Studies on the range of tissue protein synthesis in pigs: the effect of thyroid hormones

BY O. SIMON, H. BERGNER AND R. MÜNCHMEYER

Institute of Animal Nutrition, Department of Animal Production and Veterinary Medicine, Humboldt-University, Berlin, German Democratic Republic

AND TERESA ZEBROWSKA

Institute of Animal Physiology and Nutrition, Jablonna, Warsaw, Poland

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1. The effects of thyroid hormones on the range of tissue protein synthesis in growing pigs using the constant infusion technique with [^{14}C]leucine and [^{14}C]lysine were studied.

2. During a 6 h infusion, samples were taken from blood and, at the end of the infusion, from liver, pancreas, stomach, small and large intestines, kidney cortex, kidney medulla, muscle and skin.

3. Lower relative specific radioactivities of free leucine and lysine in several tissues were observed in the hormone-treated group than in the untreated one.

4. The range of protein synthesis rate and the daily amount of protein synthesized in tissues was higher in all tissues after application of thyroid hormones.

5. Assuming that the organs analysed represented 70 % of the total trichloroacetic acid-precipitable protein of the pig, the estimated range of daily protein synthesis was 251–490 and 312–880 g in untreated and hormone-treated pigs respectively.

In recent years the technique of continuous infusion of labelled amino acids has been used in experiments on the rate of tissue protein synthesis in pigs (Garlick *et al.* 1976; Edmunds & Buttery, 1978; Simon, Münchmeyer *et al.* 1978). All the calculations of tissue protein synthesis were based on the specific radioactivity (SR) of the free and the bound amino acid in the tissue at the end of the infusion period. However, the SR of the precursor amino acids for protein synthesis lies between the SR of the free amino acids of the intracellular and the extracellular space (Airhart *et al.*, 1974; Khairallah *et al.* 1977). Therefore, the rates of tissue protein synthesis estimated by the continuous infusion technique have been presented in some papers as maximum and minimum values (e.g. Garlick *et al.* 1973; Nicholas *et al.* 1977; Bohley *et al.* 1979; Lobley *et al.* 1980). For some tissues (e.g. intestine, liver) these two values differ considerably. Using a simultaneous infusion of [^{14}C]leucine and [^{14}C]lysine in rats it was possible to reduce the range between the maximum and minimum values (Bergner *et al.* 1981) compared with methods in which a single labelled amino acid was infused.

It is known that thyroxine administration increases the rate of amino acid incorporation into proteins in cell-free systems (Sokoloff *et al.* 1963, 1968; Brown, 1966; Carter *et al.* 1971; Gibson *et al.* 1977) and in vivo (Michels *et al.* 1963). Furthermore, an increased rate of tissue protein synthesis in hyperthyroid rats has been observed (Bergner *et al.* 1981).

The purpose of this paper is to estimate the range of the rate of tissue protein synthesis by means of simultaneous infusion of [^{14}C]leucine and [^{14}C]lysine in growing pigs untreated or treated with thyroid hormones.

EXPERIMENTAL

Animals and diets

The experiment was carried out on seven castrated male pigs (Polish Landrace) of 42 ± 2 kg body-weight. They were housed individually in pens except during the infusion period when they were kept in metabolism cages. Catheters were implanted into the jugular vein and into the carotid artery at least 3 d before the infusion. Additionally, two of the pigs (nos. 1 and 6) were fitted with duodenal re-entrant cannulas. The animals were fed twice daily with 700 g diet/meal. The diet was composed of (g/kg): barley 700, soya-bean oil meal 160, starch 122, vitamin–mineral mixture (Polfamix L) 5, $CaCO_3$ 10, NaCl 3.

Hormonal treatment

Four pigs were given orally 1260 μg thyroxine (Hennig-Berlin GmbH, Pharmawerke, West-Berlin) and 240 μg triiodothyronine (Thyreotom, VEB Berlin Chemie, GDR) /d for 9 d before the infusion.

Isotope administration and collection of samples

The technique was similar to that described by Simon, Münchmeyer *et al.* (1978). The infusion into the jugular vein started 6 h after a morning meal and was continued for 6 h. During infusion the animals were not fed. The rate of infusion was 4·1 ml/h using a peristaltic pump (Technicon Instruments Co. Ltd, Basingstoke, Hants) and the infusion solution contained (/ml): 0·9 mg NaCl, 0·11 μmol leucine, 900 μmol lysine.

