Alterations in the protein-synthesis, -degradation and/or -secretion rates in hepatic subcellular fractions of selenium-deficient mice

Rainer OTTER,* Rudolf REITER† and Albrecht WENDEL‡

Faculty of Biology, University of Konstanz, P.O. Box 5560, D-7550 Konstanz, Federal Republic of Germany

Single mouse livers were subfractionated by differential centrifugation and isopycnic centrifugation on sucrose or metrizamide gradients and separated into subcellular compartments. The fractionation procedure was highly reproducible and yielded essentially similar results in different preparations of livers from selenium-adequate (Se^+) and selenium-deficient (Se^-) mice that were fed on a *Torula*-yeast-based diet containing less than 10 parts per 10^9 of selenium for at least 16 weeks. Mice of both dietary groups were injected intraperitoneally with 370 kBq of L-[U- ^{14}C]leucine, and 48 h later 1.85 MBq of L-[4,5- ^3H]leucine was injected intraperitoneally. After another 1 h, the livers were removed and subjected to subcellular fractionation. Incorporation of the ^3H label into proteins of the subcellular fractions was taken as a measure of relative protein-synthesis rate. The ratio of the ^3H to the ^{14}C protein-bound label of the same fractions was used as an estimate of relative protein-degradation and/or -secretion rate. The results showed a statistically significant 180 % increase in protein-synthesis rate in the endoplasmic reticulum and a 80 % increase in relative protein-degradation and/or -secretion rate in the same compartment. A significant decrease in the $^3\text{H}/^{14}\text{C}$ ratio, by 40 and 30 % respectively, was observed in the Golgi fraction and in liver homogenate. The observed changes suggest a highly regulated hepatic response to selenium deficiency.

INTRODUCTION

Since its discovery as an essential trace element for mammals, biochemical work on the metabolic basis of selenium essentiality was focused on the selenoprotein glutathione peroxidase (EC 1.11.1.9). Very recently, another mammalian selenoprotein has been discovered and purified from rat plasma [1]. The function of this protein, however, is still unknown. The intriguing diversity of selenium-deficiency symptoms in different mammalian species [2] suggests that, at a molecular level, selenium deficiency might include much more complex metabolic phenomena than those which can be attributed to defects in hydroperoxide metabolism, i.e. glutathione peroxidase-dependent processes. We reported previously that prolonged dietary selenium deficiency in mice led to profound alterations in the specific activities of several hepatic enzymes related to drug metabolism [3]. These alterations were reversible after a single injection of trace amounts of selenium, and followed a common dose-dependence distinct from that for restoration of glutathione peroxidase activity [4]. These enzymic modulations were not associated with loss of other trace elements or micro-nutrients such as vitamin E, and could only be observed when the diet contained less than 10 p.p.b. (parts per billion) of selenium [5]. In a subsequent study we realized that selenium-dependent alterations were not confined to drug metabolism, but that enzymes of intermediary metabolism were similarly affected [6]. This raised the obvious question of the common denominator of these multiple bidirectional changes. We therefore investigated the hypothesis of whether protein-turnover changes might underlie these phenomena as far as they became manifest by activity changes of enzymically active proteins.

