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Effects of potassium deficiency on growth and protein synthesis in skeletal muscle and the heart of rats

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The effects of potassium deficiency on growth, K content and protein synthesis have been compared in 4–13-week-old rats. When maintained on K-deficient fodder (1 mmol/kg) rats ceased to grow within a few days, and the incorporation of [^{3}H]leucine into skeletal muscle protein in vivo was reduced by 28–38%. Pair-feeding experiments showed that this inhibition was not due to reduced energy intake. Following 14 d on K-deficient fodder, there was a further reduction (39–56%) in the incorporation of [^{3}H]leucine into skeletal muscle protein, whereas the incorporation into plasma, heart and liver proteins was not affected. The accumulation of the non-metabolized amino acid α-aminoisobutyric acid in the heart and skeletal muscles was not reduced. The inhibitory effect of K deficiency on ^{3}H-labelling of muscle protein was seen following intraperitoneal (10–240 min) as well as intravenous (10 min) injection of [^{3}H]leucine. In addition, the incorporation of [^{3}H]phenylalanine into skeletal muscle protein was reduced in K-depleted animals. Following acute K repletion in vivo leading to complete normalization of muscle K content, the incorporation of [^{3}H]leucine into muscle protein showed no increase within 2 h, but reached 76 and 104% of the control level within 24 and 72 h respectively. This was associated with a rapid initial weight gain, but normal body-weight was not reached until after 7 weeks of K repletion. Following 7 d on K-deficient fodder the inhibition of growth and protein synthesis was closely correlated with the K content of the fodder (1–40 mmol/kg) and significant already at modest reductions in muscle K content. In vitro experiments with soleus muscle showed a linear relationship between the incorporation of [^{3}H]leucine into muscle protein and K content, but the sensitivity to cellular K deficiency induced in vitro was much less pronounced than that induced in vivo. Thus, in soleus and extensor digitorum longus (EDL) muscles prepared from K-deficient rats, the incorporation of [^{3}H]leucine was reduced by 30 and 47% respectively. This defect was completely restored by 24 h K repletion in vivo. It is concluded that in the intact organism protein synthesis and growth are very sensitive to dietary K deficiency and that this can only partly be accounted for by the reduction in cellular K content *per se*. The observations emphasize the need for adequate K supplies to ensure optimum utilization of food elements for protein synthesis and growth.

Growth: Potassium deficiency: Protein synthesis: Rat

It is well established that potassium deficiency interferes with growth in young animals (Cannon *et al.* 1952) as well as in children (Alleyne, 1970; Waterlow, 1984). This has been related to inhibition of the synthesis of proteins, a process which depends on K both in intact cells (Ledbetter & Lubin, 1977) and in cell-free systems (Alexis *et al.* 1971). In one study, dietary K deficiency was found to cause a considerable decrease in the in vivo incorporation of [^{14}C]leucine into muscle protein of growing chickens (Rinehart *et al.* 1967). In the mature rat, only 21% inhibition of [^{14}C]leucine incorporation (not significant) could be detected following 35 d on K-deficient fodder (Gustafson *et al.* 1973). Little is known, however, about the effects of K deficiency on protein synthesis in young growing rats.

Protein synthesis is essential for growth and the restoration of tissue integrity during disease or malnutrition. Therefore, it is of interest to know by how much dietary and

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intracellular K may be reduced before interference with protein synthesis and growth can be detected, which tissues are primarily affected, and how recovery may be induced by K supplementation. The present study explores these questions by comparing changes in tissue K contents and weight gain in young rats with the incorporation of [^{3}H]leucine into muscle proteins in vivo and in vitro.

MATERIALS AND METHODS

Animals and treatment

All experiments were performed using female Wistar rats in the age range 4–13 weeks. The animals had free access to water, standard fodder or special diets and were kept at constant temperature (23°), humidity (53%) and day length (12 h).

K deficiency was induced by maintaining 4-week-old rats on distilled water and special diets containing between 1 and 40 mmol K/kg. Age-matched control rats were maintained on standard fodder containing 260 mmol K/kg. Metal frames were positioned in the bottom of the cages to prevent the animals from having access to urine or faeces.

In some experiments, the animals were pair-fed by maintaining the controls on the same amount of fodder per d as the rats on K-deficient fodder. The daily food supply was established each morning after assessment (by weighing uneaten fodder) of the food consumed by the K-depleted group during the previous 24 h. The pair-fed animals were then each given the same amount of fodder as a percentage of body-weight, as that consumed in the K-depleted group. A third group was given free access to fodder. All three groups received K-deficient fodder (1 mmol K/kg fodder) and, whereas the K-depleted group received distilled water, pair-fed and *ad lib.*-fed animals were given a potassium chloride solution (250 mmol/l).

K repletion was performed after K depletion by the administration of a single dose of KCl (36 mmol/kg body-weight) via stomach tube and giving the animals free access to normal fodder. In other instances, the K-depleted animals were K-repleted simply by giving free access to normal fodder.

In vivo experiments

In most of the experiments, the incorporation of [^{3}H]leucine into tissue proteins was determined using a modification of a previously developed technique (Young *et al.* 1975). Each animal was given an intraperitoneal injection of 10 ml/kg body-weight of a sodium chloride solution (154 mmol/l) containing [^{3}H]leucine (5 μCi/l or 20 μCi/l) together with carrier L-leucine at a final concentration of 10 mmol/l. At the indicated intervals of time after the injection (10 min–4 h), the animals were killed by decapitation, blood samples withdrawn, and tissue samples weighing about 15–40 mg (wet weight) rapidly excised and immediately frozen in liquid nitrogen. The frozen samples were weighed and placed in tubes containing ice-cold trichloroacetic acid (TCA) at a concentration of 300 mmol/l. The tissue samples were homogenized using an Ultra-Turrax Tissue homogenizer (model TD 18/10). After centrifugation at 2000 rev./min, samples of the supernatant fraction were taken for determination of K. The remaining supernatant fraction was discarded, and the protein sediment washed in 4 ml TCA (300 mmol/l). In order to facilitate the removal of [^{3}H]leucine, the TCA contained carrier L-leucine at a final concentration of 0·1 mmol/l. After recentrifugation, the supernatant fraction was discarded and the protein sediment dissolved by heating for 1 h at 55° with 1 ml sodium hydroxide (1 mol/l). After titration to neutrality with hydrochloric acid (10 mol/l), the dissolved proteins were transferred into counting vials containing 10 ml of a toluene–triton X-114 scintillation mixture. In some

experiments, the protein content of the NaOH-solubilized sediment was determined (Lowry *et al.* 1951).

