British Journal of Nutrition (1987), **57**, 319–329

Relative nutritional availability to rats of selenium in Finnish spring wheat (*Triticum aestivum* L.) fertilized or sprayed with sodium selenate and in an American winter bread wheat naturally high in Se*

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(*Received 4 March 1986 – Accepted 15 December 1986*)

1. A Finnish national programme to fertilize crops with sodium selenate led us to compare the nutritional availability to rats of selenium in two Finnish spring wheats (*Triticum aestivum* L.), either fertilized or sprayed with sodium selenate, with that in an American winter bread wheat naturally high in Se.

2. Weanling male rats were given a Se-deficient Torula yeast diet for 4 weeks followed by either continued depletion or repletion for 4 weeks with graded levels of Se as sodium selenite (standard) or wheat (test food). Plasma and liver Se levels and plasma and liver glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) activities were used as criteria of body Se status.

3. The availability of Se under these conditions was calculated with the point-slope technique at two dietary levels of Se (Expt 1) and with the slope-ratio method (Expt 2).

4. In the point-slope assay, the level of dietary Se fed had a considerable effect on the apparent availability values obtained which made interpretation of the results difficult. In the slope-ratio assay, no difference in the availability of Se from the various wheats was observed when plasma or liver Se levels were used as the response criteria.

5. The Se in the fertilized wheat was somewhat more available than that in the sprayed wheat when plasma or liver GSH-Px activities were the response criteria. Overall, availability values (%) derived by averaging all four response criteria were 86, 77 and 73 for the fertilized and sprayed Finnish wheats and the American wheat respectively (sodium selenite 100).

6. These results show that wheat is a relatively available source of Se to rats regardless of whether its Se content is naturally high or is increased by fertilization or spraying.

The selenium content of wheat in different countries varies considerably depending on the availability of Se in the soil to plants (Varo & Koivistoinen, 1981). Under the reducing conditions of Finnish soil, Se is taken up poorly by plants and consequently the Se content of domestic grain is very low. This has led to very-low dietary Se intakes in the Finnish population. However, in some years wheat has been shown to contribute as much as half the total daily Se intake in Finland because of the importation of high-Se grains (Mutanen & Koivistionen, 1983). It has also been established that the availability to rats (Douglass *et al.* 1981; Alexander *et al.* 1983) and humans (Levander *et al.* 1983) of the naturally occurring Se in wheat is good compared with that in sodium selenite or selenate. For the

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Table 1. *Composition of the diets (g/kg diet)**

Expt. no....	1		2		
Selenium group...	1a§	1b§	2a‖	2b‖	2c‖
Torula yeast of basal diet	300	300	300	300	300
Wheat supplements					
Finnish fertilized spring wheat +low-Se Finnish wheat	14·5+0	29·0+0	7·2+7·3	10·9+3·6	14·5+0
Finnish sprayed spring wheat +low-Se Finnish wheat	26·3+0	52·6+0	13·1+13·2	19·7+6·8	26·3+0
American winter bread wheat +low-Se Finnish wheat	20·8+0	41·6+0	10·4+10·4	15·6+5·2	28·3+0
Salt mix†	35	35	35	35	35
Vitamin mix‡	10	10	10	10	10
Maize oil	50	50	50	50	50
Sucrose	To total 1000				

* All diets contained (/kg diet) 100 mg vitamin E as DL-α-tocopheryl acetate, 3 g DL-methionine and 2·25 g choline.

† Williams-Briggs Salt Mix; Teklad Test Diets, Harlan Industries, Inc., Madison, WI, USA. The salt mix was adjusted to contain graded levels of Se as Na_2SeO_3 in the standard diets.

‡ Supplied (mg/kg diet): thiamin hydrochloride 3·75, riboflavin 7·5, pyridoxine hydrochloride 3·6, niacin 45, Ca pantothenate 24, cyanocobalamin 0·015, menandione 0·3, folic acid 0·5, biotin 0·2, retinyl palmitate 6·6, ergocalciferol 0·025.

§ The analysed Se concentrations for different Se levels of the diets are shown in Table 2.

