Trace Elements in Nutrition of Children,
edited by R. K. Chandra. Nestlé Nutrition,
Vevey/Raven Press, New York © 1985.

Keshan Disease: An Endemic Selenium-Related Deficiency Disease

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Keshan disease is an endemic cardiomyopathy which was first observed in Keshan County, Heilonjiang Province, Northeast China. It has a very high case-fatality rate, more than 80% in the past and around 30% at present. The disease may be acute, subacute, chronic, or insidious. Young children at the time of weaning, children below 10 years of age, and women of child-bearing age are especially vulnerable. It is seen mainly in the mountainous regions of China. Its distribution extends from northeast to southwest China, forming a wide belt across the country (1) (Fig. 1).

Because of the frequent association of Keshan disease with animal selenium (Se) deficiency diseases such as white muscle disease, it seems reasonable to suspect a connection between Se deficiency and Keshan disease.

Extremely low Se levels in tissues of patients with Keshan disease were first reported in 1973 (G. Q. Yang and G. Y. Wang, *unpublished data*). Trials were conducted to test the effectiveness of Se in the prevention of Keshan disease; although good results were consistently obtained, conclusive evidence was not obtained until 1974 and 1975 (2,3).

EPIDEMIOLOGY

Selenium Content of Soil

The distribution and availability of Se in soil is not uniform in China. The amount of water-soluble Se in Keshan disease areas is extremely low, i.e., 0.28 to 0.44 μg/100 g dry soil (Table 1).

Foodstuffs

All maize and most rice samples produced in the endemic areas contain Se at less than 0.01 ppm, and all samples from nonaffected areas contain Se not more than 0.02 ppm (4) (Figs. 2 and 3). There is a good correlation ($r = 0.830$, $p < 0.01$) between the Se content of cereals and soybeans and the amount of water-soluble Se in the soil.

Blood

The whole blood Se content of 325 subjects living in an endemic area averaged 0.018 ± 0.001 ppm (0.002–0.062 ppm), and that of 134 subjects living in a nonendemic area was 0.093 ± 0.08 ppm (0.013–0.351 ppm) (4). It is interesting to note that most of the samples from the endemic area were below 0.02 ppm, and those below 0.01 ppm were almost exclusively from the endemic area (Fig. 4). Both blood Se concentrations (5) and glutathione peroxidase (GSH-Px) activities (6) of children in the endemic area were significantly lower than those in the nonendemic area, although in the same endemic area no significant differences were found between affected children and normal children (5,6). Table 2 shows blood GSH-Px activities of individuals living in affected and nonaffected areas.

Hair

The average hair Se concentrations in endemic areas were always below 0.12 ppm, whereas those from nonendemic areas were higher than 0.16 ppm and those near the endemic areas ranged between 0.12 and 0.16 ppm. In nonendemic areas located far away from endemic areas, the levels were higher than 0.2 ppm (Fig. 5). When values from endemic and nonendemic areas were arranged in decreasing order, it was found that all affected areas were located at the extreme right of the figure with only two exceptions (4) (Fig. 6). It indicates that affected areas are consistently associated with Se insufficiency, but that the reverse is not always true. Hence low Se areas are not necessarily Keshan disease areas (Fig. 6). Factor(s) other than Se may thus be involved in the etiology of the disease.

Hair Se concentration and Se content of staple foods are significantly correlated ($r = 0.8257$, $p < 0.001$) (Tables 3 and 4). The traditional Chinese farmers' diet is basically of vegetarian origin: More than 70% of the daily Se intake comes from cereals grown on their own land. It is obvious that the Se content of the soil affects the Se status of human beings if they consume locally grown cereals. Keshan disease is found exclusively in areas producing cereals with a Se concentration lower than 0.025 ppm (7).

Population Susceptibility

Children from peasants' families are usually the population at risk. Their hair Se content is significantly lower than that of nearby families and of urban families consuming foods produced elsewhere or earning better incomes (Table 5).

Seasonal Variations

The peak seasonal prevalence of Keshan disease in northern China occurs during the winter; in southern China it is during the summer. Body stores of Se should vary with the seasons if a deficiency of Se alone is responsible for the occurrence

FIG. 1. Distribution of Keshan disease (KD) in China (From Tan et al., ref. 1.)