MATERIALS AND METHODS

Weanling male albino mice, strain NMRI han, from Lippische Versuchstierzucht, Extertal, Germany, were randomized and pair-fed on a *Torula*-yeast-based selenium-deficient diet [7] containing 10 p.p.b. of selenium for at least 4 months, and a control group received the same diet but supplemented with 330 p.p.b. of selenium as sodium selenite. The animals were kept on a 12 h day/night rhythm at 22–25 °C and a relative humidity of 50–60 %. The use of a double-label technique [8], followed by subcellular fractionation of mouse liver, allows the estimation of relative protein-synthesis and -degradation and/or -secretion rates in individual subcellular fractions of mouse liver. Selenium-deficient (Se^-) mice or selenium-adequate (Se^+) controls were injected intraperitoneally with 370 kBq (10 μCi) of L-[U- ^{14}C]leucine in a volume of 200 μl in the morning of day 1. For the next 2 days the mice had free access to drinking water. Food was limited to 4.3 g/day, i.e. the amount they consumed before the experiment. At 48 h after having received the ^{14}C label, the mice were anaesthetized (narcosis: 100 mg of Ketamine/kg, 16 mg of Xylazine/kg). Then 1.85 MBq (50 μCi) of iso-osmotic L-[4,5- ^3H]leucine in a volume of 100 μl was injected into the portal vein. Bleeding after withdrawal of the needle was stopped with a haemostypticum (Tabotamp; Johnson & Johnson). After labelling for 1 h *in vivo* with L-[4,5- ^3H]leucine, the animals were killed by cervical dislocation. The livers were perfused with iso-osmotic saline and taken for subcellular fractionation. All following steps were carried out at +4 °C. Homogenization was carried out in a Potter–Elvehjem homogenizer by five passes of a Teflon pestle (clearance 0.2 mm) at a speed of 400 rev./min. Starting from 7 ml of a 20 % (w/v) mouse liver

Abbreviation used: p.p.b., parts per billion (10^9).

* Present address: BASF A.G., D-6700 Ludwigshafen, Federal Republic of Germany.

† Present address: Boehringer-Mannheim G.m.b.H., Sandhofer Strasse 116, D-6800 Mannheim 31, Federal Republic of Germany.

‡ To whom correspondence and reprint requests should be addressed.

homogenate in isolation buffer consisting of 300 mM-sucrose, 0.5 mM-EDTA and 20 mM-Hepes, the liver was fractionated. The crude nuclear pellet, containing heavy cell fragments and non-disrupted tissue, was re-homogenized three times in 7 ml of 1 mM- NaHCO_3 solution. The final pellet was resuspended in 2.2 M-sucrose and adjusted to yield 7 ml of a solution in 1.66 mM-sucrose. It was overlaid with 1.5 ml portions of 1.48 M, 1.33 M- and 1.19 M-sucrose, and centrifuged at 190 000 *g* for 90 min in a TST-41 swing out rotor (du Pont) to yield the purified plasma membranes and nuclear fraction. Parallel to this procedure, the postnuclear supernatant was made up to 7 ml with isolation buffer and centrifuged for 10 min at 7500 *g* to yield a crude mitochondrial pellet and a post-mitochondrial supernatant. The latter fraction was included in the ultracentrifugation run described above to yield the final cytosolic and the crude microsomal fraction. The crude microsomal fraction as well as the washed mitochondrial pellet were then applied to a Metrizamide gradient for further purification. Each pellet was resuspended in 80 % (w/v) Metrizamide and diluted with the isolation buffer to yield 3 ml of a solution in 56.7% metrizamide. Discontinuous gradients consisting of 1.5 ml fractions of 32.82, 26.34, 24.53 and 19.78 % metrizamide followed by 1.0 ml portions of 15.0 and 10.0 % metrizamide solutions were overlaid. Separation was achieved by isopycnic centrifugation at 190 000 *g* for 180 min in a TST-41 swing-out rotor. Purified fractions of Golgi-vesicular, lysosomal, peroxisomal and endoplasmic-reticular origin were obtained from the crude microsomal fraction. From the second metrizamide gradient only the purified mitochondrial fraction was recovered. The gradients were unloaded with Pasteur pipettes, and portions of the fractions were stored at -20°C . Enzymic analysis of the fractions was carried out within 1 week after preparation. DNA content was used as a marker of nuclear material and was determined as described by Burton [9]. 5'-Nucleotidase activity [10] was taken as a marker of plasma membranes. It was assayed as release of P_i from AMP as described by Fiske & Subbarow [11]. Interfering acid phosphatase activity was inhibited by addition of 10 mM-tartrate [12]. Succinate dehydrogenase [13], catalase [14], acid phosphatase [15], NADPH:cytochrome *c* reductase in the presence of 100 μM -cyanide [16] and lactate dehydrogenase [17] served as marker enzymes of mitochondria, peroxisomes, lysosomes, endoplasmic reticulum and cytosol respectively. Interference with these assays by metrizamide was not observed. In contrast,