‖ The analysed Se concentrations for different Se levels of the diets are shown in Table 3.

previously-stated reasons, special attention has been given to raising the Se content of domestic wheat in order to increase the dietary Se intake in Finland. Consequently, in 1984, a national programme of fertilization of grains up to a level of 100 μg Se/kg with sodium selenate was initiated by a resolution of the Ministry of Agriculture and Forestry in Finland (Koivistoinen & Huttunen, 1986).

The purpose of the study presented here was to compare the nutritional availability of Se in Finnish spring wheat (*Triticum aestivum* L.), fertilized or sprayed with sodium selenate, to that in an American winter bread wheat naturally high in Se. Since Se availability may depend on the level of inclusion in the diet (Cantor *et al.* 1975; Mutanen *et al.* 1986), a second aim of the study was to compare two methods, the point-slope technique at two dietary levels of Se and the slope-ratio assay, in assessing the availability of Se in the three different wheats.

MATERIALS AND METHODS

In all experiments, weanling male Fischer 344 rats were housed in pairs in hanging stainless-steel-wire cages. Diet and water were provided *ad lib.* and rats were weighed weekly throughout the experiments. In all experiments, rats were first given a Se-deficient, 300 g Torula yeast/kg basal diet (Table 1) for 4 weeks. The basal diet contained 4 μg Se/kg as determined by the fluorometric method (Hoffman *et al.* 1968). During this period, the rats were brought to the same baseline Se status. The three wheats studied were two Finnish wheats which had their Se content increased either by fertilization or spraying with sodium selenate and an American winter bread wheat naturally high in Se. The fertilization was done with normal granule NPK before sowing and the spraying was done at a plant height

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of 100–150 mm under natural field conditions and following normal cultivation practice. The rate of application was 14 g Se/ha for fertilization and 30 g Se/ha for spraying. The wheats were ground to whole-wheat flour before mixing into the diets. The flour samples were analysed to contain (μg Se/g) 5·7 for American wheat, 5·7 for fertilized and 3·4 for sprayed wheat. The low Se Finnish wheat contained 0·02 μg Se/g. Sodium selenite was used as the availability standard. The diets were composed to contain graded levels of Se. The analysed Se concentrations of the diets are given in Table 2 (p. 322) for Expt 1 and in Table 3 (p. 326) for Expt 2. In Expts 1 and 2, fluorometric (Hoffman *et al.* 1968) and gas–liquid chromatographic–mass spectrometric (Reamer & Veillon, 1981) methods were used to analyse tissue Se levels respectively. The handling of plasma and liver samples as well as the glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) assay conditions have been described in detail previously (Mutanen *et al.* 1986). The substrate was 0·30 mM-*tert*-butyl-hydroperoxide. GSH-Px activity is expressed as m units/mg protein, that is nmol NADPH oxidized/min per mg protein.

The significance of differences in body-weight was determined by *t* test (Finney, 1971). Differences in the slopes of the linear regression lines (slope-ratio assays, Expt 2) were tested by pairs for significance using the contrast test (Finney, 1971).

Expt 1. This experiment was conducted to study the effect of two different dietary Se levels on the availability values as estimated by the point-slope technique. After the 4-week depletion period, rats either continued on the basal diet or were given the basal diet supplemented with graded levels of sodium selenite or with two levels of Se as different wheats. The analysed Se contents of the test diets are shown in Table 2 (p. 322). After a 4-week repletion period, the rats were killed.

The availability of Se was assessed by the ability of Se to restore liver and plasma Se levels and liver and plasma GSH-Px activities. GSH-Px activity was chosen as a response criterion because it is thought to represent the biologically active form of Se in the body (Levander, 1983). In this experiment, the availability of Se in the test diet was calculated with the point-slope technique (Cantor *et al.* 1975) as a percentage of the response generated by the test food (wheat) compared with that of the standard (selenite). In these calculations, the standard curves were smoothed by linear regression and the regression equation was used to calculate availability percentages.