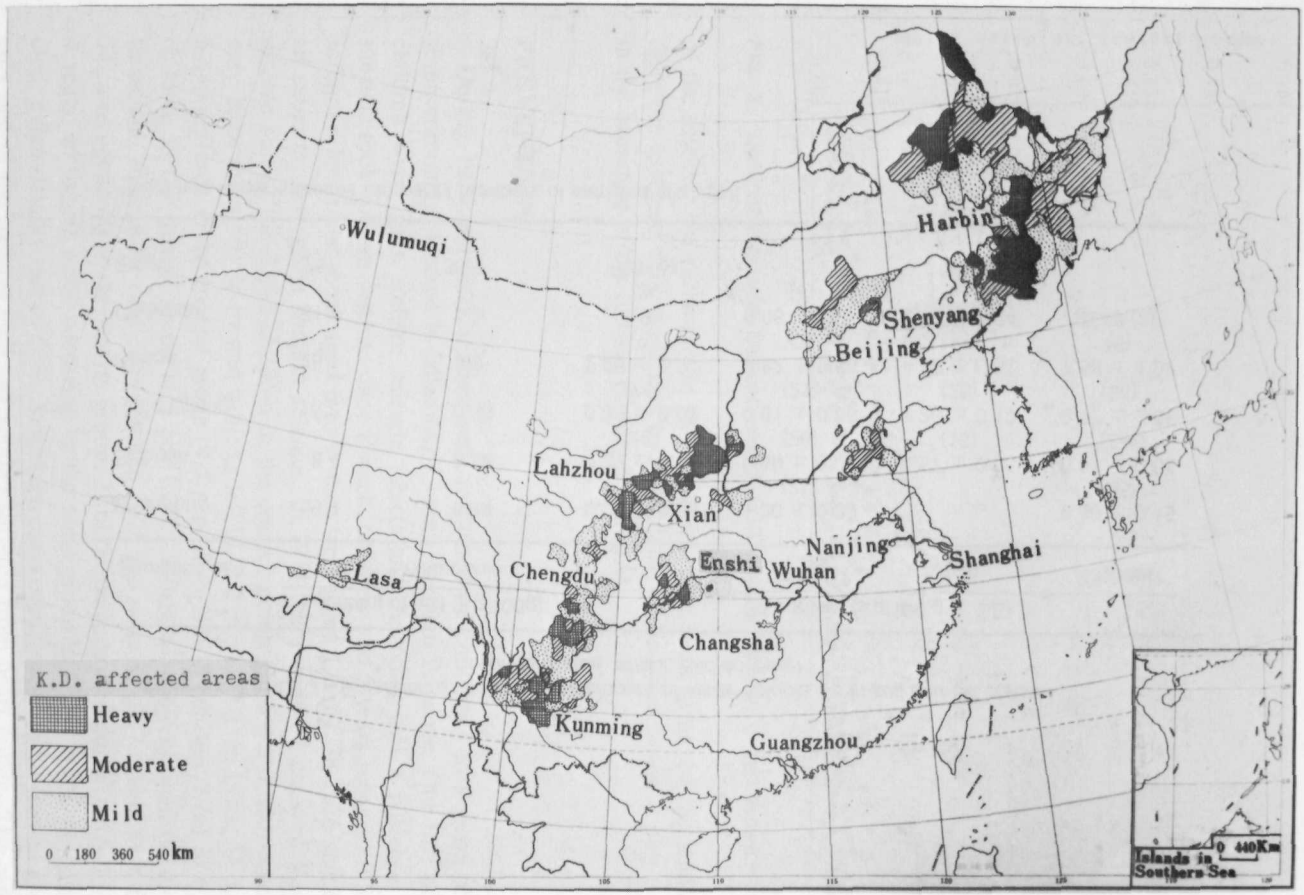
Wulumuqi
Harbin
Shenyang
Beijing
Lahzhou
Xian
Nanjing
Shanghai
Lasa
Chengdu
Enshi
Wuhan
Changsha
Kunming
Guangzhou
K.D. affected areas
Heavy
Moderate
Mild
0 180 360 540 km
0 440Km
Islands in Southern Sea