metrizamide strongly inhibited the Golgi marker enzyme galactosyltransferase [18]. Therefore 50 μl samples of the subcellular fractions were diluted 4-fold with isolation buffer and sedimented at 109 000 *g* for 15 min in a Beckman Airfuge before the assay. Protein was determined as described by Bradford [19] and when necessary corrected for the presence of metrizamide. A 500 μl portion of each fraction was analysed for ^{14}C and ^3H radioactivity. Protein of each fraction was collected after addition of 100 μl of bovine serum albumin (2 mg/ml) and precipitation with 250 μl of 25 % (w/v) trichloroacetic acid for 15 min on ice. The washed pellets were redissolved in 200 μl of 1 M-NaOH, except for homogenate and the microsomal fraction, which were redissolved by using a tissue solubilizer (TissueSol-Roth). In order to discolour the samples they were treated with H_2O_2 . The samples were mixed with 4 ml of scintillator (Atomlight; New England Nuclear), and radioactivity was determined 12 h later, after chemiluminescence had faded in a Beckman LS 6800 liquid-scintillation counter. Counting time was either 10 min or until a statistical error of less than 1 % was reached. Quenching effects were comparable in corresponding subcellular fractions of both dietary groups.

RNAase-free sucrose, AMP, glucosamine and UDP-D-galactose were supplied by Sigma Chemical Co., München, Germany. Metrizamide and Coomassie Brilliant Blue G-250 were from Serva, Heidelberg, Germany. Pyruvate, NADH, NADPH and 4-nitrophenyl phosphate were purchased from Boehringer Mannheim G.m.b.H., Mannheim, Germany. UDP-D-[6- ^3H]galactose (sp. radioactivity 565 GBq/mmol) was from New England Nuclear, Dreieich, Germany. L-[U- ^{14}C]Leucine (sp. radioactivity 2.04 GBq/mmol) and L-[4,5- ^3H]leucine (sp. radioactivity 2.14 GBq/mmol) were purchased from Amersham Buchler. All other chemicals used were of analytical grade.

Data were expressed as means $\pm$ s.d. and analysed by Student's *t* test; $P < 0.05$ was taken as significant.

RESULTS

The method described here allowed the rapid subcellular fractionation of a single mouse liver, yielding highly purified subcellular fractions. Conventional methods [20,12] were combined, and the resolution of the latter was extended by two additional metrizamide layers of different density. The different mouse liver organelles were well resolved under the experimental conditions, as

Table 1. Increase in specific marker activities or contents of various markers for different mouse liver cell compartments relative to the specific activity or content of these markers in mouse liver homogenate

Fraction	Marker	Increase	
		Mice . . .	Se ⁺ Se ⁻
Cytosol	Lactate dehydrogenase		3.1 $\pm$ 0.5 2.9 $\pm$ 0.1
Golgi	Galactosyltransferase		46.9 $\pm$ 1.5 46.7 $\pm$ 3.5
Nuclei	DNA content		6.3 $\pm$ 0.8 6.7 $\pm$ 2.6
Lysosomes	Acid phosphatase		66.8 $\pm$ 22.0 65.4 $\pm$ 13.6
Microsomal	NADPH: cytochrome <i>c</i> reductase		5.9 $\pm$ 1.4 5.7 $\pm$ 0.3
Mitochondria	Succinate dehydrogenase		4.9 $\pm$ 0.9 4.8 $\pm$ 0.1
Peroxisomes	Catalase		20.4 $\pm$ 2.7 16.2 $\pm$ 2.3
Plasma membrane	5'-Nucleotidase		32.3 $\pm$ 5.9 30.0 $\pm$ 4.2

Table 2. Specific marker enzyme activities, DNA content and protein yield of homogenate and purified subcellular fractions from mouse liver

Abbreviations: 5'-Nucl., 5'-nucleotidase; SDH, succinate dehydrogenase; Ac-Phos., acid phosphatase; NADPH-red., NADPH:cytochrome *c* reductase; Gal-trans., galactosyltransferase; LDH, lactate dehydrogenase; n.d., not detectable. Data are means $\pm$ s.d. ($n = 3$).