Expt 2. In this experiment, the availability of Se in different wheats was assessed by the slope-ratio method (Finney, 1971). The slope of the regression line generated by the dose–response relation of the test food (wheat) is divided by the slope of the regression line generated by the standard (selenite). The valid use of the slope-ratio method demands a linear dose-response over the range of dietary Se levels tested. For this reason, a preliminary experiment was conducted to test assay validity using plasma and liver GSH-Px activities as response criteria when dietary Se was varied. After 4 weeks of depletion, rats were divided into groups that continued with the unsupplemented basal diet or were given diets with 25, 50, 75 or 100 μg Se as sodium selenite/kg for an additional 2, 4 or 6 weeks. The handling of samples and the assay procedures were as described in Expt 1. To test the relation between GSH-Px activity and dietary Se level after 2, 4 and 6 weeks of repletion, the correlation coefficient was calculated.

The procedure of the second experiment utilized the results of the preliminary experiment. The study design was as in Expt 1 with the following changes. During repletion, both the standard and the test foods were given for 4 weeks at three different levels of Se. The standard diets were adjusted to contain 50, 75 and 100 μg Se/kg. The Se content of the test diets was analysed (Table 3, p. 326) and these values were used to calculate the Se availability. The total amount of wheat in the different test diets was kept constant for a given wheat by adding a low-Se Finnish wheat (20 μg Se/kg) to adjust the final Se level

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Table 2. *Expt 1. Effect of dietary selenium source on glutathione peroxidase* (EC *1.11.1.9; GSH-Px*) *activity and Se level in plasma and liver of Se-depleted rats according to point-slope assay*

(Mean values with their standard errors for five samples)

Diet	Se-content of the diet (μg/kg)	GSH-Px activity (munits/mg protein)				Se content			
		Plasma		Liver		Plasma (ng/ml)		Liver (ng/g)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Basal	4	2	0	48	2	12	1	5	1
Basal + sodium selenite	35	81	6	212	8	244	17	114	25
	85	123	9	1247	363	365	5	324	27
	167	188	13	1905	284	446	11	496	28
Finnish fertilized spring wheat	96(1a)*	163	4	1021	237	368	20	269	12
	194(1b)	165	10	1687	10	456	23	530	14
Finnish sprayed spring wheat	101(1a)	116	10	1242	173	324	21	267	27
	174(1b)	150	5	3191	298	455	14	551	39
American winter bread wheat	142(1a)	131	3	1125	156	402	8	425	46
	238(1b)	165	6	3135	488	462	10	645	18

* Se groups; for details, see Table 1.

(Table 1). To calculate the relative availability with the slope-ratio method, the regression lines were generated using the following equation (Finney 1971):

$$y = \alpha + \beta_1 X_{ST} + \beta_2 X_A + \beta_3 X_F + \beta_4 X_S,$$

where X_{ST}, X_A, X_F and X_S are the variables representing the dose values of the standard, American wheat, fertilized wheat and sprayed wheat groups respectively (0 elsewhere). The equation shows that the dose–response relation may be expressed as a multiple-regression equation. The differences between the regression lines were further tested with the regression analysis by fitting a single line for the contiguous groups (significance was tested with F test). Continuing the combining process the result model was chosen where the wheat groups could no longer be combined. The result model is the equation where one regression line represents either one wheat group or certain wheat groups together.

RESULTS

In Se availability studies, the growth rate of the animals may bias the results obtained from groups growing at different rates (Combs *et al.* 1984). Body-weights of the rats did not differ significantly in Expts 1 and 2 and thus could not influence any of the results obtained.

Expt 1. Plasma and liver Se levels as well as GSH-Px activities of rats given different wheat Se sources at two dietary levels of Se are shown in Table 2. The values for plasma and liver Se levels and plasma GSH-Px activity for the different wheat diets closely resembled those for the standard diet. However, in the case of liver GSH-Px activity, of special interest is the greater ability of Se, either in natural American wheat or in Finnish wheat sprayed with sodium selenate, to increase liver GSH-Px activity at the higher dietary level of Se compared with that at the lower dietary level of Se.