For isolation of plasma proteins, heparinized blood samples were centrifuged and 100 μl samples of the plasma mixed with 1 ml TCA (300 mmol/l) and centrifuged. Samples of supernatant fraction were counted and the protein sediment was washed twice in 4 ml TCA (300 mmol/l) containing 0·1 mmol carrier leucine/l. Finally, the sediment was dissolved in NaOH, as for muscle protein, and radioactivity counted. Within each experiment, the volume and composition of the samples added to the counting vials were adjusted so as to give the same counting efficiency (around 25%) for plasma, proteins and solutions used for injection.

The incorporation of ^{3}H activity label into total protein was expressed as counts/min (cpm) per g tissue wet weight or per ml plasma. Measurements performed 5 and 10 min after the injection of [^{3}H]leucine showed that the incorporation of ^{3}H activity into muscle protein increased at a linear rate for 10 min. In each experiment, a group of age-matched control rats were injected with the same volume of [^{3}H]leucine solution/kg body-weight.

The incorporation of [^{3}H]amino acids into proteins was also measured following intravenous injection (McNurlan *et al.* 1979). Under phenobarbital anaesthesia, the saphenous vein was exposed. Each animal was given an intravenous injection of saline (9 g NaCl/l; 10 ml/kg body-weight) containing L-leucine (100 mmol/l) and [^{3}H]leucine (200 μCi/kg body-weight). In another experiment L-phenylalanine (150 mmol/l) and [^{3}H]phenylalanine (200 μCi/kg body-weight) were injected intravenously. After 10 min, the animals were killed by decapitation and the tissues and plasma processed for determination of ^{3}H activity in protein.

In some experiments, the accumulation of [^{14}C]α-aminoisobutyric acid in the heart and skeletal muscles was measured. Each animal was given an intraperitoneal injection of 1 ml NaCl (154 mmol/l) containing [^{14}C]α-aminoisobutyric acid (2 μCi/ml). After 60 and 120 min, tissue samples were prepared and homogenized in TCA (300 mmol/l). After centrifugation, the ^{14}C activity of the supernatant fraction was measured and expressed as cpm per g tissue wet weight. The ^{14}C-counting efficiency was around 80%.

In vitro experiments

For the determination of [^{3}H]leucine incorporation in vitro, intact soleus or extensor digitorum longus (EDL) muscles from animals weighing 60–70 g were washed and incubated in Krebs–Ringer bicarbonate buffer. The procedures for preparation of these muscles have previously been described (Kohn & Clausen, 1971; Chinet *et al.* 1977). In order to reduce oxygen consumption and ensure viability, all incubations took place at 30° under continuous gassing with a mixture of 95% O_2 and 5% CO_2. In order to vary the intracellular K concentration, muscles were pre-incubated in the presence of ouabain (1 mmol/l) for 30–180 min. Then the muscles were incubated in buffer containing [^{3}H]leucine (0·4 μCi/ml, 0·5 mmol/l). After incubation, the muscles were blotted, weighed and placed in tubes containing 2 ml ice-cold TCA (300 mmol/l). Muscles were then homogenized and, after centrifugation, the K content of the supernatant fraction and the ^{3}H activity of the protein sediment were determined using the methods described previously for in vivo experiments.

The incorporation of [^{3}H]leucine into protein was also measured in soleus and EDL muscles prepared from rats which had been K-depleted for 1 week or K-depleted for 1 week and then K-repleted for 24 h.

K contents and serum proteins

As in previous studies (Clausen & Kohn, 1977) the K content of muscle tissue was determined by flame photometry of TCA extracts using a Radiometer FLM 3 flame photometer with lithium as internal standard. Plasma samples were taken for measurements of total protein and for separation of proteins by cellulose-acetate membrane electrophoresis at pH 8·6.

Amino acid analyses were performed directly on diluted plasma using Amino Quant (Hewlet–Packard).

Diets, chemicals and isotopes

All chemicals were of analytical grade. Ouabain, puromycin and cycloheximide were obtained from the Sigma Co., St Louis, MO, USA; L-[4,5-^{3}H]leucine (60 mCi/μmol) and 2-amino-[1-^{14}C]isobutyric acid (60 mCi/mmol) were obtained from Amersham International plc, Amersham, Bucks. Standard fodder as well as K-deficient fodder (containing 1 mmol K/kg) were obtained from the Altromin Co., Lage, West Germany.

Statistics

All results are given as mean values with their standard errors. The significance of difference was assessed by the two-tailed t test for groups of non-paired observations and by one-way analysis of variance. Linear correlation analysis of unweighted values was performed by the method of least squares.

RESULTS

Growth retardation

Fig. 1 shows the effects of K-deficient fodder on growth in the age range 4–8 weeks. It is evident that after 3 d on K-deficient fodder (containing 1 mmol/kg) growth is already significantly reduced, and after 14 d, weight gain amounts to only 10% of that of the controls. Pair-feeding experiments showed that this could not be attributed to reduced intake of fodder (see p. 279). In contrast, animals which had been K-depleted for 14 d and then K-repleted by the administration of KCl via a gastric tube, showed a 57% higher growth rate than the controls within the first week. In another experiment 14 d of K depletion even gave a small weight loss. K-depleted animals which in this experiment were K-repleted by giving free access to normal fodder, showed a 108% higher growth rate than the controls within the first week. During the following weeks, however, the growth rate declined, and the K-depleted and K-repleted rats did not reach the same body-weight as the controls until 5–7 weeks after K repletion had started.

In vitro experiments

The incorporation of [^{3}H]leucine into muscle protein was examined using substances blocking protein synthesis. In the isolated soleus muscle obtained from 4-week-old rats, the incorporation of [^{3}H]leucine into protein was inhibited by 87% in the presence of puromycin (100 μg/ml) and by 95% in the presence of cycloheximide (10 μg/ml).

In order to determine by how much cellular K content had to be reduced before the incorporation of [^{3}H]leucine into protein was impaired, soleus muscles were K-depleted to varying extents by pre-incubation with ouabain (1 mmol/l) for 30–180 min. As shown in Fig. 2, this gave muscles with K contents varying from 16 to 74 μmol/g wet weight (including the controls not exposed to ouabain). Over this range, the incorporation of [^{3}H]leucine into protein was reduced in proportion to the decrease in K content, and a highly significant correlation could be established (r 0·86; $P < 0·001$).

