Biochem. J. (1988) 250, 179–188 (Printed in Great Britain)

The effects of surgical stress and short-term fasting on protein synthesis *in vivo* in diverse tissues of the mature rat

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1. We measured fractional rates of protein synthesis, capacities for protein synthesis (i.e. RNA/protein ratio) and efficiencies of protein synthesis (i.e. protein-synthesis rate relative to RNA content) in fasted (24 or 48 h) or fasted/surgically stressed female adult rats. 2. Of the 15 tissues studied, fasting caused decreases in protein content in the liver, gastrointestinal tract, heart, spleen and tibia. There was no detectable decrease in the protein content of the skeletal muscles studied. 3. Fractional rates of synthesis were not uniformly decreased by fasting. Rates in striated muscles, uterus, liver, spleen and tibia were consistently decreased, but decreases in other tissues (lung, gastrointestinal tract, kidney or brain) were inconsistent or not detectable, suggesting that, in many tissues in the mature rat, protein synthesis was not especially sensitive to fasting. 4. In fasting, the decreases in fractional synthesis rate resulted from changes in efficiency (liver and tibia) or from changes in efficiency and capacity (heart, diaphragm, plantaris and gastrocnemius). In the soleus, the main change was a decrease in capacity. 5. Surgical stress increased fractional rates of protein synthesis in diaphragm (where there were increases in both efficiency and capacity) by about 50%, in liver by about 20%, in spleen by about 40%, and possibly also in the heart. In liver and spleen, capacities were increased. In other tissues (including the skeletal muscles), the fractional rates of protein synthesis were unaffected by surgical stress.

INTRODUCTION

The non-growing organism is in a state of nitrogen equilibrium. Negative nitrogen balance results from the rate of protein degradation exceeding the rate of protein synthesis, although several mechanisms can be envisaged whereby this is achieved [1]. It has long been recognized that animals which have undergone surgery or have been injured exhibit negative nitrogen balance and changes in whole-body protein turnover [2]. The degree of nitrogen imbalance may be influenced by nutritional state [3]. In spite of much previous work (reviewed in [1]), there has been much disagreement concerning the relative importance of protein synthesis and degradation in the induction of the negative nitrogen balance of surgical stress or trauma. There may be many reasons. Methodology has varied, as has the nutritional status of the subjects and the type and severity of trauma. The time period over which measurements have been made has varied. These technical problems have made it difficult to come to any definite conclusions.

We decided to measure C_s, k_s and k_{RNA} in a variety of tissues in adult, slowly growing, carefully weight-matched rats. We used a well established surgical technique, that of laparotomy. We used fasted animals, to obviate any problems that might arise because of different rates of food absorption. Perhaps most importantly, we measured k_s by probably the most reliable technique currently available [4], in which the body pools of phenylalanine are 'flooded' by large amounts of intravenously administered [4-^{3}H]phenylalanine. This ensures rapid radioisotopic equilibration between the various pools of free phenylalanine and phenylalanyl-tRNA. Many earlier studies have used constant infusion of radiolabelled amino acid to measure k_s. The problem with this technique is that the attainment of isotopic equilibrium between the extracellular and intracellular space is slow and incomplete. Calculation of k_s is thus made more difficult, and imprecision can arise. An illustration of the problems encountered is given in [5], where surgical stress was found to increase or not to alter k_s in the liver, depending on which value of the radiolabel specific radioactivity was used in the calculation.

In addition to the main project, we also of necessity studied the effects of progressive fasting on k_s. Such information has not previously been readily available, since often a single time point has been chosen for sampling, and also previous studies have tended to use young rapidly growing rats.

EXPERIMENTAL

Animals and materials

Female albino Wistar rats (body wt. about 200 g before fasting, age about 8–9 weeks) were bred in the animal house of the London Hospital Medical College.

Abbreviations used: k_s, fractional rate of protein synthesis (%/day); k_{RNA}, efficiency of protein synthesis, i.e. rate of protein synthesis relative to RNA (g of protein synthesized/day per g of RNA); k_d, fractional rate of intracellular protein degradation; S_p, plasma [4-^{3}H]phenylalanine specific radioactivity; S_t, whole-tissue [4-^{3}H]phenylalanine specific radioactivity; C_s, capacity for protein synthesis, i.e. RNA/protein ratio (μg of RNA/mg of protein); n, number of observations.

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They were maintained on a 12 h-light/12 h-dark cycle, with the light phase beginning at 08:00 h. Before the start of the experimental period, they had free access to food and water. When food was withdrawn, the rats were kept in grid-bottomed cages. Other materials were obtained from the sources described previously [6].

Surgical procedures

The rats which underwent surgical trauma were lightly anaesthetized with diethyl ether, the abdomen was shaved, and a midline incision (3 cm) was made just posterior to the xiphisternum. The skin was freed from the

Table 1. Times of immersion of tissues in ice–water, and initial and final S_t values

S_t and S_p were determined as described in the Experimental section. The time of sampling refers to the time at which the tissues that were used for determination of k_s were removed from the rats. The initial S_t values were determined on tissues of rats killed at 2 min. The final S_t values were determined on rats killed at 10 min. Statistical significance of final versus initial S_t: $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$.