Fraction	DNA content (μ g/mg)	5'-Nucl. (munits/mg)	SDH (munits/mg)	Ac-Phos. (munits/mg)	Catalase (K/mg)	NADPH-red. (munits/mg)	Gal-trans. (munits/mg)	LDH (munits/mg)	Protein (mg/g of liver)
Homogenate	31.6 $\pm$ 1.9	8.4 $\pm$ 0.9	12.7 $\pm$ 0.7	11.7 $\pm$ 4.0	26.8 $\pm$ 2.9	29.3 $\pm$ 6.5	0.08 $\pm$ 0.01	1015.6 $\pm$ 72.3	181.0 $\pm$ 11.5
Cytosol	n.d.	1.8 $\pm$ 0.3	1.2 $\pm$ 0.1	4.8 $\pm$ 0.4	8.3 $\pm$ 1.6	1.3 $\pm$ 0.6	n.d.	3163.8 $\pm$ 480.2	67.5 $\pm$ 0.3
Golgi vesicles	n.d.	3.1 $\pm$ 6.4	2.0 $\pm$ 2.8	30.8 $\pm$ 3.6	7.1 $\pm$ 1.2	6.6 $\pm$ 1.1	3.59 $\pm$ 0.16	n.d.	0.1 $\pm$ 0.0
Nuclei	198.8 $\pm$ 19.2	12.5 $\pm$ 2.3	6.3 $\pm$ 0.2	10.2 $\pm$ 3.9	5.6 $\pm$ 2.8	3.5 $\pm$ 0.6	0.06 $\pm$ 0.03	55.3 $\pm$ 21.5	2.7 $\pm$ 0.7
Lysosomes	n.d.	12.6 $\pm$ 1.6	4.8 $\pm$ 1.5	702.0 $\pm$ 58.2	9.9 $\pm$ 1.9	25.4 $\pm$ 1.8	0.52 $\pm$ 0.25	30.7 $\pm$ 17.7	0.5 $\pm$ 0.1
Microsomes	n.d.	4.8 $\pm$ 2.8	6.2 $\pm$ 0.2	34.4 $\pm$ 3.7	13.0 $\pm$ 1.5	163.6 $\pm$ 6.5	0.12 $\pm$ 0.03	5.9 $\pm$ 1.9	10.4 $\pm$ 0.8
Mitochondria	n.d.	3.3 $\pm$ 0.2	62.1 $\pm$ 8.0	33.8 $\pm$ 7.7	38.3 $\pm$ 22.7	7.7 $\pm$ 11.2	0.03 $\pm$ 0.01	13.8 $\pm$ 1.4	9.2 $\pm$ 0.2
Peroxisomes	n.d.	4.6 $\pm$ 1.6	5.1 $\pm$ 1.9	7.7 $\pm$ 1.4	543.9 $\pm$ 70.5	1.2 $\pm$ 0.1	n.d.	5.6 $\pm$ 3.7	2.7 $\pm$ 0.8
Plasma membranes	n.d.	266.5 $\pm$ 24.0	19.3 $\pm$ 0.5	40.4 $\pm$ 15.5	3.9 $\pm$ 1.8	1.0 $\pm$ 1.1	0.16 $\pm$ 0.03	42.3 $\pm$ 15.8	0.3 $\pm$ 0.1