The concentration of radioactivity was $8{\cdot}58 \times 10^5$ Bq/ml for L-[U-^{14}C]leucine and $8{\cdot}15 \times 10^5$ Bq/ml for L-[U-^{14}C]lysine (both labelled amino acids were obtained from UVVR, Prague, Czechoslovakia). The SR of the infused leucine was $7{\cdot}77 \times 10^6$ Bq/μmol and of the infused lysine 907 Bq/μmol. During the infusion period nine blood samples were taken from the carotid artery. At the end of the infusion period the animals were anaesthetized and samples from liver, pancreas, stomach, duodenum, jejunum, ileum, caecum and colon wall, kidney cortex, kidney medulla, gastrocnemius muscle, soleus muscle and skin were taken within 8–10 min.

The infusion was maintained during the sampling period. All tissues were first washed with cold saline (9 g sodium chloride/l) then placed in a plastic tube containing a known amount of cold saline and immediately frozen in a carbon dioxide–acetone bath. During the infusion of the solution containing labelled amino acids, samples of duodenal digesta from two animals were taken hourly by disconnecting the re-entrant cannula. Approximately 50 ml digesta were collected directly into 50 ml cold trichloroacetic acid solution (TCA; 200 g/l).

Preparation and analysis of samples

The samples were prepared and analysed as described by Simon, Münchmeyer *et al.* (1978). Leucine and lysine were estimated in both TCA-soluble and TCA-precipitable fractions of the tissue samples and in the TCA-soluble fraction of plasma for SR.

For measurements of ^{14}C radioactivity a liquid-scintillation system (Tricarb Spectrometer 2660; Packard Instrument Co. Inc., Illinois) was used.

The concentrations of thyroxine (T_4) and triiodothyronine (T_3) in blood serum were estimated in samples collected before starting the infusion. The T_4 concentration was estimated by competitive protein binding analysis (Horn, 1977) using the semi-automatic Columat-system (Sartorius–Membranfilter GmbH, Göttingen, FRG). T_3 concentration was measured using a commercially available kit (T_3-RIA-kit ADW and $^{125}T_4$ were purchased from Isocommerz GmbH, Berlin, GDR). The radioactivity of these samples was counted

on a dual-well type detector sample changer MAG 510 (Berthold, Wildbad, FRG) in a 15–100 keV window.

Calculations

The fractional rate of protein synthesis (k_s) was calculated using the equations derived by Garlick *et al.* (1973). Values of k_s were calculated using the extracellular SR (plasma) of free leucine and lysine as the precursor SR as well as using intracellular SR (tissue homogenates) of free leucine and lysine as the precursor SR. For the first mode of estimation the experimentally determined rate constant (λ) for the increase of SR of leucine and lysine and the SR of these amino acids at the end of the infusion (SR_{max}) in plasma was used.

The increase of SR for leucine and lysine in plasma was described by the function

$$SR = SR_{max}(1-e^{-\lambda t}), \tag{1}$$

where t is time. The parameters λ and SR_{max} were calculated for each animal by regression analysis using the principle of least squares. The sum of the squared deviations Q was considered as a function of λ and SR_{max}:

$$Q(\lambda, SR_{max}) = \sum_{j=1}^{n} (SR(t_j)-M_j)^2, \tag{2}$$

where M_j is a measured value at time t_j. The best estimates of λ and SR_{max} minimize Q and are solutions of the equations:

$$\frac{\delta Q}{\delta \lambda} = 0 \tag{3}$$

$$\frac{\delta Q}{\delta SR_{max}} = 0 \tag{4}$$

where $\delta Q/\delta\lambda$ *and* $\delta Q/\delta SR_{max}$ are partial derivatives of Q with respect to λ and SR_{max} respectively. Eqns (3) and (4) were solved by using the *regula falsi*. For these calculations a desk-top computer (Hewlett-Packard, Model 9825 A, USA) was used.

Taking the tissue SR of the free amino acids as the precursor SR for muscle and heart, the previously estimated λ values in muscle (Simon, Münchmeyer *et al.* 1978) were used whereas, for other tissues, the λ value was assumed to be the same as in plasma. The amino acid flux was calculated according to the method of Millward *et al.* (1975). Mean values are presented together with their standard errors. Student's *t*-test was used to test for significance of difference between sets of results.

