Isoleucine	7·0
Minerals and vitamins	40		

* Nitrogen × 6·25.

Fig. 2. Weight changes for pig no. 3 during the experimental period of feeding control diet and experimental diet at three levels of methionine supplementation. (↑), Change of diet and level of supplementation; (●), individual experiments.

was injected intravenously. This amount of radioactivity allowed measurements during a 2 h period.

In the experiments on pig no. 2 a gamma camera (Nuclear Chicago Pho Gamma III, Searle) was used to visualize the pattern of radioactivity distribution. Substantial radioactivity was accumulated in the regions of liver, pancreas and intestines. In all other experiments collimated detectors were used to observe the radioactivity in the liver region, the right thigh muscle and, in some experiments, also in the intestine.

Blood samples were transferred to heparinized tubes every 5 min during the first 30 min and every 10 min thereafter. After measuring radioactivity in whole blood the tubes were centrifuged to separate the blood cells and plasma. The proteins were precipitated by adding three drops of concentrated perchloric acid to approximately 3 ml blood plasma. After centrifugation and washing, the precipitate and the acid-soluble fraction were measured for radioactivity in the well detector.

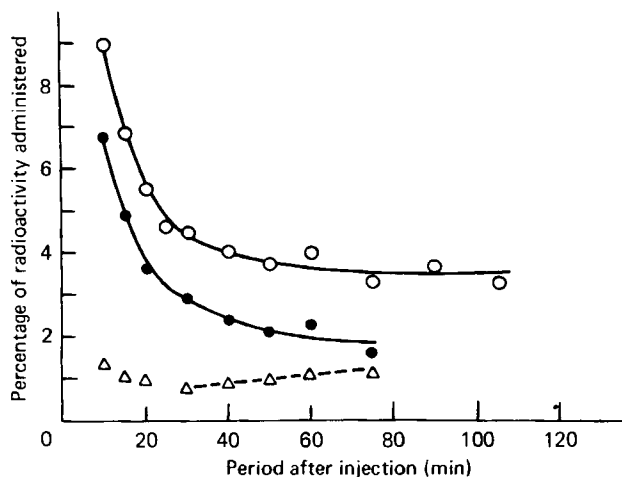Samples of exhaled $^{11}CO_2$ were collected by pumping 10 l air (flow-rate 10 l/min) from

Fig. 3. Percentage of administered radioactivity in the total blood volume and contribution of acid-soluble (●) and protein (△) fractions to whole blood (○) radioactivity at different periods after injection of L-[*Me*-^{11}C]methionine in pigs given the control diet.

the wooden box through a CO_2 absorbent (Ascarite®; Arthur M. Thomas Company, Philadelphia, PA, USA). The CO_2 trap was then measured for radioactivity in the well detector.

The ratio, radioactivity in the liver region:radioactivity in thigh muscle region, was calculated from the integrated values of the measured radioactivity within three periods after administration of the tracer.

The ratio, exhaled $^{11}CO_2$/min:radioactivity in the total blood volume, was calculated from values observed in two periods after administration of ^{11}C-labelled methionine. The cardiac output/min was considered equal to the total blood volume which was taken to be 7% of the total body-weight. This ratio is then a measure of the amount of the blood radioactivity that would be in the form of respiratory $^{11}CO_2$.

The ratio, exhaled $^{11}CO_2$/min:dietary methionine intake (mg/d per kg body-weight), was also calculated.

Occasional samples of urine and faeces were taken and their radioactivity measured.

RESULTS

The initial experiments showed that the results of external measurements of radioactivity were not substantially affected by movements of the animal or small differences in the position of the animal in relation to the detectors. The injection of the ^{11}C-methionine solution had no apparent adverse effects on the pigs.