	Fed			24 h-fasted		
Tissue group	Time of sampling (min)	Initial S_t (d.p.m./nmol)	Final S_t (d.p.m./nmol)	Time of sampling (min)	Initial S_t (d.p.m./nmol)	Final S_t (d.p.m./nmol)
Thoracic tissues						
Heart	11.60±0.04	432±6	449±4	11.72±0.06	390±17	403±6
Diaphragm	12.09±0.03	425±7	441±6	12.15±0.05	422±14	392±10
Lung	11.60±0.04	407±14	377±8	11.72±0.06	415±8	363±6^b
Skeletal muscles						
Soleus	10.82±0.04	519±7	483±8^a	10.96±0.03	487±2	481±7
Plantaris	10.82±0.04	481±9	464±8	10.96±0.03	468±2	448±11
Gastrocnemius	10.82±0.04	426±4	448±5	10.96±0.03	427±20	460±13
Smooth muscles						
Stomach	12.84±0.10	385±16	330±6^b	12.78±0.14	383±8	325±9^b
Small intestine	13.18±0.10	341±9	303±9^a	13.29±0.17	321±5	271±11^a
Uterus	13.76±0.10	455±2	435±8	13.96±0.19	443±12	409±12
Non-muscle tissues						
Liver	11.09±0.04	407±14	324±10^b	11.13±0.04	419±10	314±11^c
Kidney	12.18±0.10	348±16	302±21	12.32±0.06	385±18	255±7^c
Spleen	12.33±0.10	422±2	416±4	12.45±0.06	402±8	363±6^b
Brain	10.04±0.00	350±19	369±6	10.05±0.00	326±20	344±10
Tibia	10.82±0.04	354±10	306±7^b	10.96±0.03	356±20	290±7^b
S_p		481±10	483±8		485±18	471±8

	24 h-fasted/surgically stressed			48 h-fasted		
Tissue group	Time of sampling (min)	Initial S_t (d.p.m./nmol)	Final S_t (d.p.m./nmol)	Time of sampling (min)	Initial S_t (d.p.m./nmol)	Final S_t (d.p.m./nmol)
Thoracic tissues						
Heart	11.83±0.11	413±18	410±8	11.73±0.04	427±6	431±7
Diaphragm	12.22±0.11	414±5	403±4	12.08±0.06	435±14	421±7
Lung	11.83±0.11	419±12	362±8^b	11.73±0.04	365±13	354±12
Skeletal muscles						
Soleus	11.02±0.07	494±6	485±9	10.81±0.06	529±39	495±9
Plantaris	11.02±0.07	481±8	451±11	10.81±0.06	456±11	456±7
Gastrocnemius	11.02±0.07	456±13	454±12	10.81±0.06	473±28	476±6
Smooth muscles						
Stomach	12.79±0.12	379±13	331±7^b	12.55±0.08	368±26	341±6
Small intestine	13.43±0.19	317±7	271±15	12.97±0.12	311±16	244±13^a
Uterus	13.98±0.22	449±5	415±6^b	13.43±0.15	407±14	419±11
Non-muscle tissues						
Liver	11.19±0.08	430±18	372±9^a	11.12±0.04	437±9	345±9^c
Kidney	12.40±0.12	377±12	252±10^c	12.27±0.06	336±35	267±12^a
Spleen	12.54±0.12	382±5	371±9	12.39±0.07	374±18	351±5
Brain	10.04±0.00	345±21	350±6	10.04±0.00	303±13	360±8^b
Tibia	11.02±0.07	374±17	302±9^b	10.81±0.06	360±25	297±9^a
S_p		489±12	470±8		463±9	450±8

Table 1 (continued)

Tissue group	48 h-fasted/surgically stressed: Time of sampling (min)	Initial S_t (d.p.m./nmol)	Final S_t (d.p.m./nmol)
Thoracic tissues			
Heart	11.88±0.10	430±7	438±11
Diaphragm	12.26±0.10	414±19	413±4
Lung	11.88±0.10	387±21	375±9
Skeletal muscles			
Soleus	10.91±0.06	503±2	499±7
Plantaris	10.91±0.06	456±3	453±4
Gastrocnemius	10.91±0.06	479±29	472±6
Smooth muscles			
Stomach	12.78±0.13	388±24	356±7
Small intestine	13.22±0.16	295±5	293±10
Uterus	13.73±0.21	426±13	418±5
Non-muscle tissues			
Liver	11.19±0.64	435±11	377±6[b]
Kidney	12.40±0.10	384±6	285±12[c]
Spleen	12.52±0.11	378±3	363±7
Brain	10.04±0.00	301±3	363±4[c]
Tibia	10.91±0.06	370±8	333±8[a]
S_p		457±10	427±8

anterior abdominal muscles and the peritoneum. A similar incision was made in the abdominal wall, and the large median lobe and the smaller left lateral lobe of the liver were eventrated and re-inventrated. The abdominal wall was sutured and the skin was closed by using Michel clips. The procedure took 5–7 min. The rats were allowed to recover from anaesthesia. From then on they did not have access to food. Water was supplied *ad libitum*. Since we considered that anaesthesia was an integral part of the surgical stress, the control groups of rats did not undergo ether anaesthesia.