Expt 2. The purpose of the preliminary experiment was to determine the experimental conditions under which the GSH-Px activity in rat plasma and liver would be a linear

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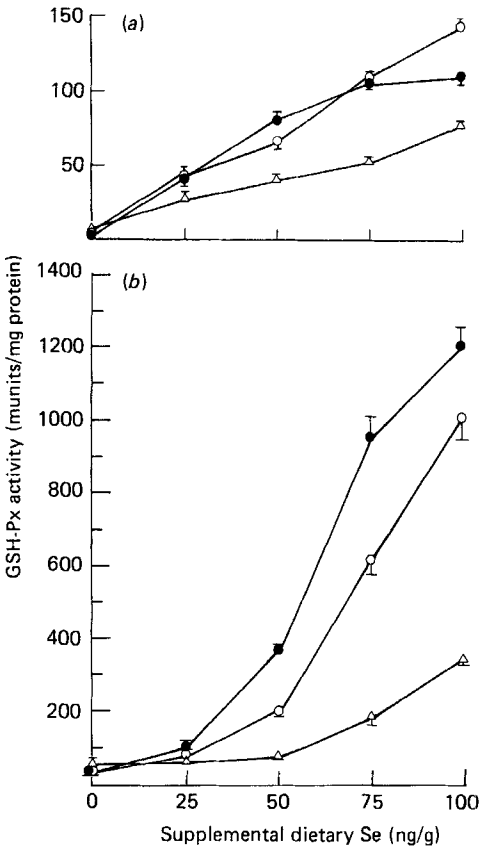

Fig. 1. Effect of length of repletion on gluthathione peroxidase (*EC* 1.11.1.9; GSH-Px; munits/mg protein) activity in (*a*) plasma and (*b*) liver of rats given graded levels of sodium selenite. Weanling male rats were given the selenium-deficient basal diet for 4 weeks and then repleted with the basal diet supplemented with various levels of sodium selenite for 2 (△), 4 (○) or 6 (●) weeks. Points are means, with their standard errors represented by vertical bars, for four or five rats.

function of Se in the diet. As shown in Fig. 1(*a*), the increase in plasma GSH-Px activity was dose-related to the Se level in the diet after various periods of repletion. Correlation coefficients for three periods of repletion were 0·96, 1·00 and 0·97 which indicate that the values for plasma were linear. The corresponding intercepts were $y = 22$, 3 and 12 (range of the observations 3–140) showing that another prerequisite, a common intercept, for the analysis was satisfactorily fulfilled. Due to the lower responses at lower dietary levels of Se as compared with those at higher Se levels, the linearity in liver GSH-Px activity over the range of supplementation was not as good as in plasma (Fig. 1(*b*)). The correlation coefficients and corresponding intercepts for liver values were r 0·85, y −130; r 0·94, y −204; r 0·98, y −247. In order to have a common positive intercept and to linearize the liver values the analysis was also done on a log scale. The correlation coefficients and intercepts for different periods of repletion were then r 0·96, y 2; r 1·00, y 1·9; r 0·98, y 1·9. Since the plasma GSH-Px activity had reached equilibrium by 4 weeks, that period was chosen to study the availability of Se with the slope-ratio technique in Expt 2. Exclusion of the 25 μg Se/kg level did not change the regression equation calculated and therefore only the three highest levels of supplementation were used in Expt 2.

Linear responses were found in plasma Se levels and plasma GSH-Px activities when

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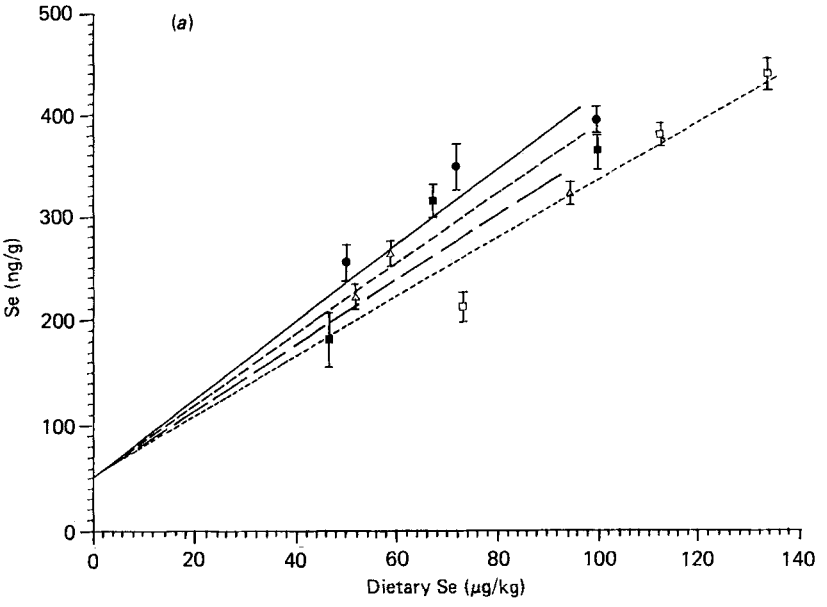