After injection, the radioactivity in the blood decreased with time as shown in Fig. 3. The radioactivities of the acid-soluble fraction and of the precipitate, containing the proteins, are also shown. The total radioactivity of the blood was approximately 3% of that injected, at the end of the control diet experiments. The rate of incorporation of radioactivity into the blood proteins (Fig. 3) was reflected by the increasing radioactivity in the precipitate.

In all experiments the radioactivity in the liver region was found to be higher than that for the muscle region.

In the last seven experiments, in which the successively less supplemented low-methionine diet was used, the radioactivity in muscle appeared to be similar to that found with the
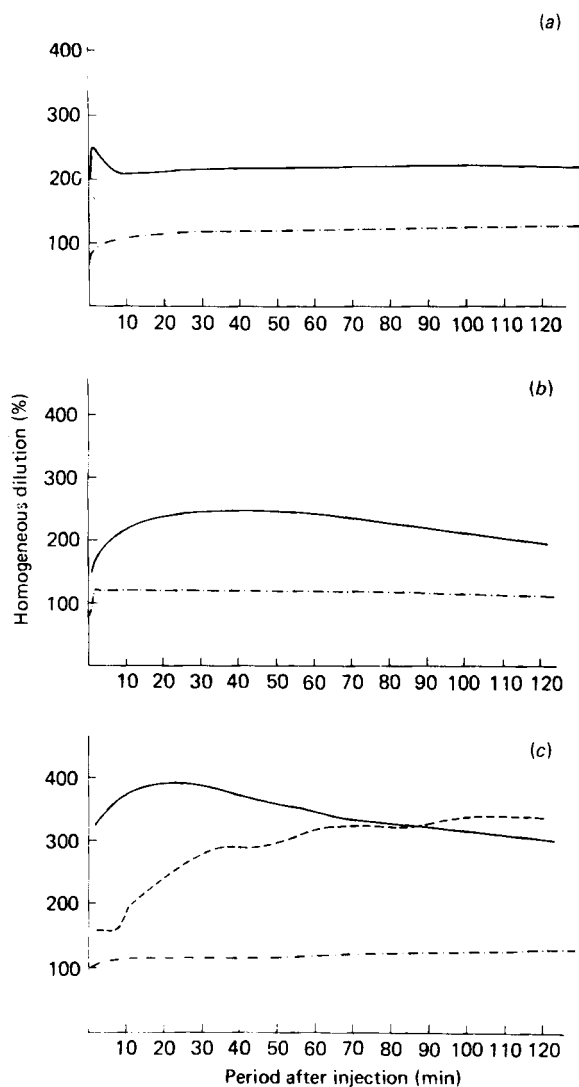
Fig. 4. External measurements of radioactivity in liver (——), muscle (– · –) and intestine (– – –) normalized to conditions of homogeneous dilution (100%) after injection of L-[*Me*-¹¹C]methionine in pigs. (a) Measured in fasting animal, control diet; (b) measured 1 h after feeding. Experimental diet supplemented with 1·2 g methionine/kg, day 14; (c) measured 1 h after feeding. Experimental diet with no supplementary methionine, day 14.

control diet. In the liver region, however, there was an increase in radioactivity during the successive experiments (Fig. 4(a), (b) and (c)).

The ratio, radioactivity in liver:radioactivity in muscle, increased (Fig. 5).