RESULTS

Concentrations of total T_3 and total T_4 in blood serum

The concentrations of T_3 (μg/l) were $0{\cdot}97 \pm 0{\cdot}120$ for untreated pigs and $1{\cdot}05 \pm 0{\cdot}050$ for treated pigs (the difference was not significant). However, the T_4 concentration (μg/l) increased significantly ($P < 0{\cdot}01$): untreated $19{\cdot}5 \pm 1{\cdot}150$, treated $28{\cdot}0 \pm 1{\cdot}49$.

SR of free leucine and lysine in plasma and other tissues

For estimation of SR of free leucine and lysine in plasma at the end of infusion a regression analysis was used. SR_{max} estimated in this way (mode B) was generally higher than estimates of SR_{max} calculated as a mean SR during the plateau phase (mode A) (Table 1). If λ values were low the differences between SR_{max} calculated by modes A and B were considerable.

The mean λ values calculated for free leucine and lysine in plasma were $31 \pm 4{\cdot}4$/d and $65 \pm 12{\cdot}3$/d respectively.

The difference between the SR of the free amino acids in several tissues and in blood

Table 1. *Comparison of the [^{14}C] specific radioactivities of free leucine and lysine in plasma at the end of an infusion calculated as a mean value (plus standard error) from seven estimates during the plateau (mode A) and calculated by the regression analysis on the basis of nine estimates together with the rate constants (λ) for the increase in specific radioactivities (mode B)*

Animal no.	Disintegrations/min per μmol							
	Mode A							
	Leucine		Lysine		Mode B		λ (/d)	
	Mean	SE	Mean	SE	Leucine	Lysine	Leucine	Lysine
1	10570	802	17870	1372	12310	19980	26	34
2	11560	865	20640	897	13680	22640	15	26
3	13150	839	21430	1157	13830	21740	42	108
4	13220	561	20590	1166	13290	20610	34	81
5	14190	845	21930	1394	15980	22400	25	64
6	10440	873	18440	713	10730	18650	49	109
7	8980	683	15260	604	10550	17010	24	36

Table 2. *Relative specific radioactivities of free leucine and lysine in tissues of pigs after 6 h intravenous infusion of L-[^{14}C]leucine and L-[^{14}C]lysine*

(Mean values with their standard errors for three untreated pigs and four hormone-treated pigs which received orally 1260 μg thyroxine and 240 μg triiodothyronine/d for at least 9 d)

Organ	Leucine				Lysine			
	Untreated		Hormone-treated		Untreated		Hormone-treated	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver	0·39	0·080	0·30	0·060	0·36	0·062	0·24	0·068
Pancreas	0·39	0·026	0·30	0·057	0·52	0·026	0·44	0·048
Stomach	0·39	0·042	0·36	0·093	0·34	0·041	0·21	0·015*
Duodenum	0·45	0·020	0·28	0·057	0·34	0·012	0·23	0·056
Jejunum	0·39	0·095	0·28	0·057	0·27	0·052	0·17	0·023
Ileum	0·37	0·103	0·33	0·099	0·24	0·043	0·22	0·048
Caecum	0·48	0·015	0·36	0·049	0·30	0·021	0·18	0·023*
Colon	0·42	0·018	0·35	0·064	0·25	0·055	0·15	0·036
Kidney cortex	0·63	0·110	0·51	0·089	1·14	0·042	0·60	0·116**
Kidney medulla	0·64	0·117	0·45	0·109	1·03	0·110	0·63	0·173
Gastrocnemius muscle	0·56	0·021	0·65	0·108	0·52	0·060	0·51	0·085
Soleus muscle	0·54	0·053	0·66	0·111	0·46	0·003	0·43	0·054
Heart	0·95	0·130	0·80	0·062	0·65	0·100	0·61	0·076
Skin	0·34	0·046	0·24	0·006	0·35	0·046	0·30	0·025

Differences between values for treated and untreated pigs were statistically significant: *$P < 0·05$, **$P < 0·01$.