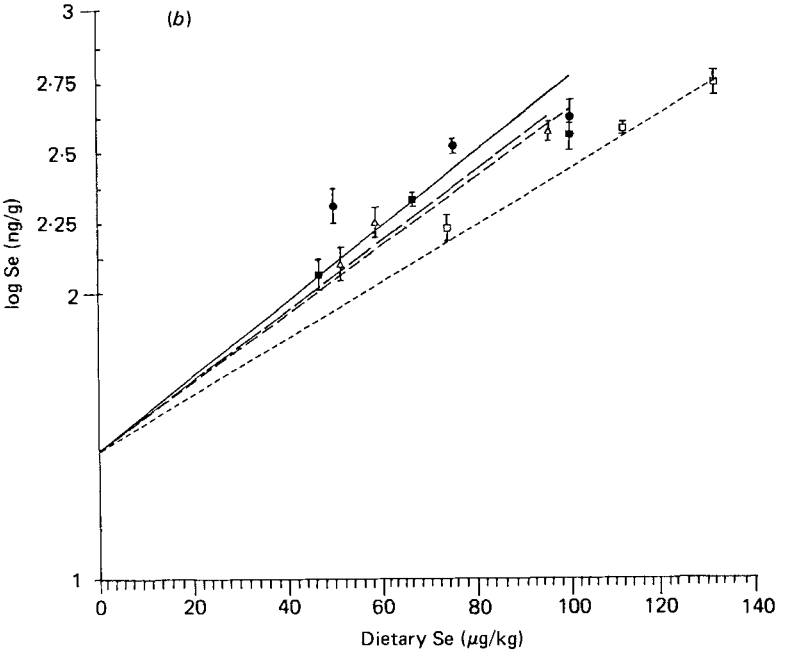

Fig. 2.