judged by established marker enzymes (Table 1). Specific cross-contaminations, expressed as % residual marker-enzyme activity in the contaminated fraction relative to the maximal enzyme activity in the highly purified subcellular fraction from which the enzyme originated, were greatly diminished. The specific cross-contaminations of the fractions were below 10%, except for the Golgi and the lysosomal fractions. In the Golgi fraction a considerable overlap of galactosyltransferase and 5'-nucleotidase activity occurred. Galactosyltransferase and NADPH:cytochrome *c* reductase activity were the major contaminants of the lysosomal fraction. However, these contaminations did not exceed 15% of the specific enzyme activities found in the plasma membranes, Golgi or microsomal fraction respectively. All purified fractions were recovered in sufficient quantities for biochemical analysis, with an overall yield of about 50% of total liver protein. Recoveries of the purified Golgi, lysosomal and plasma-membrane fractions ranged from 0.1 to 0.5 mg of protein per fraction, starting from 180 mg of total liver protein (Table 2). Therefore this fractionation method seems specially suitable for analytical purposes which need to be quickly performed. This complete cell fractionation of single mouse livers was now used to study selenium-dependent changes in protein turnover. We had previously reported that animals in this nutritional state are characterized by multiple enzyme modulations affecting drug metabolism [3-5] as well as intermediary metabolism [6,21]. A prerequisite to applying this fractionation technique was that the recovery as well as the purity of the individual fractions must not be influenced by the nutritional status of the animals. Data in Table 1 demonstrate that this condition was satisfied. In the short-term labelling experiments with [3 H]leucine, significantly more protein-bound radioactivity was found only in the microsomal fraction (Table 3). The smaller increase in this parameter in mitochondria was statistically insignificant. All other fractions showed essentially no difference between the amount of [3 H]leucine incorporated into liver protein of Se $^-$ or Se $^+$ animals. These results indicate that the relative

Table 3. Estimation of the cumulative relative protein-synthesis rates (c.p.m./mg of protein) in different liver compartments from selenium-deficient and -adequate mice *in vivo* after labelling by [3 H]leucine

Data were obtained 1 h after intraportal administration of 1.85 MBq of L-[4,5- 3 H]leucine. Results are means $\pm$ s.d. ($n = 4$ for Se $^+$ and 3 for Se $^-$): ** $P \leq 0.01$.

Fraction	Mice ...	Cumulative protein-synthesis rate (c.p.m./mg)	
		Se $^+$	Se $^-$
Homogenate		39 324 $\pm$ 8276	40 981 $\pm$ 5218
Cytosol		10 919 $\pm$ 2881	9902 $\pm$ 5302
Golgi		124 984 $\pm$ 5224	113 699 $\pm$ 27 946
Nuclei		22 996 $\pm$ 7390	18 705 $\pm$ 7298
Lysosomes		55 703 $\pm$ 9190	52 441 $\pm$ 8543
Microsomal		22 181 $\pm$ 13 522	62 573 $\pm$ 7688**
Mitochondria		15 958 $\pm$ 2761	23 111 $\pm$ 4784
Peroxisomes		9163 $\pm$ 2228	9275 $\pm$ 2009
Plasma membrane		23 187 $\pm$ 4788	24 711 $\pm$ 12 355

Table 4. Estimation of protein-bound [^{14}C]leucine (c.p.m./mg of protein) in different liver compartments from selenium-deficient and -adequate mice 49 h after *in vivo* labelling with an intraperitoneal injection of 370 kBq of L-[U- ^{14}C]leucine

Results are means $\pm$ s.d. ($n = 4$ for Se^+ and 3 for Se^-):
* $P \leq 0.05$.

Fraction	Mice...	Protein-bound [^{14}C]leucine (c.p.m./mg)	
		Se^+	Se^-
Homogenate		3273 $\pm$ 188	5128 $\pm$ 1215*
Cytosol		1213 $\pm$ 126	1841 $\pm$ 291*
Golgi		1864 $\pm$ 124	2351 $\pm$ 399
Nuclei		1796 $\pm$ 128	2265 $\pm$ 828
Lysosomes		2620 $\pm$ 182	2906 $\pm$ 671
Microsomal		3641 $\pm$ 598	4656 $\pm$ 438
Mitochondria		1503 $\pm$ 276	2768 $\pm$ 453*
Peroxisomes		713 $\pm$ 158	812 $\pm$ 405
Plasma membrane		3272 $\pm$ 542	3684 $\pm$ 1101

Table 5. $^3\text{H}/^{14}\text{C}$ ratio of protein-bound radioactivity derived from [^3H]leucine (after 1 h) or [^{14}C]leucine (after 49 h) incorporation into liver proteins of selenium-deficient and -adequate mice

Results are means $\pm$ s.d. ($n = 4$ for Se^+ and 3 for Se^-):
* $P \leq 0.05$.