Measurement of rates of protein synthesis

Rates of protein synthesis were measured by the flooding-dose method of Garlick *et al.* [4], as described previously [6]. [4-^{3}H]Phenylalanine (150 mM; sp. radioactivity about 0.25 Ci/mol) was administered (1 ml/100 g body wt.) to conscious restrained rats by injection into a lateral tail vein. After the injection, the rat was returned to her cage unrestrained. After 2 min (in experiments to determine S_p, S_t, protein content and C_s only) or 10 min (in experiments to determine k_s and k_{RNA} in addition), the rat was decapitated, a sample of blood was collected into a heparinized tube for the determination of S_p, and tissues were removed into ice–water in a predetermined order as quickly as possible. The time of immersion of the tissue was taken to be the time at which protein synthesis ceased. The order of immersion of the various tissues after decapitation was head, right leg (i.e. tibia, soleus, gastrocnemius, plantaris), liver, heart and lungs, diaphragm, left kidney, spleen, stomach, small intestine (which was subsequently flushed with ice-cold water to remove the contents) and uterus. The times of immersion of the tissues in ice-water are given in Table 1. After dissection was complete, the carcass was skinned. The coefficients of variation for removal times were about 2%. Further dissection was carried out at 0 °C, after which tissues were blotted, weighed, frozen in liquid N_2 and stored at −80 °C until processing as described previously [4,6]. In some tissues, the values of k_s that we obtained could be slightly lower than true values *in vivo* because of the possibility of some decrease in k_s over the period between decapitation and immersion of the tissues in ice–water. However, because the times that a given tissue was immersed were identical for the various experimental groups (Table 1), k_s values for each individual tissue under the various experimental conditions should be strictly comparable with one another.

Calculation of rates of protein synthesis

For each tissue, an initial S_t was determined by using rats decapitated at 2 min ($n = 3$ for each experimental group; see Table 1). The final S_t in the individual tissues was determined by using rats decapitated at 10 min ($n = 7$–8 for each experimental group; see Table 1). If, for a given tissue, the final S_t value was not significantly different from the initial S_t under any of the experimental conditions, then the final S_t was used in the calculation of k_s. This method of calculation of k_s was only used for the striated muscles. In the remaining tissues, S_t often altered significantly over the course of the experiment (Table 1). Here, the change in S_t was assumed to be linear over the experimental period, and a mean S_t was calculated (i.e. an S_t calculated for the time half-way between injection and immersion of the tissue in ice–water). A k_s in units of %/day was then calculated from the formula:

$$k_s = \frac{S_B}{S_t} \cdot \frac{100}{t}$$

where S_B is the specific radioactivity of [4-^{3}H]-phenylalanine bound in protein and t is the time between injection and immersion of the tissue in

ice–water. Rates of protein synthesis were also expressed relative to RNA (i.e. as efficiencies) from the formula:

$$k_{RNA} = \frac{10k_s}{C_s}$$

Absolute rates of protein synthesis were calculated from the tissue protein content and k_s.

Other methods

Protein was measured as in [7] and RNA as in [8]. Statistical significance was determined by analysis of variance, with $P < 0.05$ being taken as indicating a significant difference.

RESULTS

General comments

All rats used in this study were apparently healthy and were slowly gaining weight (up to 10 g in the week preceding the experiment). Rats were carefully weight-matched. The initial weights and the final weights of the groups of rats used in this study (with the number of observations and weight loss as a percentage of initial weight given in parentheses) were as follows: (i) control (fed) group, initial wt. = 218±4 g ($n = 7$); (ii) 24 h-fasted group, initial wt. = 220±3 g, final wt. = 202±3 g ($n = 7$, percentage loss of weight = 8.1±0.6%); (iii) surgically stressed/24 h-fasted group, initial wt. = 221±3 g, final wt. = 205±3 g ($n = 7$, percentage loss of weight = 7.7±0.3%); (iv) 48 h-fasted group, initial wt. = 221±4 g, final wt. = 197±3 g ($n = 8$, percentage loss of weight = 10.8±0.7%); (v) surgically stressed/48 h-fasted group, initial wt. = 218±5 g, final wt. = 197±4 g ($n = 7$, percentage loss of weight = 9.2±0.5%). There was thus no statistically significant difference between the initial weights of the rat groups, nor between the weight losses of comparable groups. Most of the weight loss occurred within 24 h after removal of food. However, part of this was probably the result of loss of faeces.

We paid careful attention to S_t over the course of the experiments (Table 1). Tissues could be broadly divided into four groups: (i) the initial S_t always attained > 75% of the initial S_p, and S_t did not decrease significantly over the course of the experiment (heart, diaphragm, plantaris, gastrocnemius); (ii) the initial S_t always attained > 75% of the initial S_p, but S_t often decreased significantly over the course of the experiment (lung, stomach, uterus, liver, spleen); (iii) the initial S_t was always < 75% of S_p, and S_t did not decrease significantly over the course of the experiment (brain); (iv) the initial S_t was not always > 75% of S_p, and S_t often decreased significantly over the course of the experiment (small intestine, kidney, tibia). In the soleus muscles from fed rats, there was a significant but relatively small (7%) decrease in S_t over the course of the experiment. However, we chose to classify this muscle with the other striated muscles in group (i) above for the purpose of calculation of k_s, i.e. the final S_t value was used. In the brains of 48 h-fasted animals, the initial S_t seemed to be lower than in fed animals, and S_t increased significantly over the course of the experiment (Table 1). Presumably fasting could decrease the rate of phenylalanine uptake by the brain. In some tissues, the decrease in S_t over the course of the experiment was relatively large. For example, in the kidney, the final S_t sometimes decreased by as much as 33% of the initial S_t. This decrease is much greater than that observed in the kidney by Goldspink & Kelly [9]. Thus, of the tissues studied here, it is justifiable to use the final S_t to calculate k_s only for striated muscles (see also the Experimental section). These results emphasize that great care must be taken with respect to variation of S_t over the course of the experiment if accurate k_s values are to be obtained.