Table 3. *Example of the estimation of the range of fractional rate of protein synthesis* (k_s) *in tissues when two* ^{14}*C-labelled amino acids were infused simultaneously into a pig*

Organ	k_s (%/d) estimated with: L-[^{14}C]leucine Minimum*	L-[^{14}C]leucine Maximum*	L-[^{14}C]lysine Minimum	L-[^{14}C]lysine Maximum	Range for k_s (%/d)
Liver	11·3	20·6	10·8	21·5	11·3–20·6
Pancreas	77·5	200·0	48·0	91·0	77·5–91·0
Stomach	16·5	37·0	11·3	35·0	16·5–35·0
Duodenum	22·0	46·0	12·0	35·5	22·0–35·5
Jejunum	20·5	52·0	14·5	55·0	20·5–52·0
Ileum	10·0	30·0	6·5	42·0	10·0–30·0
Caecum	24·3	45·5	15·0	45·0	24·3–45·0
Colon	13·5	30·7	8·6	28·0	13·5–28·0
Kidney cortex	16·5	20·1	12·8	10·4	16·5–10·4
Kidney medulla	17·5	29·0	14·7	15·4	17·5–15·4
Gastrocnemius muscle	3·0	6·3	1·8	3·6	3·0–3·6
Soleus muscle	2·9	6·2	1·9	5·0	2·9–5·0
Heart	5·3	5·7	3·4	6·0	5·3–5·7
Skin	4·5	12·6	1·9	7·0	4·5–7·0

* For details of procedures, see p. 573.

Table 4. *Range of the fractional rate of tissue protein synthesis* (k_s)† *in untreated pigs and pigs treated with thyroid hormones*

(Mean values with their standard errors for the three untreated pigs and four hormone-treated pigs which received orally 1260 μg thyroxine and 240 μg triiodothyronine/d for at least 9 d)

Organ	Range of k_s (%/d) Untreated Mean	SE	Mean	SE	Hormone-treated Mean	SE	Mean	SE
Liver	11·4	1·10 Leu‡	28·0	4·16 Lys‡	19·0	4·20 Leu	80·4	30·2 Leu
Pancreas	75·3	5·73 Leu	88·0	7·94 Lys	74·3	6·75 Leu	151·0	44·5 Lys
Stomach	13·3	4·43 Leu	22·9	8·87 Lys	15·1	1·04 Leu	41·3	10·4 Lys
Duodenum	23·0	1·83 Leu	45·2	5·13 Lys	37·6	9·58 Leu	106·0	15·1* Leu
Jejunum	25·0	2·75 Leu	70·0	16·0 Lys	24·7	6·13 Leu	120·0	41·9 Leu
Ileum	17·7	3·86 Leu	42·5	6·61 Leu	23·1	7·22 Leu	81·3	34·8 Lys
Caecum	27·0	1·65 Leu	57·2	6·93 Leu	27·6	5·39 Leu	88·9	25·1 Leu
Colon	17·5	2·18 Leu	44·0	8·33 Leu	28·0	6·89 Leu	100·0	40·5 Leu
Kidney cortex	9·4	0·09 Lys	15·1	1·42 Leu	17·2	2·26* Leu	37·9	12·6 Lys
Kidney medulla	11·8	1·84 Lys	15·7	1·67 Leu	19·6	4·02 Leu	34·7	14·6 Lys
Gastrocnemius muscle	2·5	0·26 Leu	3·6	0·23 Lys	2·6	0·66 Leu	4·8	1·20 Leu
Soleus muscle	2·4	0·24 Leu	4·7	0·85 Lys	2·8	0·71 Leu	4·4	0·91 Leu
Heart	4·6	0·41 Leu	5·9	0·33 Leu	6·0	0·92 Leu	7·5	0·66 Leu
Skin	3·7	0·56 Leu	8·6	3·25 Leu	7·5	0·73** Leu	17·1	3·35 Lys

Differences between values for treated and untreated pigs were statistically significant: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.

† For details of range-estimation of k_s, see p. 576 and Table 3.

‡ The index Leu or Lys indicates the ^{14}C-amino acid by which the k_s value was estimated.

Table 5. *Amount of protein synthesized daily in selected organs of untreated pigs or pigs treated with thyroid hormones**