Fraction	Mice...	$^3\text{H}/^{14}\text{C}$ ratio	
		Se^+	Se^-
Homogenate		12.1 $\pm$ 2.5	8.1 $\pm$ 0.8*
Cytosol		8.9 $\pm$ 2.5	5.5 $\pm$ 2.4
Golgi		68.4 $\pm$ 3.0	48.1 $\pm$ 8.2*
Nuclei		12.6 $\pm$ 4.4	8.0 $\pm$ 1.7
Lysosomes		22.0 $\pm$ 3.1	18.4 $\pm$ 2.2
Microsomal		7.6 $\pm$ 3.2	13.5 $\pm$ 2.1*
Mitochondria		11.6 $\pm$ 0.8	8.5 $\pm$ 2.0
Peroxisomes		13.9 $\pm$ 3.0	12.5 $\pm$ 2.8
Plasma membrane		6.8 $\pm$ 1.9	6.7 $\pm$ 1.0

protein-synthesis rate is increased in the endoplasmic reticulum of Se^- mouse liver. In the long-term-labelling experiments with [^{14}C]leucine, a significant increase in protein-bound radioactivity was observed in homogenate and the cytosolic fraction as well as in mitochondria (Table 4). These data suggest that decreases in the relative rates of protein degradation and/or secretion had occurred in these compartments. The most informative parameter, however, is the ratio of protein labelling by ^3H and ^{14}C (Table 5). The data indicate significant changes in Golgi fraction, most prominent changes in the microsomal fraction and the cumulative reflection of these events in the homogenate. The observed changes in protein synthesis and degradation and/or secretion were then expressed relative to the conditions in control livers in Fig. 1, which demonstrates significant prominent changes in protein synthesis as well as degradation and/or secretion in the endoplasmic reticulum of Se^- mouse

Fig. 1. Relative rates of protein synthesis (a) and degradation and/or secretion (b) in selenium-deficient mouse liver subcellular fractions as compared with selenium-adequate controls (= 100 %)

The maximum statistical error of the calculated data in the synthesis panel (a) for a single compartment was $\pm 50\%$ (s.d.) for fraction N, and the mean error over all fractions was $\pm 26\%$. In the degradation and/or secretion panel (b) the greatest statistical error was 30% for fraction C, with a mean error of $\pm 19\%$ (* $P \leq 0.05$, $n = 3$). Subcellular fractions: H, homogenate; C, cytosol; G, Golgi apparatus; N, nuclei; L, lysosomes; ER, endoplasmic reticulum; M, mitochondria; P, peroxisomes; PM, plasma membranes.

liver. As shown in Table 4, an enhanced relative degradation and/or secretion rate is also observed in the Golgi fraction and in homogenate. These results show that, although the total protein content of mouse liver shows no significant changes in selenium deficiency, different organelles were subject to large alterations in hepatic protein synthesis, degradation or secretion, or a combination of these processes.

DISCUSSION

The integral method described here for the fractionation of single mouse livers on an analytical scale provided sufficient material and reproducibility to apply it to experimental approaches to the study of endogenous or exogenous influences on distribution of compounds or their metabolic fate in liver cell compartments.

Using an established double-labelling method [8], we compared relative protein-synthesis and -degradation and/or -secretion rates in selenium-deficient and -adequate mice in order to assess possible selenium-dependent changes on a subcellular level. Alterations were evident

already in the homogenate. As shown in Fig. 1, the relative protein-synthesis rate was only increased marginally, while at the same time the relative degradation and/or secretion rate was decreased by 30%. As the estimates of both rates were derived from different protein populations and pool sizes, which are hardly representative of total hepatic protein, quantitative changes expressed as mg of protein/g wet wt. of liver cannot be extrapolated. A large change in the synthesis or degradation and/or secretion rate of a small protein pool may readily be compensated by a comparatively small change in a major pool to give an undetectable overall effect. Our finding that total hepatic protein content was independent of the selenium status of mice is not contradictory to the observed changes when the possibility is considered of quantitative changes in hepatic protein composition and/or an altered hepatic secretion during deficiency.