Changes in tissue protein content in response to fasting and surgical stress

Fasting for 48 h decreased body weight by 9–11% (see above). In the liver, the small intestine and the stomach, there were significant losses of protein after 24 h of fasting (18%, 18% and 8% respectively; see Table 2). After 48 h of fasting, these tissues had lost 20%, 24% and 11% of their initial protein content respectively. After 48 h of fasting, there were significant decreases in protein contents of heart (8%), spleen (17%) and tibia (14%). No decrease in the protein content of the other tissues studied (diaphragm, skeletal muscles, lung, uterus, kidney, brain and skin) could be detected at any stage.

The superimposition of surgical stress in addition to fasting did not greatly alter the above pattern (Table 2). In the liver after 24 h, surgical stress caused a significant change in protein content compared with the fasting group (increasing it by 10%). Again, there were no significant changes in protein contents of the diaphragm, skeletal muscles, lung, uterus, kidney, brain or skin. Whereas in some cases there had been significant differences between fed and fasted groups, significance was occasionally lost after the superimposition of surgical stress (stomach at 24 h, heart, spleen and tibia at 48 h). The result for the tibia at 24 h attained significance.

Changes in C_s in response to fasting and surgical stress

Only a minority of tissues (striated muscles and possibly the uterus) showed decreases in C_s on fasting (Table 2). When surgical stress was imposed additionally, C_s in the stomach was also decreased compared with the fed control. In other tissues, there were no decreases in C_s with fasting or with fasting plus surgical stress compared with fed controls. Only in the gastrocnemius at 24 h did surgical stress cause an additional decrease in C_s compared with fasting. In three tissues (diaphragm, liver and spleen) there were significant increases (11–24%) in C_s induced by surgical stress relative to the appropriately fasted control groups.

Changes in k_s and k_{RNA} in response to fasting and surgical stress

In striated muscles, uterus, liver and spleen, k_s was consistently (i.e. at both 24 and 48 h) decreased by fasting (Table 3). The 'anaerobic' striated muscles (plantaris, gastrocnemius) were proportionately more severely affected than the 'aerobic' muscles. In other tissues, k_s was unaffected (lung, stomach), or decreased only after 24 h of fasting (small intestine, kidney, brain) or decreased only after 48 h of fasting (tibia). The lack of or inconsistency in response suggests that k_s in many tissues is not especially sensitive to fasting. The gastrointestinal tissues show consistent protein losses during fasting (Table 2). Since k_s is maintained, this suggests either that there was an increase in k_d or an increase in the proportion of protein secreted, or that cells were lost

Table 2. Protein contents and capacities for protein synthesis in various tissues

Statistical significance of results is as follows: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, versus results obtained in fed rats; [d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$, for the surgically stressed group versus rat fasted for the same length of time.

Tissue group	Protein content (mg)				
	Fed	24 h-fasted	24 h-fasted/ surgically stressed	48 h-fasted	48 h-fasted/ surgically stressed
Thoracic tissues					
Heart	136.5±3.6	132.1±3.0	128.4±2.8	125.9±3.3[a]	131.5±3.6
Diaphragm	70.0±2.1	70.1±1.2	69.3±1.5	69.0±1.3	67.3±1.7
Lung	294.3±24.1	346.4±29.0	347.1±29.5	302.1±12.3	349.8±27.9
Skeletal muscles					
Soleus	17.2±0.3	17.8±0.4	17.6±0.3	18.0±0.5	17.5±0.5
Plantaris	40.9±0.7	41.9±1.0	40.4±1.0	40.4±1.1	41.1±1.5
Gastrocnemius	220.1±3.5	216.4±3.4	215.8±6.0	220.4±5.9	210.5±5.3
Smooth muscles					
Stomach	145.5±2.3	134.4±3.2[a]	136.8±5.4	129.2±1.8[b]	133.0±3.5[a]
Small intestine	861.7±28.5	704.2±48.5[b]	717.2±19.0[b]	655.9±30.9[c]	635.7±17.6[c]
Uterus	40.9±2.9	43.7±2.3	44.3±3.6	38.8±2.7	42.1±3.6
Non-muscle tissues					
Liver	1282±32	1049±34[c]	1149±23[b,d]	1024±36[c]	1044±21[c]
Kidney	117.9±3.9	110.4±2.7	109.3±2.0	111.2±4.2	111.3±2.8
Spleen	113.9±7.6	102.5±3.8	109.0±9.2	94.8±4.2[a]	99.5±6.5
Brain	185.6±3.8	186.8±2.2	190.8±2.7	191.7±2.8	190.0±2.9
Tibia	33.3±1.6	32.0±1.0	29.5±0.9[a]	28.8±1.2[a]	31.4±1.2
Skin	3959±832	3942±161	3417±252	3405±230	3773±179
	C_s (μg of RNA/mg of protein)				
	Fed	24 h-fasted	24 h-fasted/ surgically stressed	48 h-fasted	48 h-fasted/ surgically stressed
Thoracic tissues					
Heart	7.24±0.14	6.38±0.06[c]	6.56±0.09[c]	6.09±0.09[c]	6.06±0.06[c]
Diaphragm	5.16±0.23	4.29±0.08[b]	5.07±0.11[d]	4.21±0.08[b]	5.07±0.12[e]
Lung	9.18±0.52	8.04±0.58	7.78±0.59	7.90±0.48	8.22±0.75
Skeletal muscles					
Soleus	4.32±0.23	3.68±0.21	3.61±0.16[a]	3.37±0.11[b]	3.36±0.12[b]
Plantaris	3.01±0.16	2.56±0.09[b]	2.44±0.10[c]	2.19±0.11[c]	2.26±0.09[c]
Gastrocnemius	2.68±0.15	2.39±0.13[a]	2.06 ±0.06[c,d]	2.02±0.06[c]	1.95±0.07[c]
Smooth muscles					
Stomach	26.22±0.47	25.16±0.44	24.10±0.38[a]	25.00±0.85	23.00±0.58[a]
Small intestine	50.46±0.34	50.39±0.79	50.33±1.03	49.53±0.81	49.71±0.82
Uterus	27.73±2.24	24.71±1.80	24.92±1.76	22.74±1.56[a]	22.33±1.34[a]
Non-muscle tissues					
Liver	40.07±0.63	40.29 ±0.69	45.28±0.59[c,f]	39.41±0.31	43.84±0.67[c,f]
Kidney	22.04±0.33	21.31±0.33	21.59±0.34	20.96±2.55	21.22±0.12
Spleen	31.01±0.92	28.72±0.63	33.04±2.50[d]	27.84±1.06	34.51±1.00[e]
Brain	12.61±0.11	12.45±0.13	12.36±0.15	12.39±0.10	12.43±0.09
Tibia	28.90±1.37	27.25±1.56	28.12±0.89	28.05±1.39	27.52±1.13
Skin	9.52±1.03	8.26±0.28	9.78±0.75	9.25±0.95	8.04±0.80