In one experiment with low-methionine diet (Fig. 4c) a third detector placed over the small intestine region showed a slow but steady increase in radioactivity from initially 150%, as compared to homogeneous dilution, to more than 300% 2 h later. Occasional measurements of urine and faeces samples gave values of 10×10^{-6}/ml and 0.2×10^{-6}/g respectively of the administered radioactivity.
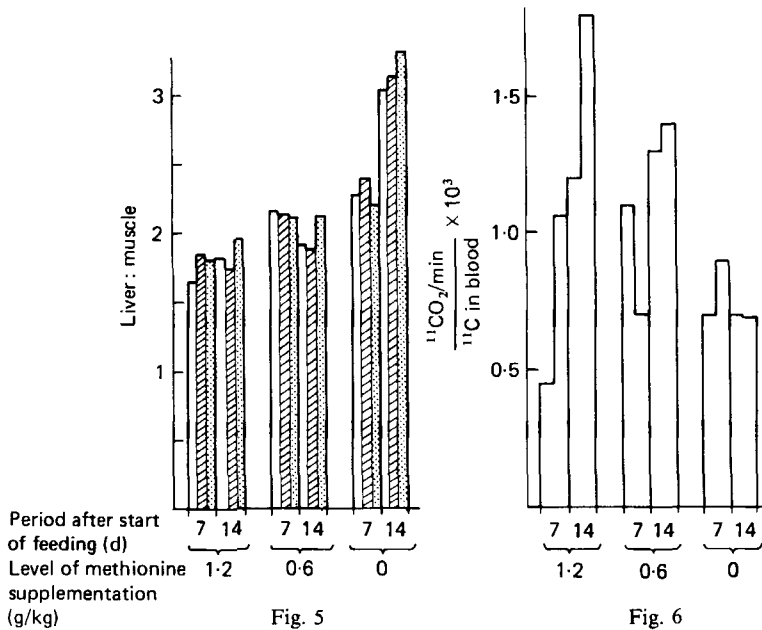
Fig. 5. Ratio, radioactivity in liver:radioactivity in muscle, from measurements on days 7 and 14 after starting on the experimental diet at each of three levels of methionine supplementation. The values were calculated as integrated means for three different periods after injection of L-[Me-^{11}C]methionine (5–20 (□), 20–60 (■) and 60–120 (▨) min).

Fig. 6. Ratio, exhaled $^{11}CO_2$/min:radioactivity in blood, from measurements on days 7 and 14 after starting on the experimental diet at each of three levels of methionine supplementation. The values represent one measure of the relative amount of blood radioactivity that was in the form of $^{11}CO_2$. The values were calculated as integrated means for two different periods after injection of L-[Me-^{11}C]methionine (20–80 and 80–115 min respectively).

Table 2. *Relative amount of the administered radioactivity exhaled (/min) during two different periods after administration of L-[Me-^{11}C]methionine to pigs given diets supplemented with methionine at three levels*

Level of methionine supplementation (g/kg)	Day of treatment	Exhaled $^{11}CO_2$/min:administered radioactivity	
		20–80 min	80–115 min
1·2	7	2·0	1·5
	14	8·3	6·8
0·6	7	5·6	2·2
	14	7·2	3·9
0	7	4·2	3·3
	14	2·1	1·2

The ratio, exhaled $^{11}CO_2$:radioactivity in blood, is given for days 7 and 14 respectively on each level of supplementation of the diet (Fig. 6). Values were calculated for periods of 20–80 min and 80–115 min respectively after administration. Table 2 shows the measured $^{11}CO_2$ in different experiments.

Methionine intake (mg/d per kg body-weight) decreases in a stepwise manner (Table 3).

Table 3. *Explicit summary of body-weight (kg), diet intake (kg/d), daily methionine intake/body-weight (special diet + supplementary DL-methionine) (mg/d per kg body-weight), the ratio, relative amount exhaled $^{11}CO_2$/min:daily methionine intake/kg body-weight*

Level of methionine supplementation (g/kg)	Day of treatment	Weight (kg)	Diet intake (kg/d)	Methionine intake (mg/d per kg body-weight)	$10^6 \times \dfrac{\text{exhaled } ^{11}CO_2/\text{min}}{\text{administered } ^{11}C}$: methionine intake	
					20–80 min	80–115 min
1·2	7	65	2·3	113	0·02	0·01
	14	75	2·5	107	0·08	0·04
0·6	7	84	2·7	84	0·07	0·03
	14	91	2·8	80	0·09	0·05
0	7	100	3·0	60	0·07	0·04
	14	105	3·1	69	0·04	0·02

The ratio, exhaled $^{11}CO_2$:methionine intake (mg/d per kg body-weight) indicates decreases for the 14th day measurements in the 80–115 min measuring period.