Organ	Relative weight (% body-weight)	TCA-precipitable protein (mg/g tissue)				Protein content (g)		Range of protein synthesis† (g/kg body-weight per d)	
		Untreated		Hormone-treated					
		Mean	SE	Mean	SE	Untreated	Hormone-treated	Untreated	Hormone-treated
Liver	2·97[a]	160	1·38	173	5·56	211	202	0·54–1·33	0·96–4·11
Pancreas	0·33[a]	144	3·62	156	7·19	21	20	0·36–0·41	0·38–0·76
Stomach	0·91[a]	122	1·69	112	9·25	49	40	0·16–0·25	0·15–0·43
Small intestine	3·32[a]	92‡	7·25	112‡	5·75	135	146	0·70–1·60	0·04–3·78
Caecum	0·25[b]	76	6·25	96	5·31	8	9	0·05–0·11	0·05–0·20
Colon	1·62[b]	76	7·38	105	17·9	54	67	0·20–0·54	0·46–1·70
Kidney	0·53[a]	114‖	12·44	123‖	6·94	27	26	0·07–0·09	0·13–0·23
Muscle	45·0[c]	142§	11·56	146§	5·31	2828	2581	1·56–2·64	1·78–3·02
Heart	0·43[a]	122	7·88	133	1·44	23	22	0·02–0·03	0·03–0·04
Skin	7·0[d]	129	11·25	125	35·94	399	345	0·34–0·77	0·66–1·50
Total						3755	3458	4·00–7·77	4·64–15·77

From [a] Wiesemüller *et al.* (1975); [b] own results; [c] Munro (1972); [d] Schmidt *et al.* (1933).

* For details of hormonal treatment, see Table 4.

† Protein synthesis was calculated using the protein content and the fractional rates of protein synthesis as shown in Table 4. The body-weight of the untreated animals was (mean ± SE): 44 ± 1·9 kg (*n* 3) and of the hormone-treated animals 39 ± 3·0 kg (*n* 4).

‡ Mean of TCA-precipitable protein content of duodenum, jejunum and ileum.

‖ Mean of TCA-precipitable protein content of kidney cortex and kidney medulla.

§ Mean of TCA-precipitable protein content of gastrocnemius and soleus muscles.

plasma is characterized by the relative SR (Table 2), where the SR of free leucine and lysine in plasma were the reference values. A lower relative SR of both amino acids was observed in the hormone-treated group than in the untreated one; this was associated with an increase in the concentrations of free leucine and lysine in most tissues. In addition, the contents of TCA-precipitable lysine and leucine in all sections of the intestine were higher in T_3- and T_4- treated animals than in untreated animals. With the exception of kidney the relative SR of lysine was lower than the relative SR of leucine in all organs.

The range of the rate of protein synthesis

Using the results it was possible to calculate four values for the fractional rate of protein synthesis (k_s) in each tissue. With each labelled amino acid a maximum k_s resulted from the ratio of SR of protein bound amino acid (SR_b):SR of the intracellular (homogenate) free amino acid (SR_i) at the end of the infusion. A minimum k_s resulted from the ratio SR_b:SR of free amino acid in plasma (SR_p) at the end of the infusion.

In the case of overlapping of the k_s values estimated with [^{14}C]leucine and [^{14}C]lysine, the range of the rate of tissue protein synthesis was determined by the lowest of the maximum values and the highest of the minimum values. The procedure of estimating the range of k_s is demonstrated in Table 3 using the values from one pig. With this procedure the lower and upper limits of k_s were estimated in each tissue of each animal. The results are summarized in Table 4. The range of protein synthesis rate was generally shifted to higher values in animals treated with thyroid hormones. The most pronounced changes were observed in kidney, heart and skin but some changes in other tissues were seen (e.g. liver, duodenum, stomach).

Table 6. *Percentage contribution of tissues* to the total body protein synthesis† in untreated pigs or pigs treated with thyroid hormone‡*

	Untreated		Hormone-treated	
	Minimum k_s	Maximum k_s	Minimum k_s	Maximum k_s
Gastrointestinal tract‖	19·4	22·6	21·1	27·1
Liver + kidney + pancreas	17·0	16·5	18·2	22·6
Muscle + heart	27·6	24·1	22·5	13·6
Skin	6·0	6·9	8·2	6·7
Other	30	30	30	30

* Calculated on the basis of tissue protein synthesis (g/kg body-weight per d, see Table 5).
† Total body protein synthesis was calculated using the sum of protein synthesized in tissues (see Table 5) and assuming that these organs represent only 70% of total trichloroacetic acid-precipitable protein in the body.
‡ For details of hormonal treatment, see Table 4.
‖ Sum of stomach, small intestine, caecum and colon (see Table 5).

Table 7. *Leucine and lysine flux in three untreated pigs or four pigs treated with thyroid hormones**

			μmol/min				μmol/kg $W^{0·68}$ per min				g/kg body-weight per d			
	Body-weight		Leucine		Lysine		Leucine		Lysine		Leucine		Lysine	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Untreated	44·3	1·9	264	6·5	155	4·2	20·1	1·1	11·8	0·7	1·13	0·08	0·74	0·05
Hormone-treated	39·4	3·0	267	24·0	169	12·3	22·3	2·3	14·0	0·9	1·30	0·15	0·91	0·06

* For details of hormonal treatment, see Table 4.