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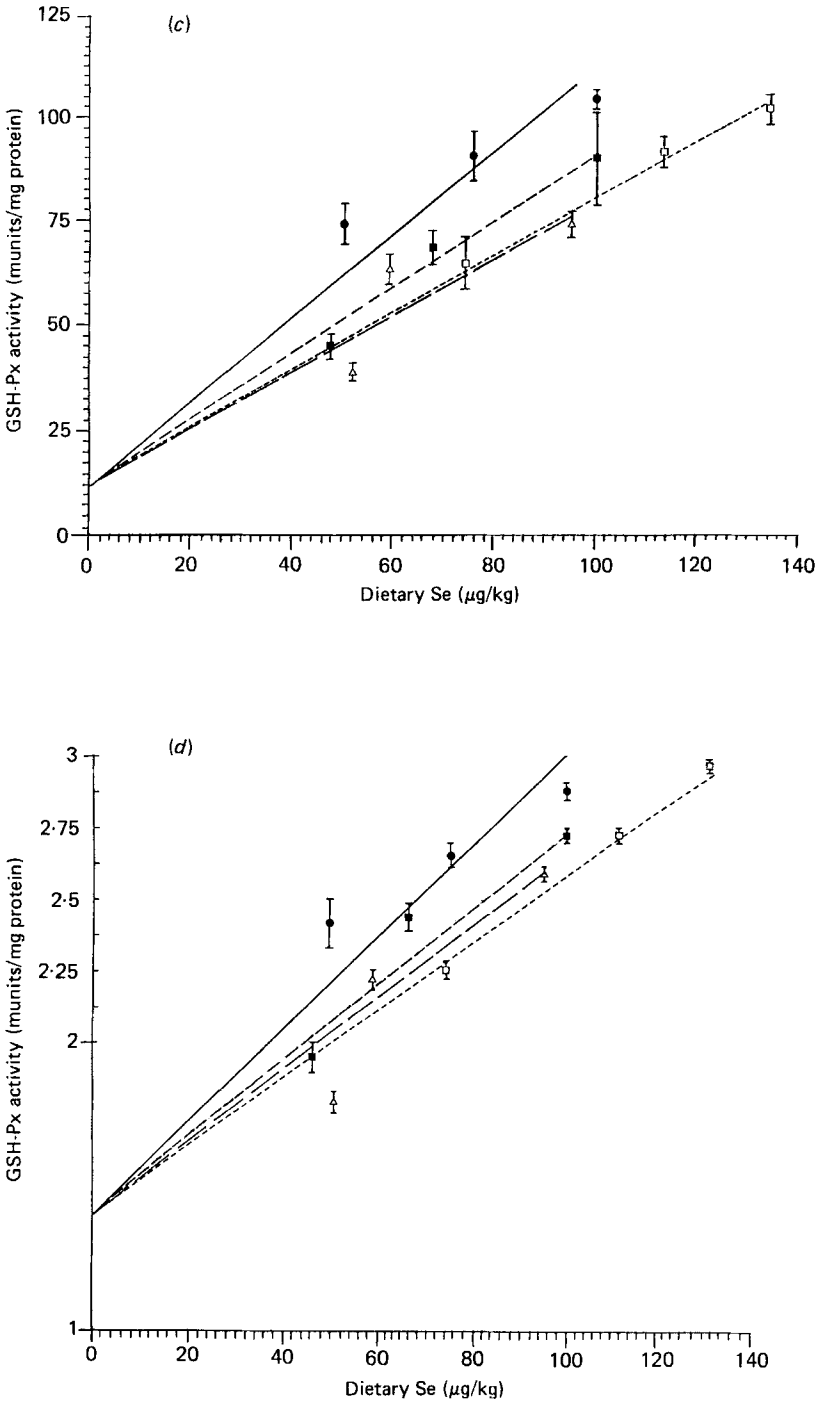

Fig. 2. Expt 2. Regression lines used in the slope-ratio bioassay to calculate availability of selenium in fertilized Finnish wheat (■---■), sprayed Finish wheat (△——△), American wheat (□---□), the selenite standard (●——●) by the criteria of (*a*) plasma and (*b*) liver Se levels and (*c*) plasma and (*d*) liver glutathione peroxidase (*EC* 1.11.1.9; GSH-Px; munits/mg protein) activities. Points are means, with their standard errors represented by vertical bars, for five rats.

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Table 3. *Percentage availability of wheat selenium sources relative to selenite measured by glutathione peroxidase* (EC *1.11.1.9; GSH-Px*) *restoration or elevation of Se in plasma and liver of rats*

(Mean values with their standard error for five samples)*

Diet and method of assessment	Se content of the diet (μg/kg)	Availability (%)							
		Plasma				Liver			
		GSH-Px		Se		GSH-Px		Se	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Finnish fertilized spring wheat									
Point-slope	96 (1a)†	133	4	144	7	90	24	88	5
	194 (1b)	73	5	82	5	73	1	88	3
Slope-ratio	47, 67, 100 (2a,b,c)	78	7	90	6	83	5	91	5
Finnish sprayed spring wheat									
Point-slope	101 (1a)	91	9	97	7	62	18	83	10
	174 (1b)	73	2	89	3	154	15	102	8
Slope-ratio	57, 59, 95 (2a,b,c)	68	5	78	5	72	4	91	5
American winter bread wheat									
Point-slope	142 (1a)	76	3	93	3	66	16	95	17
	238 (1b)	60	26	70	2	110	25	88	3
Slope-ratio	74, 113, 134 (2a,b,c)	68	5	78	5	72	4	75	5

* The 100% values are those predicted for 100 or 200 μg Se as sodium selenite/kg diet by regression analysis of responses over the linear ranges on standard curves (point-slope, Expt 1) or represent the ratios × 100 of the slope from the regression line generated from tissues of rats given the test diets divided by that generated from tissues of rats given the diets with selenite (slope-ratio, Expt 2).