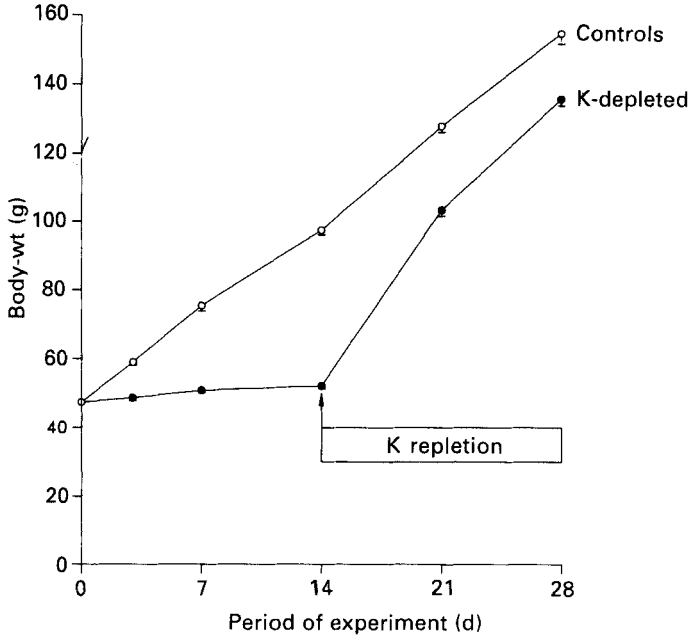

Fig. 1. Effect of potassium depletion and K repletion on body-weight gain. Groups of 4-week-old rats were maintained on standard fodder or K-deficient fodder. After 2 weeks, the rats on K-deficient fodder were K-repleted by the administration of potassium chloride via gastric tube (36 mmol/kg body-weight). Henceforth, both groups of animals were maintained on the K-deficient fodder (Altromin) enriched with KCl so as to contain the same amount of K as the standard fodder (260 mmol/kg). Points are the mean weights of six to eighteen animals, with their standard errors represented by vertical bars. The difference between the body-weight of the controls and that of the K-depleted or repleted rats was significant at all points ($P < 0.001$).

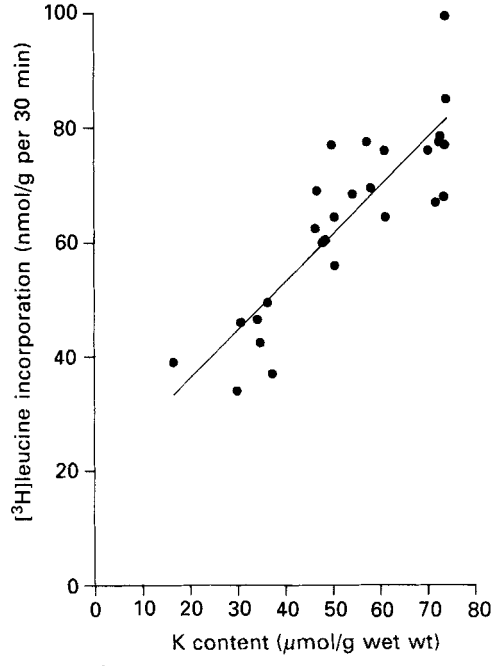

Fig. 2. The potassium content and [^{3}H]leucine incorporation into protein of soleus muscle following K depletion in vitro. Soleus muscles were K-depleted by pre-exposure to ouabain (1 mmol/l) for 30–180 min and then incubated for 30 min in buffer containing [^{3}H]leucine. Each point represents one soleus muscle, and the rate of incorporation of ^{3}H activity is expressed as nmol/g wet weight per 30 min. The correlation line was constructed using the method of least squares. r 0·86, $P < 0.001$.

Table 1. *Effect of 1 week of potassium depletion or repletion on the in vitro incorporation of [^{3}H]leucine into muscle protein*†

(Values are means and standard errors for four to eight muscles)

	[^{3}H]leucine incorporation (nmol/g per 30 min) into protein					
	Control		K-depleted		K-depleted/repleted	
Muscle	Mean	SE	Mean	SE	Mean	SE
Soleus	81·9	1·7	57·6***	4·9	91·9**	1·1
Extensor digitorum longus	56·3	1·9	30·0***	1·6	55·4	2·1

Significantly different from control values: ** $P < 0.01$, *** $P < 0.001$.

† Rats (4 weeks old) were maintained for 1 week on K-deficient fodder (K-depleted) or on the same fodder enriched with potassium chloride to a final concentration of 260 mmol/kg (controls). The third group was K-depleted for 1 week and then K-repleted by giving free access to the K-enriched fodder for 24 h (K-depleted/repleted). Soleus and extensor digitorum longus muscles were prepared and incubated for 30 min in buffer containing [^{3}H]leucine.

In order to determine whether the exposure to K-depletion in vivo would cause an impairment of protein synthesis which could be detected in vitro, the incorporation of [^{3}H]leucine into protein was measured in soleus and EDL muscles obtained from rats which had been K-depleted for 1 week, or K-depleted for 1 week and then K-repleted for 24 h. It should be noted that in the soleus and EDL muscles prepared from 1-week K-depleted animals, the K-content following the incubation was reduced by 10 and 11% respectively compared with age-matched controls. In spite of this relatively modest decrease, the incorporation of [^{3}H]leucine into protein was inhibited by 30 and 47% in soleus and EDL respectively (Table 1). This impairment of protein synthesis could be restored by giving the animals access to fodder with normal K-content for 24 h before the muscles were prepared (Table 1).