TABLE 1. *Relationship between the amount of water-soluble Se in soil and Se content of corn, rice, wheat, and soybean*

Sampling site	Se content of soil (μg/100g)		Se content (μg/100 g ± SE)			
	Total	Water-soluble	Corn	Wheat	Rice	Soybean
Shandong	5.9	0.44	0.98 ± 0.03 (19)[a]	1.30 ± 0.09 (21)		2.60 ± 0.15 (25)
Sichuan	8.4	0.28	0.32 ± 0.04 (16)	0.59 ± 0.07 (20)	0.57 ± 0.05 (18)	0.61 ± 0.04 (24)
Heilongjiang	18.2	0.33	0.36 ± 0.02 (76)	0.61 ± 0.06 (21)	2.26 ± 0.19 (26)	0.77 ± 0.06 (54)
Beijing	28.1	3.9	2.26 ± 0.21 (7)	3.82 ± 0.66 (7)	4.12 ± 0.31 (11)	7.58 ± 1.37 (6)
Chengdu	31.8	1.1	1.36 (2)	6.06 ± 1.01 (4)	2.99 ± 0.24 (4)	3.48 (2)
Enshi	787	35.4	633 (5)		148 (4)	

[a]Numbers in parentheses represent numbers of samples analyzed.

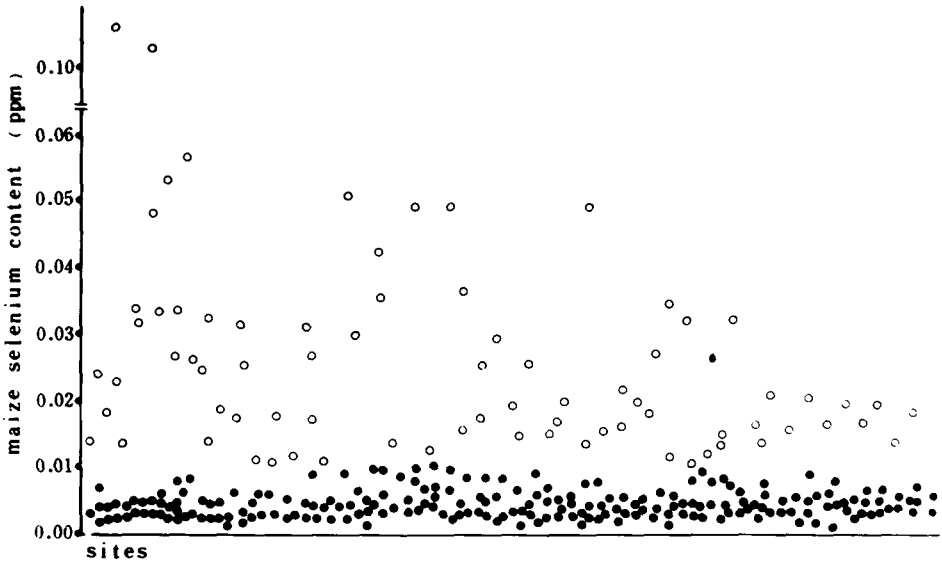

FIG. 2. Distribution of maize Se concentration in KD-affected (•) and KD-nonaffected (○) areas.

of the disease. No variations in hair Se levels have been observed (8), and it is therefore assumed that in addition to a deficiency of Se other factor(s) play a role in the causation of the disease (Tables 6 and 7).

PREVENTION

During 1974 and 1975 studies were conducted on children 1 to 9 years old in seven communes located in Mianning County, Sichuan Province. One-half of the children were given sodium selenite tablets and the other half a placebo. The subjects took sodium selenite once a week; children 1 to 5 years received 0.5 mg sodium selenite and those aged 6 to 9 years were given 1.0 mg. In 1976 and 1977 all subjects were given sodium selenite. In 1974 there were 54 cases of Keshan disease among the 3,985 children in the control group (13.5 per 1,000) whereas only 10 of the 4,510 Se-treated subjects fell ill (2.2 per 1,000). In 1975, 52 out of 5,445 children in the control group (9.5 per 1,000) fell ill but only 7 out of 6,767 children in the treated group (1.0 per 1,000). When all subjects received the Se treatment, there were only four cases in 1976 and none in 1977 (Table 8). Of the 54 cases occurring in the control group, 27 of the patients died during the following 4 years; of the 52 cases occurring in the same group in 1975, 26 of the patients died, a case-fatality rate of 50%. Seventeen cases occurred in the Se-treated group; only one of the patients died and another suffered from heart failure. The cardiac function of the remaining 15 patients recovered, although in 3 cases the heart was