at an increased rate during fasting. It should be noted that we measured the average k_s in the small intestine. There is regional variation in k_s in this tissue, with the mucosal k_s being about twice the serosal k_s [10].

In four tissues (diaphragm, liver, spleen and possibly heart), surgical stress caused an increase in k_s compared with the appropriate fasted group. In no tissue did the superimposition of surgical stress lead to a significant decrease in k_s in addition to that which could be attributed to fasting alone. In lung, superimposition of surgical stress in addition to 24 h of fasting caused a significant decrease in k_s compared with the fed control.

In some tissues, the changes in k_s between the various experimental groups were relatively small (Table 3). We therefore inspected the values of S_t used in the calculation of k_s to ensure that the observed changes in k_s did not

Table 3. Protein-synthesis rates in various tissues

Protein-synthesis rates were measured as described in the Experimental section. Statistical significance of results is as follows: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, versus results obtained in fed rats; [d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$ for the surgically stressed group versus the group fasted for the same length of time.

	k_s (%/day)				
Tissue group	Fed	24 h-fasted	24 h-fasted/ surgically stressed	48 h-fasted	48 h-fasted/ surgically stressed
Thoracic tissues					
Heart	8.58±0.14	6.72±0.22[c]	7.39±0.29[c,d]	6.48±0.21[c]	6.67±0.14[c]
Diaphragm	5.74±0.32	4.24±0.14[a]	6.26±0.38[e]	3.93±0.16[b]	5.96±0.31[e]
Lung	21.59±1.06	17.21±1.58	16.20±1.83[a]	17.52±1.32	19.56±2.56
Skeletal muscles					
Soleus	6.74±0.40	5.55±0.32[b]	5.78±0.16[a]	5.72±0.26[b]	5.43±0.23[b]
Plantaris	4.59±0.21	2.67±0.24[c]	3.07±0.11[c]	2.51±0.13[c]	2.75±0.32[c]
Gastrocnemius	4.69±0.16	2.56±0.24[c]	2.76±0.12[c]	2.42±0.16[c]	2.50±0.17[c]
Smooth muscles					
Stomach	51.32±1.86	45.91±2.49	45.50±0.88	49.02±2.49	45.40±2.17
Small intestine	75.57±2.04	69.81±2.50[a]	71.60±2.51	71.28±3.47	76.09±4.02
Uterus	34.18±3.59	25.51±2.71[a]	23.93±2.73[a]	25.35±3.05[a]	24.52±2.79[a]
Non-muscle tissues					
Liver	80.52±3.50	61.92±4.55[c]	75.09±4.04[e]	57.79±1.93[c]	67.59±2.65[a,d]
Kidney	35.07±3.51	28.56±1.74[a]	33.58±1.82	33.91±1.47	31.77±1.04
Spleen	38.25±2.97	31.58±1.01[a]	43.97±2.43[f]	30.44±2.33[a]	43.55±1.58[f]
Brain	12.11±0.44	10.67±0.34[b]	11.66±0.39	11.47±0.46	11.91±0.31
Tibia	21.15±1.79	17.86±1.26	17.37±1.37	14.59±1.03[b]	15.37±1.80[a]
	k_{RNA} (g of protein/day per g of RNA)				
	Fed	24 h-fasted	24 h-fasted/ surgically stressed	48 h-fasted	48 h-fasted/ surgically stressed
Thoracic tissues					
Heart	12.30±0.21	10.55±0.34[c]	11.16±0.34[a]	10.66±0.36[c]	10.96±0.30[b]
Diaphragm	11.90±0.35	9.95±0.31[b]	12.32±0.56[f]	9.29±0.29[c]	11.35±0.45[f]
Lung	23.58±0.74	21.43±0.82	21.97±0.83	22.01±0.79	22.83±0.35
Skeletal muscles					
Soleus	16.80±0.64	15.73±0.76	16.04±0.61	16.89±0.70	16.99±0.99
Plantaris	16.34±0.71	10.64±0.88[c]	12.08±0.55[c]	11.61±0.94[c]	11.86±0.79[c]
Gastrocnemius	18.81±0.87	10.82±1.12[c]	13.14±0.50[c]	12.15±0.68[c]	12.86±1.01[c]
Smooth muscles					
Stomach	19.75±0.42	18.22±0.75	18.88±0.19	19.29±0.45	18.88±0.77
Small intestine	15.00±0.47	13.89±0.29	14.57±0.72	14.11±0.65	15.03±0.59
Uterus	11.81±0.55	9.86±0.55[a]	10.18±0.65	10.31±0.82	10.49±0.76
Non-muscle tissues					
Liver	20.41±0.87	15.72±1.06[c]	16.45±0.96[b]	14.59±0.41[c]	15.43±0.64[c]
Kidney	15.99±1.48	13.63±0.93	15.36±0.79	16.22±0.66	15.04±0.55
Spleen	12.48±0.59	11.37±0.45	13.82±1.19[e]	11.03±0.50	12.73±0.18
Brain	9.61±0.33	8.56±0.23[b]	9.29±0.22[d]	9.20±0.18	9.54±0.21
Tibia	7.54±0.74	6.89±0.65	6.27±0.37	5.37±0.46[a]	5.44±0.60[a]