The stimulating effect of the thyroid hormones on k_s in tissues was associated with a higher content of TCA-precipitable proteins in most tissues (Table 5).

Because both k_s and TCA-precipitable proteins in tissues increased under the action of thyroid hormones the daily amount of protein synthesized in tissues was higher in all tissues after application of thyroid hormones (Table 5). The contributions of individual tissues to total protein synthesis (Table 6) shows that protein synthesis in skeletal muscle and heart accounts for only 20–30% of total synthesis. A similar proportion of total protein synthesis was seen in tissues of the gastrointestinal tract or viscera.

Leucine and lysine flux rates

From the SR of free leucine and lysine in plasma at the end of the infusion period the flux rate of these amino acids was calculated (Table 7). The flux of an amino acid is the sum of its loss from blood plasma by its incorporation into protein and by its catabolism. Related to body-weight or metabolic body-weight ($W^{0·68}$) the flux rates of leucine and lysine were increased in animals treated with thyroid hormones; however, the differences were not significant. The daily flux rates of both amino acids were 3·5 times higher than their dietary intakes.

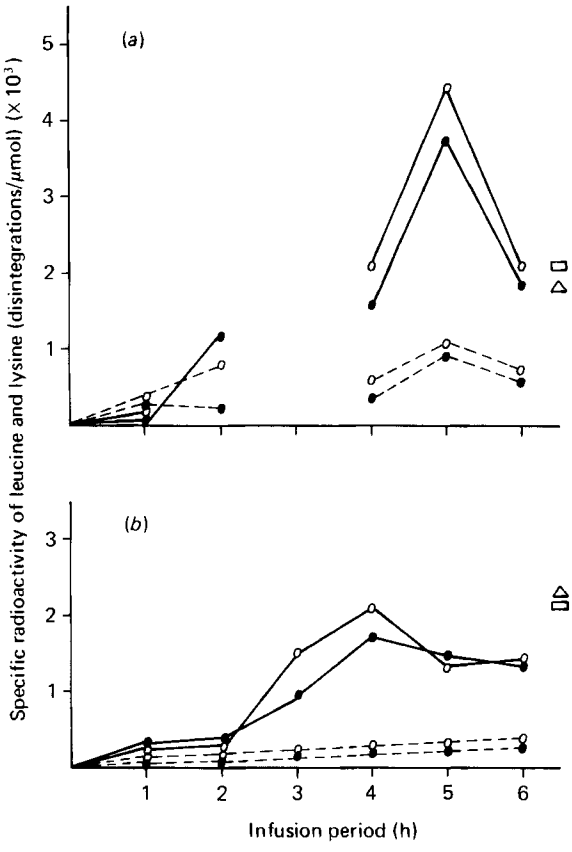

Fig. 1. Specific radioactivities (SR) of [^{14}C]leucine (○) and [^{14}C]lysine (●) in the trichloroacetic acid (200 g/l; TCA)- precipitable (——) and TCA-soluble (---) fractions of duodenal digesta of (*a*) pig no. 1 and (*b*) pig no. 6 during continuous infusions of [^{14}C]leucine and [^{14}C]lysine. The SR of [^{14}C]leucine (△) and [^{14}C]lysine (□) in the TCA-precipitable fraction of pancreatic tissue was estimated at tl. : end of the infusion period.

SR of [^{14}C]leucine and [^{14}C]lysine in the duodenal digesta (Fig. 1)

The SR of both amino acids in the TCA-precipitable fraction of duodenal digesta was similar or higher during the infusion period than that in the same fraction of the pancreatic tissue at the end of the infusion. On the other hand the SR of leucine and lysine in the TCA-soluble fraction of digesta was lower than in the TCA-precipitable fraction, and lower than the TCA-precipitable fraction of pancreatic tissue after 6 h of infusion.

DISCUSSION

In experiments with farm animals, sampling of blood during the infusion period is possible. Because of fluctuations of the SR of free amino acids in plasma, a calculated SR for the end of infusion should be used in further estimations of the rate of protein synthesis, on the basis of the extracellular SR of the respective amino acids. As shown in Table 1, higher SR_{max} result if the regression analysis was applied compared with a mean value of SR during the plateau. The plateau for SR represents only a quasi steady-state situation and is influenced by the λ value. Therefore, the more reliable SR_{max} in plasma results from the regression analysis.