In vivo experiments

The effect of K-deficiency on [^{3}H]leucine incorporation into proteins in vivo was assessed using two routes of administration. Table 2 shows the ^{3}H activity in skeletal muscles 10 min after intravenous or intraperitoneal injection of the same quantity of [^{3}H]leucine (200 μCi/kg body-weight). Generally, the intravenous administration gave somewhat lower and more uniform ^{3}H levels in all the tissues tested than the intraperitoneal administration. This is probably caused by the difference in specific radioactivity in the injections (2 μCi/μmol *v.* 0·2 μCi/μmol). In the experiments with intraperitoneal injection, the ^{3}H activity of diaphragm protein was higher than that of the other muscles, presumably because the [^{3}H]leucine had more ready access to this muscle. Both types of experiments showed, however, the same trend, namely that in the K-deficient animals, the incorporation of ^{3}H activity into protein was significantly reduced in skeletal muscle. Following intraperitoneal injection the incorporation of [^{3}H]leucine in the K-depleted animals into soleus, EDL and diaphragm muscles was reduced by 25, 38 and 32% respectively (Table 2). This should be compared with the values obtained following intravenous injection, where K-depletion was accompanied by a 24, 38 and 28% reduction in the same three muscles respectively (Table 2). Both types of experiment cause a considerable rise in plasma leucine and there was no significant difference between the plasma ^{3}H activity level in controls and K-deficient animals. Therefore, the inhibitory effect of K-deficiency on [^{3}H]leucine incorporation cannot be attributed to differences in specific activity.

Table 2. *Effect of potassium depletion on [^{3}H]amino acid incorporation following intraperitoneal (i.p.) or intravenous (i.v.) administration*†

(Values are means with their standard errors for six to ten animals)

	Muscle	[^{3}H]amino acid incorporation (counts/min per g wet wt)				Effect of K-depletion (%)	Statistical significance of difference: P
		Control		K-depleted			
		Mean	SE	Mean	SE		
(*a*) i.p. injection of [^{3}H]leucine	Soleus	23642	1752	17777	1244	−25	< 0·025
	EDL	14769	1201	9214	621	−38	< 0·005
	Diaphragm	55809	3352	37989	2880	−32	< 0·005
(*b*) i.v. injection of [^{3}H]leucine	Soleus	14026	678	10679	799	−24	< 0·01
	EDL	11017	491	6885	367	−38	< 0·001
	Diaphragm	13490	1010	9715	718	−28	< 0·01
(*c*) i.v. injection of [^{3}H]phenylalanine	Soleus	3007	290	2256	179	−25	< 0.05
	EDL	2210	275	1331	87	−40	< 0·001
	Diaphragm	2796	326	1795	176	−36	< 0·02

EDL, extensor digitorum longus.

† Groups of 4-week-old rats were maintained for 1 week on K-deficient fodder or the same fodder enriched with K. (*a*) Incorporation of [^{3}H]leucine into skeletal muscles 10 min after an intraperitoneal injection of [^{3}H]leucine (200 μCi/kg body-weight with 10 mmol carrier leucine/l). (*b*) Values obtained 10 min after an intravenous injection of [^{3}H]leucine (200 μCi/kg body-weight with 100 mmol carrier leucine/l). (*c*) Values obtained 10 min after an intravenous injection of [^{3}H]phenylalanine (200 μCi/kg body-weight with 150 mmol carrier phenylalanine/l).

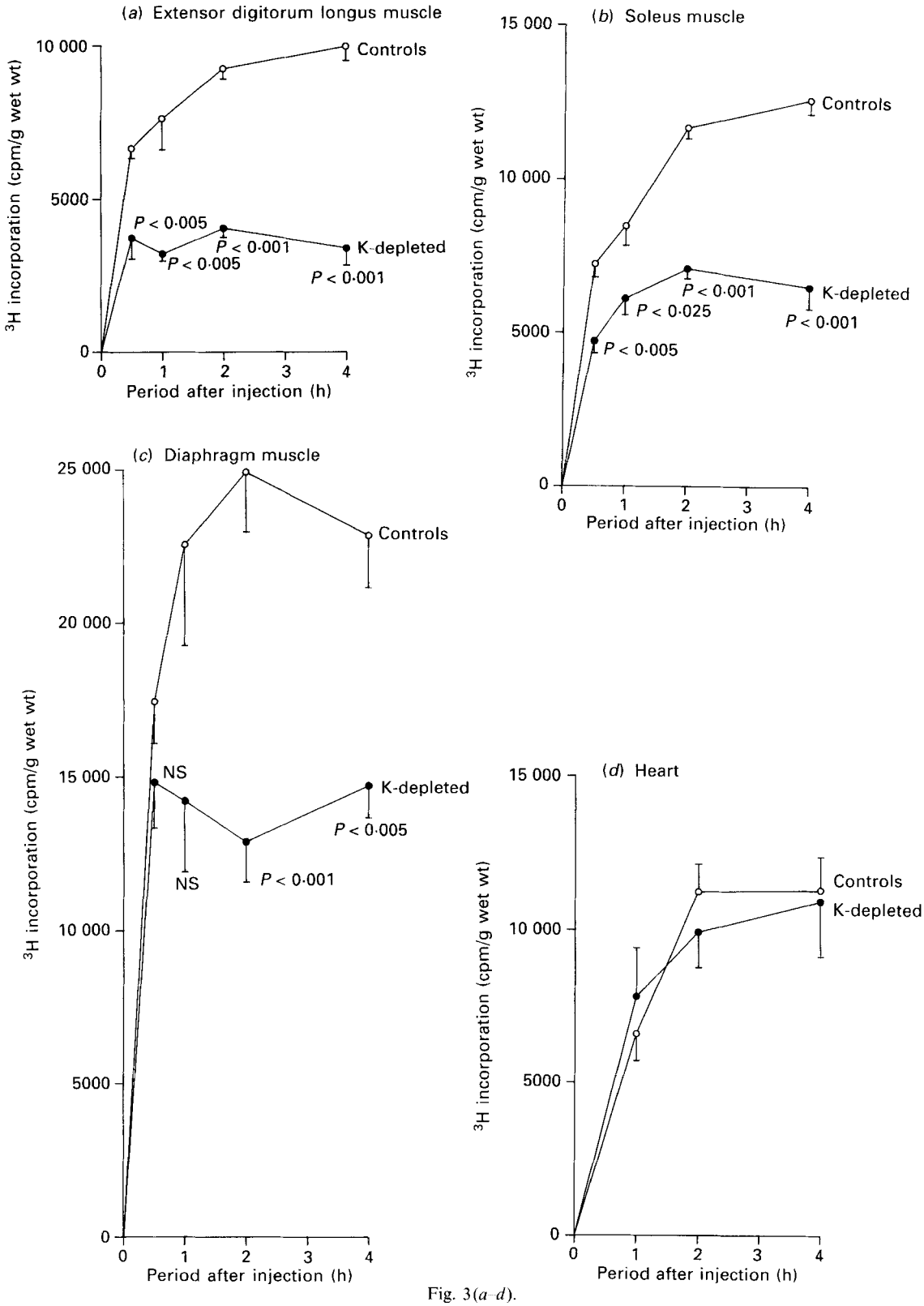

Fig. 3(*a–d*).