result from differences in S_t. Under none of the conditions could a change in k_s be explained by variation in S_t.

We examined the mechanisms for the decreases in k_s values in tissues which showed a consistent response (i.e. a decrease in k_s at 48 h or at both 24 h and 48 h) to fasting. In heart, diaphragm, plantaris and gastrocnemius, the decreases in k_s resulted from changes in both k_{RNA} and C_s. Thus, because of the changes in C_s, the effects of fasting are partly attributable to changes in RNA turnover. This pattern is by no means universal, since, in the liver and the tibia, fasting decreased k_s primarily by decreasing k_{RNA} in the absence of any change in C_s. In the soleus, the decrease in k_s was primarily attributable to a decrease in C_s. In spleen and uterus there were small decreases in both k_{RNA} and C_s, but, because the decreases were small, it was difficult to be certain as to the mechanism for the decrease in k_s.

Of the three tissues where surgical stress clearly and

Table 4. Published rates of protein synthesis in rat tissues *in vivo*

k_s values *in vivo* were measured by the 'flooding dose' method [4] in fed male rats, with [4-^{3}H]phenylalanine as radiolabel. The Wistar strain of rats was used for the experiments described in [4] and [18]. Except for the value for the tibia, which was taken from [18], the remaining values in that column were taken from [4]. For experiments described in [9], [15] and [16], the CD strain of rats was used. For experiments described in [17], the Sprague–Dawley strain was used. Key: [a] extensor digitorum longus, [b] jejunal mucosa, [c] jejunal serosa, [d] tibialis anterior, [e] plantaris.

	k_s (%/day)				
Reference(s)...	[4], [18]	[9], [15], [16]			[17]
Tissue group	Body wt. 130 g	Body wt. 211 g (8 weeks old)	Body wt. 584 g (44 weeks old)	Body wt. 604 g (105 weeks old)	Body wt. 160 g
Thoracic tissues					
Heart	20.0	12.0	10.3	6.6	14.6
Diaphragm	–	–	–	–	11.3
Lung	32.7	–	–	–	24.7
Skeletal muscles					
Soleus	21.3	14.8	9.6	5.7	12.9
Fast twitch	18.0[a]	9.1[d]	4.5[d]	3.8[d]	10.0[e]
Gastrocnemius	16.9	–	–	–	9.5
Smooth muscles					
Small intestine	119.2[b]/54.4[c]	82.4	–	64.4	–
Non-muscle tissues					
Liver	83.3	58.8	42.5	48.0	104
Kidney	–	31.8	30.8	23.5	65.6
Spleen	68.3	–	–	–	–
Brain	–	–	–	–	16.8
Tibia	90.4	–	–	–	53.7

consistently increased k_s, i.e. diaphragm (increased by about 50%), liver (about 20%) and spleen (about 40%), the effect in the diaphragm was definitely the result of an increase in both k_{RNA} and C_s. Because of the smaller increases in k_s in the liver and spleen, attribution of the effects of surgical stress were not clear, although increases in C_s were definitely involved in the response.

DISCUSSION

General comments

Although other workers (e.g. [11]) have examined rates of protein turnover in rat tissues, the range of tissues selected for study has been relatively restricted. However, the constant-infusion technique used in many earlier studies may be criticised on the basis of slow equilibration between S_p and S_t leading to errors (particularly in tissues with a high rate of protein turnover) [12], and on the basis that immobilization of the rat during infusion itself decreases k_s in some tissues [13]. A careful study [14] showed that the flooding-dose technique [4] gave consistently higher k_s values than the constant-infusion technique, presumably because of avoidance of problems associated with precursor specific radioactivity.

Protein turnover in fed rats

Protein synthesis *in vivo* in young rapidly growing rats has been extensively studied (e.g. [11]). However, because of the quantities of radioisotope required, protein synthesis has been much less well studied in mature rats. An advantage in using female rats is that when mature they grow relatively slowly compared with male rats, and furthermore their body weight at maturity is less.