† Se groups, for details see Table 1.

dietary Se from different wheat sources increased from 47 to 134 μg Se/kg (Fig. 2(*a*, *c*)). The regression analysis gave the result equation $y = \alpha + \beta_1 X_{ST} + \beta_2 X_{AS} + B_3 X_F$, where X_{AS} is American and sprayed wheat combined, both for plasma Se levels and plasma GSH-Px activities. Fertilized wheat was more active ($P < 0{\cdot}05$) than sprayed or American wheat in increasing plasma Se level or plasma GSH-Px activity, but the Se in all three wheats was less available ($P < 0{\cdot}05$) than selenite when judged by these criteria also (Fig. 2(*a*, *c*)). The ability of Se in all three wheats studied to elevate liver Se levels and liver GSH-Px activities was less effective ($P < 0{\cdot}05$) than selenite (Fig. 2(*b*, *d*)). By the criterion of liver Se level, the Se in American wheat was less available ($P < 0{\cdot}05$) than that in fertilized or sprayed Finnish wheats (Fig. 2(*b*)). On the other hand, by the criterion of liver GSH-Px activity, the availability of the Se in fertilized wheat was significantly different ($P < 0{\cdot}05$) from that in sprayed or American wheat (Fig. 2(*d*)).

In agreement with the preliminary experiment, the response of liver Se level or GSH-Px activity to dietary Se was not as linear as that of plasma Se level or GSH-Px activity. Therefore as in the preliminary experiment the liver values for different Se sources were analysed on a log scale. The equations were $y = \alpha + \beta_1 X_{ST} + \beta_2 X_A + \beta_3 X_{FS}$ where X_{FS} is fertilized and sprayed wheat combined, for liver Se values and $y = \alpha + \beta_1 X_{ST} + \beta_2 X_{AS} + \beta_3 X_F$ for liver GSH-Px values.

The relative availabilities of the various wheat sources of Se as assessed by the two different methods from Expts 1 (point-slope) and 2 (slope-ratio) are summarized in Table 3. There is generally good agreement between these two methods if the criterion used is either plasma or liver Se level. However, if the criterion is plasma or liver GSH-Px activity, there is poor agreement within the point-slope method at the two dietary Se levels fed. When the criterion is plasma GSH-Px activity, the point-slope method gives a high value for the availability of the Se in fertilized and sprayed wheat at the lower dietary Se level compared with that at the higher dietary Se level. On the other hand, if the criterion is liver GSH-Px activity, the point-slope method gives a high value for the availability of the Se in sprayed or American wheat at the higher dietary Se level compared with the lower dietary Se level. The availability value based on the slope-ratio assay usually agrees with the lower estimate of Se availability provided by the point-slope technique regardless of the criterion used or dietary Se level fed.

DISCUSSION

Two distinct experimental conditions must be met for a valid Se availability study. First, valid indicators of body Se status must be used as response criteria and second, assay conditions must be used which fulfill the requirements of statistical validity. These conditions cannot be met entirely until the general validity of different criteria in reflecting body Se status has been clarified. The liver has the largest labile pool of Se in the body, whereas plasma Se level reflects short-term changes in Se intake (Levander, 1983). Therefore plasma and liver criteria were chosen as indicators of body Se status in our study.

The availability values obtained by point-slope analysis at two dietary Se levels should have been similar. This was not true in the case of all indicators used in Expt 1. The different levels of wheat in the diets may partly explain the variability of the apparent availability of Se. According to this experiment it was impossible to estimate an average value for the availability of the Se in the three wheats studied. Therefore, another approach, the slope-ratio assay, was taken in Expt 2. The requirements for the valid use of the slope-ratio technique are the linearity of the response over the dose range studied and the common intercept of the regression lines generated. The response in plasma GSH-Px activity was found to meet those requirements in the preliminary study of Expt 2. Previously, a linear

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dose response in plasma GSH-Px activity was also reported in chicks repleted with graded dietary levels of sodium selenite (20–120 μg Se/kg) (Gabrielsen & Opstvedt, 1980). As shown by us previously, the dose–response curves for plasma Se level and plasma GSH-Px activity in rats plateau above the 100 μg dietary Se/kg level (Mutanen *et al.* 1986). Consequently, availability percentages calculated by these criteria above the 100 μg/kg dietary level may not be valid for estimating an average availability value.