Furthermore, measurement of plasma leucine concentration before injection (167 μM (SE 14) in control *v.* 114 μM (SE 9) in K-depleted animals (n 6/6), $P < 0{\cdot}02$) indicates that differences in plasma leucine were not responsible for the inhibition of [^{3}H]leucine incorporation seen in K-depleted animals. Finally, the incorporation of [^{3}H]phenylalanine into protein was inhibited in K-depleted animals, confirming the general trend (Table 2).

The effect of K deficiency on the time-course of labelling was assessed in rats which had been given K-deficient fodder (1 mmol/kg) for 14 d. They were injected intraperitoneally with the same dose of [^{3}H]leucine (100 μmol and 50 μCi/kg body-weight), and it should be noted that at the various times of measurement, the TCA-soluble ^{3}H activity in plasma was not significantly different from that of the control rats. In spite of this, the ^{3}H activity of the total protein isolated from all the skeletal muscle samples was clearly reduced. Total muscle protein concentration (mg/g wet weight), however, was not reduced in the K-deficient animals.

Fig. 3(*a–d*) shows the time-course of the incorporation of [^{3}H]leucine into the proteins of various types of skeletal muscle and the heart. In all the tissues studied, the maximum incorporation level was reached in about 4 h, followed by a relatively slow decline over the next 20 h (not shown). In the diaphragm muscle, the ^{3}H activity reached a level about two-fold higher than that of the heart and hind-limb muscles, presumably because part of the [^{3}H]leucine reached the muscle fibres by diffusion directly from the peritoneal cavity.

The most pronounced effect of K deficiency was seen in EDL. As shown in Fig. 3*a*, the inhibition varied from 44% within the first 30 min to 66% over 4 h. In soleus (Fig. 3*b*), the inhibition varied between 28 and 49% over the first 4 h, whereas in diaphragm (Fig. 3*c*) between 15 and 48% reduction was found. At variance with skeletal muscle, the heart ventricles showed no significant response to K deficiency (Fig. 3*d*). In the K-deficient rats, the decrease in ^{3}H activity from 4 to 24 h in soleus, diaphragm and the heart was considerably slower than in the controls. This possibly reflects a reduced rate of protein degradation.

Since a 2-h interval allowed the detection of a clear-cut response to K deficiency, this time-period was used in the following experiments which were designed to compare the changes in K content and incorporation of leucine into protein.

In the proteins isolated from the liver and plasma, ^{3}H activity was the same in controls and K-deficient rats. Total plasma proteins were 48·3 (SE 1·0) and 52·2 (SE 2·1) g/l in controls and K-deficient rats respectively. Only the α_2-macroglobulin fraction showed a significant change with K depletion (+107%).

Fig. 4(*a–b*) shows the K content and the incorporation of [^{3}H]leucine into proteins of soleus and EDL following various periods on K-deficient fodder. After 3 d, the K content and [^{3}H]leucine incoporation in EDL were reduced by 22 and 38% respectively. In soleus, reductions of 16 and 28% were found. During the folowing 11 d, somewhat more pronounced reductions in K content and [^{3}H]leucine incorporation into skeletal muscle were observed. Again, the heart showed no significant decrease in [^{3}H]leucine incorporation, and its K content underwent a smaller reduction (17%) than that of the EDL and soleus muscles (27 and 40% respectively).

Fig. 3(*a–d*). Effects of K depletion on the incorporation of ^{3}H activity from [^{3}H]leucine into muscle protein. Groups of 4-week-old rats were either maintained on standard fodder or on K-deficient fodder for 2 weeks and then given an intraperitoneal injection (10 ml/kg body-weight) of a solution containing 154 mmol sodium chloride/l, 10 mmol L-leucine/l and 5 μCi [^{3}H]L-leucine/ml. At the indicated time-intervals after the injection, the animals were decapitated and samples of the tissues indicated excised, frozen and prepared for the isolation and determination of ^{3}H in protein. The incorporation of ^{3}H is expressed as counts/min (cpm) per g wet weight. Points are means of values obtained in six to twenty animals, with their standard errors represented by vertical bars. The significance of the difference between controls and experimental animals is indicated by P except in Fig. 3*d*, where none of the differences reached statistical significance. NS, not significant.