The statistical errors in the external radioactivity measurements given by the standard deviation of the radioactivity values were less than 7% for the period up to 2 h after injection. The low radioactivity in samples taken made these measurements uncertain during the last hour of measurements (SD > 10%).

DISCUSSION

The intravenous injection of the ^{11}C-methionine preparation did not cause any adverse reactions. Precautions were taken to obtain a non-toxic, sterile, isotonic and neutral solution of high specific radioactivity (400 MBq/μmol) suitable for injection. No change in the physiological blood plasma concentration should be expected. The total amount of methionine injected was less than 100 nmol (< 15 μg) in each experiment.

The specially prepared diet was designed so as to give a methionine intake as low as practically possible without using a totally synthetic diet. An adequate intake of nutrients was given as judged by the weight gain and thrift of the animal. Thus none of the diets was growth limiting. The initial plateau on the weight-gain curve (Fig. 2) was due to the fact that the pig refused food for a few days after the operation of the indwelling catheter. No signs of wound infection or dysenteria were observed.

In order to make a more thorough evaluation of the distribution measurements the possible influences of non-methionine-dependent changes in distribution volumes, and changes in blood flow should be excluded.

There are several different types of positron emitting labelled tracers that could be used for this purpose [^{11}C-carboxy]haemoglobin (Hnatowich et al. 1979) for blood volume, $H_2^{15}O$ (Ter-Pogossian et al. 1969) for passive distribution dynamics. This also means that the passive distribution dynamics can be substracted from the active uptake. These types of corrections have not been performed in this work.

However, we still noticed that the ratio, radioactivity in liver:radioactivity in muscle, increases (Fig. 5). This is in accordance with experiments using ^{14}C-labelled methionine in rats (Aguilar et al. 1974).

The $^{11}CO_2$ measurements were not continued for any longer time and therefore could not be compared with the results of other investigators who have measured the oxidized fraction for 6 h or more (Aguilar et al. 1974; Newport et al. 1976). The measured $^{11}CO_2$ values are, however, lower than expected. This could be explained by incomplete gas sampling due to the low rate of gas flow from the partially open sampling box. The flow

rate was limited by the trapping efficiency of the CO_2 absorber that had to fit the detector system. This technique has, however, been improved upon by the use of a specially designed scintillation detector which does not limit the flow and permits continuous measurements.

There are variations in the $^{11}CO_2$ based values (Tables 2 and 3, Fig. 6). However, the measurements in the 80–115 min period on the 14th day at each level of methionine supplementation showed a decrease as the intake of methionine decreased. Thus the initial period (20–80 min) of the $^{11}CO_2$ measurements may not be preferable for this type of study.

We conclude that the distribution and dynamics of the administered radioactivity can be studied using the experimental procedures described. No identification of ^{11}C-labelled compounds of low molecular weight was performed in the present study in blood and urine although this may be possible using fast LC-techniques.

With acceptance of the activities of ^{11}C in exhaled air and urine, the external end compartments, as measurements of amino acid utilization, the study indicates that the technique described is useful for studies in intact animals. The distribution of radioactivity between, for example, liver and muscular tissue, may also present a useful criterion, as well as the blood measurements. The duration of the measuring period may be increased by administering greater doses of the labelled compound. A doubling of the initial radioactivity allows another 20 min of measurements.

More detailed information on ^{11}C distribution in single organs or even parts of organs can be obtained by measurements with positron emission tomography (Ter-Pogossian et al. 1980).

Systems including production accelerators, labelling chemistry and 'positron cameras' are now commercially available and installed at many medical centres in increasing numbers. This may open possibilities for nutritional researchers to apply the type of technique described here, or preferably the more sophisticated positron emission-tomography technique, in investigations in both large animals and humans.

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