If no biopsy material is taken from tissues during infusion, the SR_{max} of free amino acids in tissues can be estimated by analysis of one sample only at the end of the infusion period. Furthermore, the assumption that λ for SR of free amino acids in intestine and liver is the same as in plasma may result in an underestimation of the maximum k_s. However, taking into consideration the wide range between the minimum and maximum values of k_s (Table 4) the error introduced by this assumption is small, e.g. if in a given case a λ value of 60/d is used instead of 26/d the k_s would be reduced by only 7%.

The problem of which precursor SR of amino acids should be used for calculations of k_s in tissues has been frequently discussed. We think that the best way to present k_s values is to calculate a maximum k_s value based on the SR of free amino acids in tissues and a minimum k_s value based on the SR of free amino acids in plasma for each tissue; this has been done by some other authors (see p. 571). However, if the relative SR of free amino acids is low, the range between the minimum and maximum k_s values estimated with one amino acid is large (Table 3). The use of two labelled amino acids simultaneously enables us to reduce the range of possible k_s values by limiting the range to the lowest of the two maximum values and the highest of the two minimum values for k_s (see Table 3).

The solution infused in the present experiment contained unlabelled lysine as well as the labelled amino acids. The amount of lysine infused during 6 h corresponded to 25% of the daily lysine intake of the pigs. The aim of the lysine supplementation was to reduce fluctuations of lysine concentration in plasma and to increase the relative SR of free lysine in the tissues. Compared with experiments in which infusion solutions without supplementary lysine were used (Simon, Münchmeyer *et al.* 1978), the relative SR increased in most tissues; however it was still smaller than the relative SR of leucine (Table 2). The exception was the relative SR of [^{14}C]lysine in kidney. Values exceeding 1·0 are possible in our procedure because the maximal SR of free plasma amino acids were computer calculated on the basis of all samples collected during infusion; on the other hand, the SR of free amino acids in tissues were estimated only at the end of the infusion.

The same technique was successfully used in a similar experiment with rats (Bergner *et al.* 1981) in which k_s for proteins in gastrocnemius muscle were 4·5–5·1%/d in a control group and 5·5–6·7%/d in a group treated with thyroid hormone. Although other combinations of labelled amino acids may be even more suitable than leucine and lysine, the best combination and the influence of the mode of infusion still need to be studied.

Studies of factors influencing protein synthesis should include estimates of the range of k_s. In this experiment the general effect of increased thyroid hormone levels was a shift of the range of k_s toward higher values in all tissues (Table 4). Even with the values in Table 4, where the range of k_s is narrowed by the use of two amino acids, the interpretation of only the minimum or maximum k_s values would lead us to different conclusions about the magnitude of the effect of thyroid hormones on protein synthesis.

The use of only maximum k_s values would indicate for most tissues an increase of protein synthesis by 100% in hormone-treated animals while changes in minimum k_s were much lower.

It needs to be mentioned that the continuous infusion method in general probably yields low estimates of k_s in tissues with very high turnover rates, such as liver and intestine (Waterlow *et al.* 1978; McNurlan *et al.* 1979; McNurlan & Garlick, 1980). However, for studying a large variety of tissues in farm animals this method seems to be the best one.

Other problems arise when proteins are secreted by such tissues as the pancreas and liver. According to Corring (1975) 18 g protein is secreted daily in pancreatic juice. As shown in Fig. 1, a larger amount of the label was found in proteins of duodenal digesta than in pancreatic tissue. The SR of leucine and lysine in the TCA-precipitable fraction of digesta reached or exceeded that of the SR of leucine and lysine in proteins of pancreatic tissue

(while the SR in the tissue protein fraction increases continuously during the infusion period). However, the protein fraction of digesta was mixed with an unknown amount of exogenous proteins. The secretion of free [^{14}C]leucine and [^{14}C]lysine in pancreatic juice seems to be insignificant.

The stimulatory effect of thyroid hormones on tissue protein synthesis can be shown more clearly by the total amount of protein synthesized in tissues than by the fractional rate of protein synthesis, because of a higher content of TCA-precipitable proteins/g tissue in thyroid-hormone-treated animals (Table 5). Similar results were obtained in experiments on rats (Bergner *et al.* 1981).