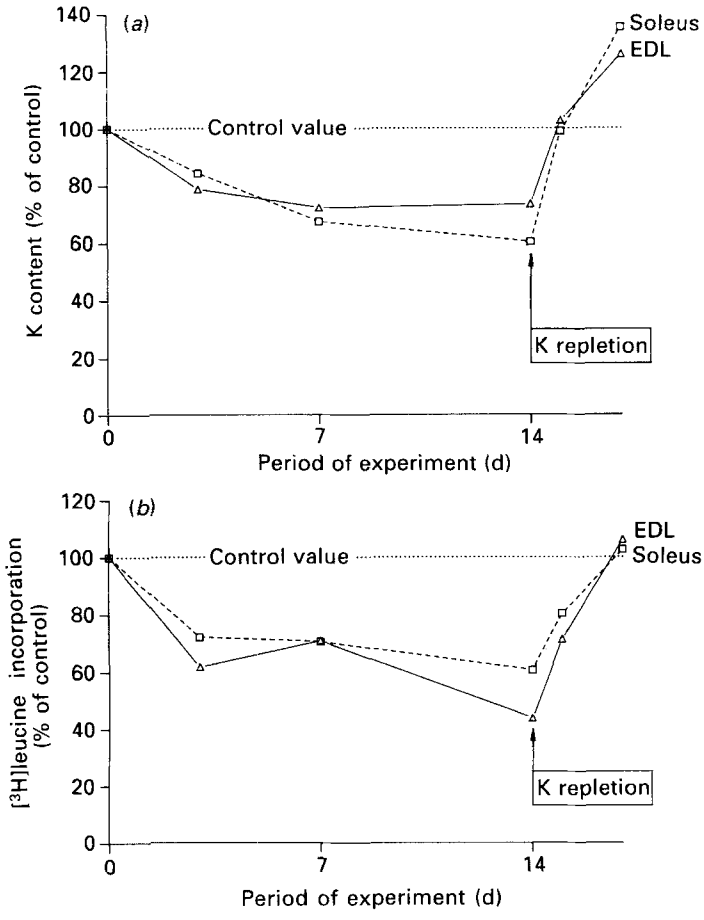

Fig. 4. Time-course of the changes (*a*) in potassium content and (*b*) the incorporation of ^3H from [^3H]leucine into muscle protein during K depletion or repletion. Rats (4 weeks old) were maintained on standard fodder or on K-deficient fodder for 2 weeks and then K-repleted (see p. 270). At the indicated time-intervals, each animal was given an intraperitoneal injection (10 ml/kg body-weight) of a solution containing 154 mmol sodium chloride/l, 10 mmol L-leucine/l and 5 μCi [^3H]L-leucine/ml. After 2 h, the animals were decapitated and soleus and extensor digitorum longus (EDL) prepared for determination of K content and the incorporation of ^3H activity into protein. All values are given as a percentage of the control value determined using the age-matched rats on standard fodder. Points are means of values determined in five to twenty animals.

This would indicate that the inhibition of [^3H]leucine incorporation was mainly related to the reduction in K content. If the impaired protein synthesis were entirely due to decreased intracellular K, it should be possible to obtain complete normalization by restoring the normal K content. In rats which had been K-depleted for 14 d and then K-repleted by the administration of KCl via stomach tube, the K content of the soleus and EDL muscles reached values even slightly above the control level within 2 h. In spite of this, the incorporation of [^3H]leucine into the protein of soleus and EDL muscles was not significantly different from that measured in age-matched 14 d K-depleted rats (5834 (SE 250) *v.* 6908 (SE 629) cpm/g in soleus and 4517 (SE 375) *v.* 4170 (SE 805) cpm/g in EDL, *n* 6). Even 24 h after this K repletion, the incorporation of [^3H]leucine into soleus and EDL was still suppressed by 20 and 29 % respectively. At 72 h after the onset of K repletion, the incorporation of [^3H]leucine reached a level slightly above the control value (Fig. 4*b*). Thus,

Table 3. *Effect of potassium depletion on weight gain and [^{3}H]leucine incorporation of rats subjected to pair-feeding for 1 week*‡

(Values are means and standard errors for six to twelve animals)

	Ad lib.-fed		Pair-fed		K-depleted	
	Mean	SE	Mean	SE	Mean	SE
(*a*) Wt gain (g)	27·4	0·7	25·1*	0·5	4·7†††	0·6
(*b*) [^{3}H]leucine incorporation (counts/min per g wet wt)						
Soleus muscle	9141	582	8667	272	7023†††	266
Extensor digitorum longus	8756	779	8585	295	5069†††	455

Significantly different from *ad lib.*-fed group: * $P < 0{\cdot}05$.
Significantly different from pair-fed group: ††† $P < 0{\cdot}001$.
‡ Groups of 4-week-old rats were given free access to K-deficient fodder and distilled water (K-depleted) or K-deficient fodder and potassium chloride (250 mmol/l) (*ad lib.*-fed controls). The third group was given the same amount of K-deficient fodder (relative to body-weight) as that consumed by the K-deficient group during the previous 24 h and given free access to KCl (250 mmol/l) (pair-fed controls). (*a*) Weight gain in 1 week, (*b*) incorporation of [^{3}H]leucine into soleus and extensor digitorum longus muscles following intraperitoneal injection.

the impaired protein synthesis could not be attributed to the cellular K deficiency as such, but also seemed to depend on some other regulatory factor.

In order to examine the possibility that the effects of K-deficient fodder might have been caused by other anomalies in composition than the lack of K, control experiments were performed using K-deficient fodder in which the K content had been normalized (to 260 mmol/kg) by the addition of KCl. It was found that the weight gain of 3-week-old rats maintained on this fodder was 85% of that of rats on standard fodder, and the [^{3}H]leucine incorporation into muscle protein showed no significant difference. After K depletion for 2 weeks, the administration of the K-enriched fodder gave the same weight gain and restoration of [^{3}H]leucine incorporation as the standard fodder used for the control rats.

It could be envisaged that the administration of K-deficient fodder would lead to reduced energy intake and ensuing impairment of growth and protein synthesis. As shown in Table 3, however, pair-feeding only caused a modest (10%) reduction in growth and no change in the incorporation of [^{3}H]leucine into muscle protein compared with *ad lib.* feeding. The same experiment showed a considerable reduction in growth (81%) and [^{3}H]leucine incorporation (19–41%) in the K-deficient animals compared with the pair-fed controls.

A more detailed analysis of the importance of muscle K content for growth and protein synthesis was performed by maintaining 4-week-old rats for 1 week on K-deficient fodder which had been K-enriched by the addition of graded amounts of KCl (1–260 mmol/kg). As can be seen from Fig. 5, fodder containing 40 and 20 mmol K/kg caused no significant change in the K content of soleus muscle, but significant reductions in body-weight gain of 15 and 26% respectively. In rats maintained on fodder containing 10 mmol K/kg the K content of soleus was only reduced by 9%, but the body-weight gain and [^{3}H]leucine incorporation had dropped by 76 and 34% respectively.

Varying the K content of fodder from 40 to 1 mmol K/kg, body-weight gain was inhibited in proportion to the fodder K content, and a significant correlation could be established (r 0·87, $P < 0{\cdot}001$). Also the [^{3}H]leucine incorporation was significantly correlated with the K content of fodder in the range 1–40 mmol/kg (r 0·78, $P < 0{\cdot}001$).