For comparison, we have shown some published k_s values obtained by the 'flooding-dose' technique in fed male rats with [4-^{3}H]phenylalanine as radiolabel in Table 4. For k_s values taken from [4], [9], [15], [16] and [17], the S_t values used in the calculation of k_s were corrected for the decline in S_t that occurred over the course of the experiment. Values taken from [17] were not corrected in this manner, and thus may overestimate k_s in tissues other than striated muscles. Comparison with previously published k_s values is difficult, because of differences in age, sex, rat strain and range of tissues studied. However, k_s values in mature fed female rats (Table 3) exhibit the same general pattern as young and older male rats (Table 4). Striated muscles and brain show relatively low k_s values, whereas smooth muscles and tissues such as the liver show relatively high k_s values. Comparison of k_s values in Table 3 with other studies on surgically stressed rats is even more problematical, because other workers have used the constant-infusion technique and different radioisotopes to measure k_s, and isotopic equilibrium between S_p and S_t was not attained (see, e.g. [5]).

There is a considerable variation in k_{RNA} between tissues (Table 3, see also, for example, [18]). In fed rats k_{RNA} is lowest in the tibia, and is 2.5–3-fold higher in the liver. It could be argued that these findings could be affected by the interval between decapitation and the time of immersion of tissues in ice–water. However, differences in k_{RNA} can still be observed in tissues immersed at almost the same times, e.g. liver and tibia. It has been proposed that the basal k_s is largely set by C_s [19,20]. Our results would seem to argue against this.

Table 5. Absolute rates of protein synthesis in various tissues

Absolute rates of protein synthesis were calculated as described in the Experimental section. The results for skeletal muscles and the tibia refer to tissues taken from a single leg. The result for the kidney refers to the left kidney. Statistical significance of results is as follows: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, versus results obtained in fed rats; [d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$, for the surgically stressed group versus the group fasted for the same length of time.

Tissue group	Absolute rates of protein synthesis (mg/day per rat)				
	Fed	24 h-fasted	24 h-fasted/ surgically stressed	48 h-fasted	48 h-fasted/ surgically stressed
Thoracic tissues					
Heart	11.6±0.5	8.72±0.46[c]	9.77±0.44[b]	8.11±0.37[c]	8.74±0.37[c]
Diaphragm	4.05±0.31	2.99±0.13	4.32±0.27[d]	2.72±0.14[a]	4.01±0.31[d]
Lung	62.7±4.6	56.1±3.9	57.9±4.4	52.4±4.4	67.9±4.8[d]
Skeletal muscles					
Soleus	1.18±0.08	0.98±0.07[a]	1.04±0.04	1.06±0.06	0.94±0.06[a]
Plantaris	1.88±0.07	1.13±0.10[c]	1.22±0.05[c]	1.02±0.06[c]	1.15±0.19[c]
Gastrocnemius	10.4±0.4	5.6±0.6[c]	5.9±0.2[c]	5.4±0.4[c]	5.4±0.5[c]
Smooth muscles					
Stomach	74.6±3.3	61.3±4.0[a]	62.9±3.4[a]	62.6±2.7[a]	59.5±4.7[b]
Small intestine	631±33	472±51[b]	528±17[a]	473±28[b]	463±34[b]
Uterus	15.3±2.5	10.9±1.3	11.3±2.2	10.1±1.8	10.6±2.3
Non-muscle tissues					
Liver	1004±63	662±71[c]	890±46[e]	599±34[c]	688±34[c]
Kidney	40.7±3.7	31.2±2.2[a]	36.9±2.1	37.0±2.5	34.7±1.3
Spleen	47.5±7.3	33.2±2.2[a]	53.0±7.4[e]	29.9±3.5[a]	46.4±2.9[d]
Brain	22.4±0.7	19.8±0.6[a]	22.1±0.8[d]	21.9±0.8	22.8±0.7
Tibia	7.19±0.89	5.62±0.60	5.26±0.45	4.15±0.33[b]	4.68±0.56[a]

If it is taken [18] that skeletal-muscle soluble protein constitutes a maximum of about 50% of body soluble protein, that the protein concentration in female rats is the same as in male rats of about 200 g body wt. (125 mg of protein/g body wt. [9]), and that most of the skeletal muscle is white ($k_s = 4.69\%$/day; Table 3), the absolute rate of protein synthesis in skeletal muscle in the fed rats used in this study would be approx. 630 mg/day per rat. Thus, in mature rats used here, the absolute rate of protein synthesis in skeletal muscle resembles that in the small intestine and is considerably less than that in the liver (Table 5). The importance of the liver as a site of protein synthesis in the mature rat contrasts with the situation in the growing rat, where the absolute rate of skeletal-muscle protein synthesis was some 65% greater than that in liver [18]. In the mature fed female rat, the absolute rates of synthesis in the small intestine were also considerable. If the rates of protein synthesis in the tibia are typical for all bones (which may not be the case, since the long bones are the site of haemopoiesis and may therefore be expected to exhibit higher k_s values than non-haemopoietic bone tissue), it can be calculated that the absolute rate of bone soluble-protein synthesis (bone representing 2.8% of whole-body soluble protein [18]) is about 160 mg/day per rat. Bone is thus an important site of protein synthesis in relation to whole-body nitrogen balance.