Assuming that the organs analysed represented 70% of the total TCA-precipitable protein of the pigs, and knowing that the body-weight of the untreated pigs was $44 \pm 1{\cdot}9$ and that of the treated ones 39 ± 3 kg, the estimated range of daily protein synthesis was 251–490 and 312–880 g in untreated and hormone-treated pigs respectively. It was shown that T_3 therapy in thyroidectomized rats stimulates in vivo protein synthesis in skeletal muscle (Millward *et al.* 1979). The daily rate of thyroxine secretion in 30–60 kg pigs has been estimated to be 10–12·5 μg/kg body-weight (Bergner *et al.* 1969; Münchow & Bergner, 1970; Münchmeyer *et al.* 1974).

The dose of thyroid hormones used in this experiment was approximately 3 times the normal daily secretion rate. If it is assumed that only 70% thyroid hormones were absorbed (Read *et al.* 1970) the actual amount entering the blood stream was only 2·5 times the normal secretion rate. Therefore, the concentrations of T_3 and T_4 were within physiological ranges. Ingram & Evans (1980) found in 2–3 month old pigs fed with various diets that the concentrations of T_3 and T_4 in plasma ranged from 0·44 to 1·49 and from 23 to 35 μg/l respectively.

The energy cost for peptide bond synthesis has been estimated to be 3·5 kJ/g protein (Millward *et al.* 1979). The energy cost of protein synthesis, however, is difficult to estimate and appears to be in the range 27–29 kJ/g protein synthesized, including the energy content of protein (Müller & Kirchgessner, 1979; Thorbek, 1980). When protein synthesis was regressed against heat production the slope of the line was approximately 21 kJ/g protein synthesized (Lobley & Reeds, 1980; Reeds *et al.* 1980). More exact estimates are available for the energy cost of protein deposition, which vary between 1·3 and 2·8 kJ/g protein (Hoffmann *et al.* 1977; Müller & Kirchgessner, 1979). It means that deposition of 1 g protein will require 31–67 kJ. Because of the lack of accurate values for the energy cost of protein synthesis and of the relatively large range of the possible amount of synthesized protein, further calculations using values from the present experiment would not be meaningful. However, our results support the hypothesis of Sokoloff & Kaufmann (1959) and Weiss & Sokoloff (1963) that hypermetabolism induced by large doses of thyroxine is secondary to stimulation of protein synthesis and that even treatment with small amounts of thyroid hormones stimulates protein turnover rate and consequently increases energy expenditure. The contribution of muscle protein synthesis to total protein synthesis (Table 6) accounted for 24–28% in the control group and 14–23% in the hormone-treated group and was in the same range as in other pigs of various body-weights and in cattle (Buttery, 1980; Lobley *et al.* 1980). Despite the much lower total protein content of the gastrointestinal tract and viscera than in muscle, the proportions of protein synthesized by the gastrointestinal tract, viscera and muscle were similar.

The problems connected with the calculation of total protein synthesis flux rates of amino acids were recently reviewed by Reeds & Lobley (1980). Despite the different results obtained with different labelled amino acids for interspecies comparisons, the use of [^{14}C]-leucine was recommended. This amino acid was infused in men (Golden & Waterlow, 1977; Garlick *et al.* 1980) and pigs (Reeds *et al.* 1980) and gave comparable results under similar

conditions. Using U-[^{14}C]leucine we calculated the flux rate to be 1·13 g leucine/kg body-weight per d in the untreated animals and 1·30 g/kg body-weight per d in the hormone-treated animals. In our previous experiment on pigs (Simon, Bergner *et al.* 1978) we estimated a leucine flux of 1·21 g/kg body-weight per d, which supports the conclusions of Reeds & Lobley (1980). Lysine flux is excluded from this comparison because, in contrast to other experiments, we infused unlabelled lysine and it is known that lysine oxidation is very sensitive to the dose, especially if the requirement is exceeded (Bergner & Simon, 1976; Simon, Bergner *et al.* 1978). In our experiment the oxidation of leucine and lysine was not measured and therefore total body protein synthesis was not calculated from the flux rates. However, the hormonal treatment induced and increased the flux rates of leucine and lysine by 15 and 23% respectively (see Table 7). This stimulation is of the same order as the increase of the estimated total minimum protein synthesis (251 *v.* 312 g/d = 24% increase).

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