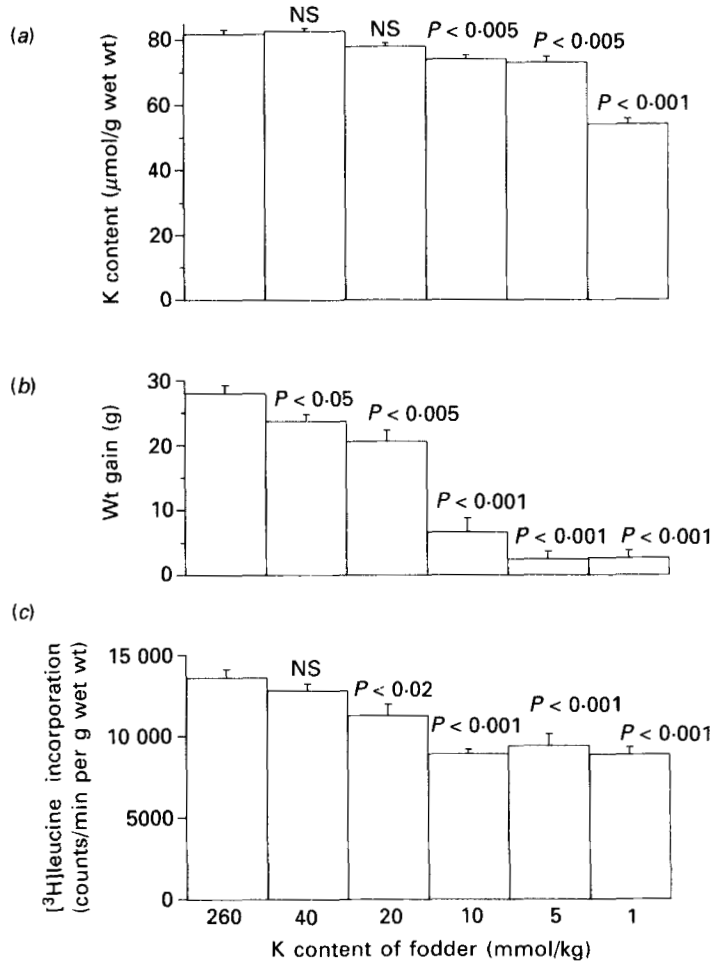

Fig. 5. Effects of graded potassium-deficient fodder on (*a*) K content, (*b*) weight gain and (*c*) incorporation of ^{3}H from [^{3}H]leucine into soleus muscle protein. Rats (4 weeks old) were maintained for 1 week on K-deficient fodder which had been enriched by the addition of graded amounts of KCl to a final content of 1–260 mmol/kg. Values are means of results obtained with six to twelve animals with their standard errors represented by vertical bars. Statistical significance levels for differences between controls (kept on fodder containing 260 mmol K/kg) and K-depleted animals are expressed by *P*. NS, not significant.

Somewhat weaker correlations were obtained between muscle K content and [^{3}H]leucine incorportion (r 0·64, $P < 0·001$) as well as between muscle K content and growth (r 0·71, $P < 0·001$).

The inhibitory effect of K depletion on leucine incorporation into protein might in part be attributed to a reduction in the intracellular accumulation via a sodium-gradient-dependent transport mechanism. The significance of this transport process was assessed in experiments with α-aminoisobutyric acid, a non-metabolized amino acid, which is accumulated in rat skeletal muscle via Na-dependent transport (Kipnis & Parrish, 1965). Measurements performed in twenty-four rats (twelve which had been K-depleted for 14 d and twelve age-matched controls) showed that 60 or 120 min after intraperitoneal injection of [^{14}C]α-aminoisobutyric acid, the ^{14}C activity accumulated in the heart ventricles,

diaphragm, soleus and EDL was not significantly reduced in the K-deficient animals compared with the controls.

DISCUSSION

The present study was primarily undertaken with the purpose of obtaining quantitative information about the K requirement for growth and protein synthesis. A second aim was to develop a simple procedure allowing the detection of protein synthesis defects under in vivo conditions.

Growth retardation

The results confirm and extend earlier observations that dietary K is essential for optimal growth (Kornberg & Endicott, 1946; Cannon *et al.* 1952; Sapir *et al.* 1976). This is perhaps most strikingly illustrated by the fact that already within the first 3 d on K-deficient fodder (1 mmol K/kg), there was virtually no weight gain (Fig. 1). Furthermore, during the first week after K repletion, weight gain was between 57 and 108% above that for age-matched controls. In spite of this early growth acceleration, normal weight was not attained until 5–7 weeks after the return to control fodder. Thus, K deficiency seems to cause a serious set-back in growth, which is not readily compensated. It would be expected that this growth retardation was mainly the result of decreased intracellular K in the muscles. However, the experiments with moderately K-deficient fodder (40–20 mmol/kg) indicate that growth is very sensitive to inadequate K supplies. Already at very modest reductions in muscle K (0–5%), there was a highly significant inhibition of weight gain (15–26%). All variables were, however, severely affected when the animals received fodder containing less than 20 mmol K/kg. This minimum dietary K level is in keeping with the studies of Kornberg & Endicott (1946), who found a dietary K concentration of 1·7 g/kg (about 40 mmol/kg) to be the minimum for optimum growth.

As body-weight of the animals decreased during graded K depletion, the wet weight of the soleus and EDL muscles showed the same relative decrease, indicating that the inhibited growth of the animals can be accounted for largely by the reduced growth of the muscles.

Earlier studies have demonstrated immediate cessation of growth when K supply was insufficient, but this has been explained by a reduction in the intracellular concentration of K (Cannon *et al.* 1952; Alexis *et al.* 1971). To our knowledge it has not previously been demonstrated that even minute reductions in muscle K could be associated with growth retardation. Taken together, the very early and marked growth responses to alterations in dietary K supplies and the highly significant correlation between K content of fodder and weight gain indicate that regulatory factors other than cellular K are of importance in determining the rate of protein turnover and weight gain.

Recent studies performed in our laboratory demonstrated that in young rats, the administration of K-deficient fodder leads to a decrease in the plasma levels of growth hormone and somatomedin C (Flyvbjerg *et al.* 1988). This reduction is related to the decrease in K content of the fodder and was shown to be fully reversible within 24 h of the return to standard fodder with normal K content (Flyvbjerg *et al.* 1988).

In vitro experiments

The rate of [^{3}H]leucine incorporation into total protein of untreated normal soleus muscles amounted to 157 (SE 7) nmol/g wet weight per h. This should be compared with the value of 140 nmol/g wet weight per h obtained by Odessey & Goldberg (1972) in the isolated rat soleus muscle at 37° using [^{14}C]leucine at a concentration of 0·1 mmol/l. The physiological plasma level of leucine is 0·1 mmol/l in the rat (Banos *et al.* 1973). Recently, Hood & Terjung (1987) found in a perfused rat hind-limb muscle preparation that the incorporation

of [^{14}C]leucine into soleus muscle amounted to 102 nmol/g per h using a leucine concentration of 0·5 mmol/l in the perfusate. As shown in Table 1, the incorporation of [^{3}H]leucine into protein was about 45% higher in soleus than in EDL muscle. This is in keeping with the difference observed in measurements of [^{3}H]phenylalanine incorporation (49%) into protein (Maltin & Harris, 1985).