Effects of fasting

Protein-synthesis rates were decreased by fasting in most of the tissues studied. It is, however, interesting that in some tissues (lung, stomach, small intestine, kidney, brain, tibia) k_s did not seem to be especially sensitive to fasting. Decreases in k_s were greatest in relatively anaerobic skeletal muscles (the plantaris and gastrocnemius show decreases of about 45%). In fed rats, k_s in soleus was about 44% greater than in plantaris, and it was much less sensitive to fasting. The reasons for these differences are not understood, but could result from differential sensitivity or responsiveness of the two muscle types to hormones, to metabolites or to differences in mechanical activity.

The liver exhibited the highest absolute rates of protein synthesis in the fasted mature rat (Table 5). In fasting generally, the relationships between tissues in terms of their absolute rates of protein synthesis resembled those in the fed rats, except that the absolute rate in skeletal muscle (310 mg/day per rat) was now less than the absolute rate in small intestine, even though the small intestine showed a loss of protein, whereas skeletal muscle did not.

We attempted to determine whether fasting caused any change in k_d. We calculated k_d from the difference between k_s and the fractional rate of protein accretion. Although this method gives at best qualitative information concerning the direction of changes in k_d, isotopic methods for the determination of k_d are fraught with methodological problems. Calculations of k_d are only appropriate when there is a significant change in protein content over the course of the experiment (heart, stomach, small intestine, liver, tibia; see Table 2). Except for the liver, there was a tendency for k_d to increase with fasting (results not shown). However, calculated changes in k_d may not necessarily indicate the rate of intracellular protein hydrolysis to amino acids, because tissues such as liver, small intestine and tibia export proteins, and tissues such as the small intestine are very actively dividing, with cells being continually lost and replaced.

This study showed a decrease in cardiac protein content after 48 h of fasting, with no detectable change in the protein content of the skeletal muscles studied. These findings confirm our earlier work [6] and support the findings of Goodman & Ruderman [21], who showed that protein loss from the heart after 5 days of fasting was proportionately greater than that from skeletal muscles. It therefore cannot be claimed with any validity that the heart is protected against protein loss during fasting.

In agreement with the findings of Millward *et al.* [22], we did not detect any change in the protein contents of the skeletal muscles after 2 days of fasting. As reviewed in [23], in the first few days of fasting the major loss of protein is from the liver and gastrointestinal tract. Thereafter, the major loss is from skeletal muscle (and, in the rat, from the skin).

The plasma concentrations of many hormones alter on fasting. The concentrations of insulin and the thyroid hormones decrease, whereas the concentrations of glucagon, the glucocorticoids and growth hormone all increase. Insulin, glucagon and the glucocorticoids all have effects on protein turnover [24–26]. Alterations in the concentrations of the pancreatic hormones during fasting are such as to decrease k_s. Glucocorticoids inhibit skeletal-muscle k_s *in vivo*, but hepatic k_s is at least maintained for several days [26]. Thyroid hormones also increase skeletal-muscle k_s by increasing C_s rather than by increasing k_{RNA} [27]. It is worth noting that the striated muscles show decreases in C_s on fasting (Table 2). However, there are large numbers of specific binding sites for thyroid hormones in the nuclei and mitochondria of tissues such as the liver and the kidney, in which C_s does not alter during fasting [28]. Although growth hormone may affect protein synthesis, its effects should be anabolic rather than catabolic.

Effects of surgical stress

The metabolic response to surgery or trauma is associated with a range of metabolic disturbances, including insulin resistance and changes in insulin secretion (see [29] for a review). In the animal model utilized here, there is a distinct shift towards oxidation of lipid substrates from oxidation of carbohydrate fuels [30]. Ketogenesis is decreased in the face of increased fatty acid mobilization.

There were significant increases in k_s in the liver, diaphragm, spleen and possibly the heart following surgical stress. In experiments where the constant-infusion technique was used, synthesis of non-secreted protein is increased in the liver after trauma or infection [5,31–34]. Because of the prolonged time course of infusion, it is generally accepted that this technique measures mainly synthesis of non-secreted protein, since the contribution of proteins which are being continuously secreted is likely to be small. The flooding-dose method measures the synthesis of both secreted and non-secreted proteins, although we are unable to estimate the relative contributions of each pool to the overall k_s in our experiments. Pomposelli *et al.* [14] have shown that liver total (i.e. secreted + non-secreted) protein synthesis is increased by bacteraemia in young rats by using a 'flooding dose' technique. Thus the increase in liver k_s may be a generalized response to stress rather than a specific response to surgery.

The increase in spleen k_s after surgical stress was presumably related to the importance of this tissue in the mediation of the immune response. We do not know why the diaphragm k_s was increased by surgical stress, unless there was some increase in the frequency of respiration or tidal volume resulting in a workload-induced increase in k_s.

Whether there is any alteration in skeletal-muscle (other than diaphragm) k_s after trauma or infection etc. is controversial. Different workers have shown an increase [14,32], no change [5,34] or a decrease [31,35]. These differences may be attributable to the different models of trauma or infection studied and the different muscle tissues investigated. We failed to detect any alterations in protein content or k_s in the three leg muscles that we studied here.

In those tissues (liver, diaphragm and spleen) in which surgical stress stimulated k_s, there was a complementary significant increase in absolute rates of protein synthesis (Table 5). Additionally, in the lung and the brain, there were increases in the absolute rates of protein synthesis, which achieved significance from the combination of small non-significant increases in protein content and k_s. The increase in the lung may be of relevance to the results obtained in the diaphragm.

This work was supported by grants from the Medical Research Council and the British Heart Foundation.

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Received 2 July 1987/17 September 1987; accepted 22 October 1987