Findings concerning liver GSH-Px activity did not comply as well with the requirements of the slope-ratio method. The slopes of the liver curves became steeper with higher dietary levels of Se from the American wheat (Expt 2). Even though liver criteria may be valid indicators of body Se status, this phenomenon may weaken the use of these criteria when studying overall Se availability. All the findings discussed previously show the difficulties that exist when comparing values from different studies and assay conditions.

Our results also support the hypothesis (Cantor *et al.* 1975; Mutanen *et al.* 1986) that the availability of Se may be dependent on the Se content of the diet. The reason for this is not clear. Usually the change in Se level of a test diet causes changes in the contents of other nutrients also and it has been shown that Se availability may be affected by several other nutrients (Yasumoto *et al.* 1979; Sunde *et al.* 1981; Zhou *et al.* 1983). These factors could not be operating in the present Expt 2 in which Se was the only variable among different test diets made up with a given type of wheat. However, it may explain to some extent the differences in Se availabilities between different wheat diets.

In previous studies (Cantor *et al.* 1975; Douglass *et al.* 1981; Alexander *et al.* 1983) the availability to rats or chicks of the naturally occurring Se in wheat has been shown to vary from 70 to 217% depending on the methods, supplementation levels and response criteria used. With experimental conditions similar to ours, Douglass *et al.* (1981) found values of 83 and 86% for the availability of wheat Se at the 200 μg/kg dietary level when the indicators used were either liver GSH-Px or liver Se level. Those values are close to the corresponding results of our slope-ratio assay of American wheat. On the other hand Alexander *et al.* (1983) used the slope-ratio method over a dietary range of 50–150 μg Se/kg and found values as high as 217% for the availability of Se in whole-wheat flour as assessed by liver GSH-Px activity, almost three times as much as our corresponding value.

In our slope-ratio assay, statistically significant differences were observed in the availability of Se from the three wheats tested by some criteria, but the differences were relatively small and likely to be of only minor significance from the practical nutritional point of view. In fact, if the availability values for each type of wheat were averaged over all four response criteria (plasma GSH-Px and Se, liver GSH-Px and Se) to give a general estimate of Se availability, the values are 86, 77 and 73% for the fertilized and sprayed Finnish wheats and the American wheat respectively (sodium selenite 100%).

The Se compounds present in wheat are not fully characterized. Most of the Se that occurs naturally in wheat is thought to exist as organoselenium compounds, presumably mainly selenomethionine (Olson *et al.* 1970; Beilstein & Whanger, 1984). Se, either in the form of wheat or as selenomethionine, is retained better in the body than sodium selenite or selenate (Robinson *et al.* 1978; Levander *et al.* 1983). Recently Gissel-Nielsen (1986) has shown in his experiments with barley and ryegrass grown to maturity that there was no significant difference in the stored Se whether Se was added as selenite or selenate, and whether it was applied to the roots or leaves. Therefore it is reasonable to assume that the American wheat naturally high in Se does not contain much more organically-bound Se than the Finnish wheat either fertilized or sprayed with sodium selenate.

The Se content of the wheats tested was considerably higher than that of commercial wheats originating from high-Se areas (Lorenz, 1978). There is, however, no reason to believe that the availability of Se in wheat would be a function of its Se content. Our results

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show that the Se in wheat is nutritionally available to rats and its availability to this species is not markedly dependent on whether the Se in the wheat occurs naturally or is deliberately added by man.

Marja Mutanen was a Fellow of the W. K. Kellogg Foundation during the academic year 1983–4. Mrs Daisy M. Wills provided competent animal care.

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Printed in Great Britain

https://doi.org/10.1079/BJN19870040 Published online by Cambridge University Press