The most pronounced effect in our experiments was observed in the microsomal fraction. Within the endoplasmic-reticular compartment, which is responsible for the synthesis and the discharge of proteins, the ^3H incorporation rate was much increased, while simultaneously the $^3\text{H}/^{14}\text{C}$ ratio was also significantly increased in selenium-deficient mouse liver. This means that in selenium deficiency an increased flow of protein through the endoplasmic reticulum occurs. Two explanations seem conceivable. (1) The changes could reflect alterations in the synthesis and degradation rates of proteins of the endoplasmic reticulum. The previously reported selenium-dependent modulations of certain enzyme activities related to microsomal drug metabolism [3–5], as well as selenium-responsive defects in haem utilization [22], could be explained by such a mechanism. A report describing an altered expression of cytosolic glutathione *S*-transferase subunits in selenium-deficient rat liver supports this view [23]. Furthermore the defect in synthesis of selenoproteins during deficiency might contribute to an increased protein turnover. Selenocysteine is coded for by the nucleotide triplet TGA [24], which was formerly regarded as a stop codon. During deficiency this could lead to the enhanced discharge of immature polypeptide fragments of selenoproteins, which are rapidly degraded. (2) Alternatively, changes in the translocation rates of hepatic proteins into other cell compartments or into the bloodstream must be considered. A differentiation between the two alternatives is beyond the scope of these experiments.

Besides the endoplasmic reticulum, the Golgi apparatus was the only cell compartment investigated that was significantly affected by selenium deficiency. A roughly 30% decrease in relative protein-degradation and/or -secretion rate without any significant alteration in the corresponding synthesis parameter was found in this compartment. These changes might reflect a slowing-down in newly synthesized integral proteins of the Golgi stack. However, effects on proteins processed and distributed by this cell organelle must also be considered.

Our data demonstrate changes in subcellular compartments which are competent for the synthesis and/or the distribution of proteins. However, the absence of significant alterations in other cell compartments does not exclude the possibility that the fate of individual proteins in these compartments was also affected.

The basic question as to the cause of the increase in both the endoplasmic-reticulum protein synthesis rate

and the rate of transfer of proteins from this cell organelle and/or their degradation during selenium deficiency remains to be answered. Two possible rationales may be provided by the following considerations: our results strongly suggest that the observed changes are not a general effect in the organ, but are rather specific for certain cell compartments. Thus these changes may reflect a successful adaptation of the liver to a deficiency equilibrium in order to counteract harmful effects of deficiency or to ensure unimpaired metabolic support of other tissues. Indeed, it has been shown that during deficiency a redistribution of selenium from the liver towards other tissues [25] takes place. In agreement with this explanation, we found previously an essentially normal plasma protein profile [26] and urinary metabolite pattern [6], but no evidence of hepatic damage in selenium-deficient mice. It is recalled that 'dietary liver necrosis' was found only in triply deficient rats, i.e. lacking dietary vitamin E, sulphur-containing amino acids and selenium [27].

A second hypothesis is based on the assumption that the decreased $^3\text{H}/^{14}\text{C}$ ratio is due to an enhanced protein degradation. The lack of selenium-dependent glutathione peroxidase activities may lead to less hydroperoxide reduction. An enhanced steady state of reactive oxygen species, a situation which is hardly analytically detectable *in vivo*, would also result in a faster oxidation of susceptible amino acids in proteins. In model systems it has been shown for several proteins that oxidation of a single amino acid can trigger the attack of proteinases, resulting in an instantaneous degradation of this protein [28,29]. Thus an increase in the turnover of susceptible proteins may occur under certain conditions.

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