Treatment with ouabain decreased the K content of muscle and a significant correlation could be demonstrated between this variable and [^{3}H]leucine incorporation. Incubation of soleus muscles for 180 min in the presence of ouabain reduced incorporation by 58%. In a similar way, Odessey & Goldberg (1972) found that incubation with ouabain for 90 min inhibited the incorporation of labelled leucine into proteins of rat diaphragm by 44%.

It has been shown previously that ouabain can inhibit the transport of certain amino acids into muscle cells. Odessey & Goldberg (1972) found, however, that leucine oxidation and accumulation into the intracellular pool were not significantly altered by incubation with ouabain. Taken together, these results indicate that the inhibition of [^{3}H]leucine incorporation into protein is induced by K depletion and that protein synthesis depends on K, confirming the observations of Alexis *et al.* (1971) and Ledbetter & Lubin (1977).

The influence of K on protein synthesis is, however, different in the in vitro and in vivo system. Reducing the K content of muscle in the vitro system by 10% reduced [^{3}H]leucine incorporation by 7%, whereas a similar reduction in the in vivo system inhibited incorporation by 34%. This much more pronounced inhibition in vivo indicates that other regulatory factors are of importance.

The inhibition of [^{3}H]leucine incorporation observed in the in vivo experiments could also be demonstrated in muscles prepared from K-deficient animals (1 week on K-deficient fodder) and incubated in Krebs–Ringer bicarbonate buffer with a normal K content (5·9 mM). In these mucles, the K content was reduced by only 10–11%, indicating that the defect in protein synthesis is maintained in spite of almost complete K repletion. The observation that the defect could only be restored by K repletion in vivo (24 h) suggests that the K content is less important than endocrine factors controlling the overall capacity for protein synthesis. Following a longer K depletion period (2 weeks), the incorporation of [^{3}H]leucine into protein in vivo was not even fully restored after 24 h of K repletion in vivo (see Fig. 4).

In vivo experiments

In the K-deficient rats, the incorporation of ^{3}H activity from [^{3}H]leucine and [^{3}H]phenylalanine into protein was clearly inhibited in all the muscles tested. As shown in Fig. 3 the relative reduction was almost the same in diaphragm, soleus and EDL 2 h after the injection of [^{3}H]leucine. If the inhibition were due to reduced absorption from the peritoneal cavity, more pronounced actions would have been detected in the hind-limb muscles, and no inhibition would have been detected in the experiments with intravenous injection. Furthermore, the incorporation of ^{3}H into the proteins of the heart, liver and plasma was relatively unaffected by K deficiency. This may be related to the fact that in the heart the K content was only reduced by 17% after 14 d on fodder containing 1 mmol K/kg. It has been shown previously that the K content of rat liver is unaffected by 4–8 weeks of K depletion (Heppel, 1939; Kjeldsen *et al.* 1984). In keeping with these observations, the total concentration of proteins in plasma was not reduced by K depletion. Gustafson *et al.* (1973) evaluated protein synthesis by the in vivo incorporation of [^{14}C]leucine into organs and muscle tissue of K-depleted rats and found only a 21% decrease in the ^{14}C activity of skeletal muscle protein (not significant). This could be attributed to the fact that the animals used were larger (200–220 g) and likely to have a slower growth rate and turnover rate of muscle proteins.

The reduced incorporation of [^{3}H]leucine into muscle tissue seen in K-depleted animals

could be due to an impaired Na-gradient-dependent transport of amino acids into the cell. However, acute K repletion by the administration of KCl via stomach tube leading to normal muscle K values (and normal transmembrane Na-gradient) caused no significant increase in leucine incorporation within the first 2 h and incomplete restoration even after 24 h. Furthermore, the accumulation of [^{14}C]α-aminoisobutyric acid, which depends on the transmembrane Na-gradient, was not reduced in the muscles and the heart of the K-depleted animals, presumably because the decrease in Na-gradient was not sufficient to bring about any major interference with [^{14}C]α-aminoisobutyric acid accumulation.

It could be argued that the amount of leucine used in the experiments with intraperitoneal injection would be inadequate to flood the precursor pools to the same extent in the controls and the K-deficient animals. When [^{3}H]leucine was given intravenously in a ten-fold larger dose, there was the same relative inhibition of the incorporation into muscle proteins in the K-deficient animals. Also the in vitro experiments indicate that the impairment of protein synthesis seen in muscles isolated from K-deficient animals cannot be attributed to reduced availability of precursor.

During sustained K depletion, the food intake declines (Alexis *et al.* 1971). This reduction is, however, small during the first week on the K-deficient diet. In further support of this view Rinehart *et al.* (1967) found that there was no significant difference between the in vivo incorporation of amino acids into muscle protein of control chicks fed *ad lib.* and those pair-fed to K-depleted chicks. The present experiments showed that control rats given the same amount of fodder as those on K-deficient fodder only had a modest reduction in body-weight and no signficant change in incorporation of [^{3}H]leucine into muscle protein.

In young chicks Rinehart *et al.* (1967) found that the incorporation of [^{14}C]leucine into a mixed skeletal muscle sample was significantly reduced when the fodder contained less than 40 mmol K/kg. However, the K content in the skeletal muscles was not measured, and inhibition of protein synthesis was attributed to reduced concentration of intracellular K.

Our experiments with graded K depletion (Fig. 5) indicate that in young rats protein synthesis responds to a similar reduction in the K content of the fodder. All variables were, however, severely affected when the animals received fodder containing less than 20 mmol/kg. The major new observation is that [^{3}H]leucine incorporation is considerably reduced already at modest reductions in cellular K content.

A clinical implication of suboptimal K supplies is accentuation of the growth inhibition seen in protein or energy malnutrition (Waterlow, 1984). Cannon *et al.* (1952) demonstrated that in protein-depleted rats the omission of K from the repletion diet caused failure to gain weight adequately, and Leach *et al.* (1959) found that optimum K supplementation gave increased growth at a certain dietary protein content. Thus a diet adequate in K optimizes the utilization of perhaps already limited supplies of dietary protein.

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