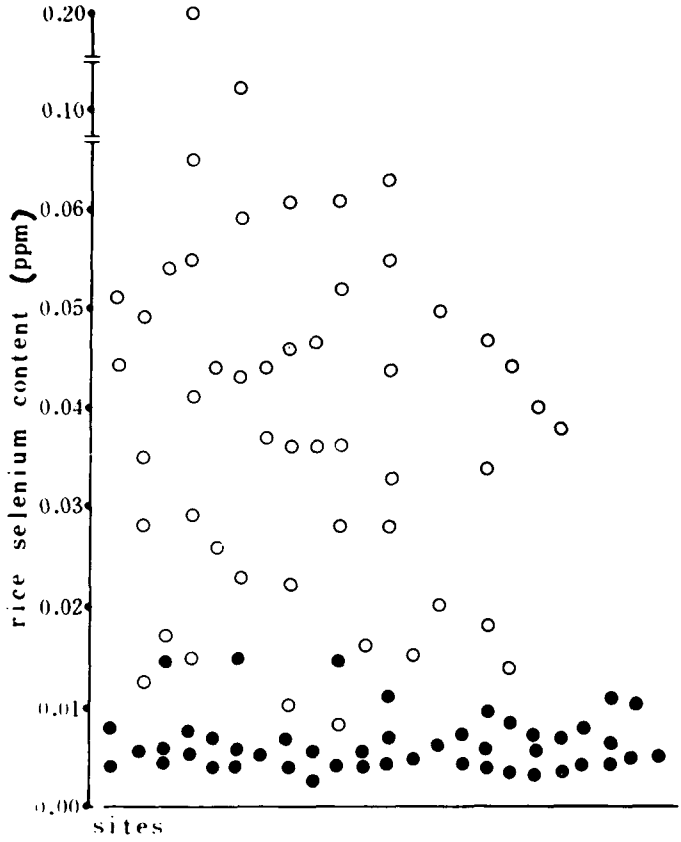

FIG. 3. Distribution of rice Se concentration in KD-affected (•) and KD-nonaffected (○) areas.

still slightly enlarged. It appears therefore that Se-treated subjects not only have a lower incidence but also a lower fatality rate and a better life prognosis.

Etiological Factors

Keshan disease is endemic, and a deficiency of Se together with other unknown factor(s) appears to be responsible for the occurrence of the disease. A deficiency of Se alone does not appear to be responsible for the occurrence of Keshan disease for the following reasons: (a) the absence of relationship between seasonal prevalence and changes in Se concentration in the hair; (b) Keshan disease is exclusively observed in areas where Se deficiency occurs but not all areas deficient in Se are necessarily endemic; and (c) no differences in blood concentrations and GSH-Px

FIG. 4. Distribution of individual blood Se concentration in KD-affected (•) and KD-nonaffected (○) areas. Affected area: 325 subjects; av. 0.018 ppm ± 0.001 SD. Nonaffected area: 134 subjects; av. 0.093 ppm ± 0.081 SD.

Blood Se level (ppm)
Individuals from different regions

TABLE 2. *Whole blood GSH-Px activities of individuals in affected and nonaffected areas*

Area	No. of subjects	Average GSH-Px activity (unit)[a]
Outskirts of Beijing (nonaffected)	22	77.5 ± 2.1
Outskirts of Chengdu (nonaffected)	20	73.6 ± 3.1
Mianning County, Sichuan (affected)	63	60.5 ± 0.7
Zhouxian County, Shandong (affected)	20	61.9 ± 2.8

[a]One unit is defined as the activity of 8 μl of whole blood which causes after incubation for 5 min at 37°C a decrease of 1 μM GSH, after correction for nonenzymatic activity. Results are given as the mean ± SE.

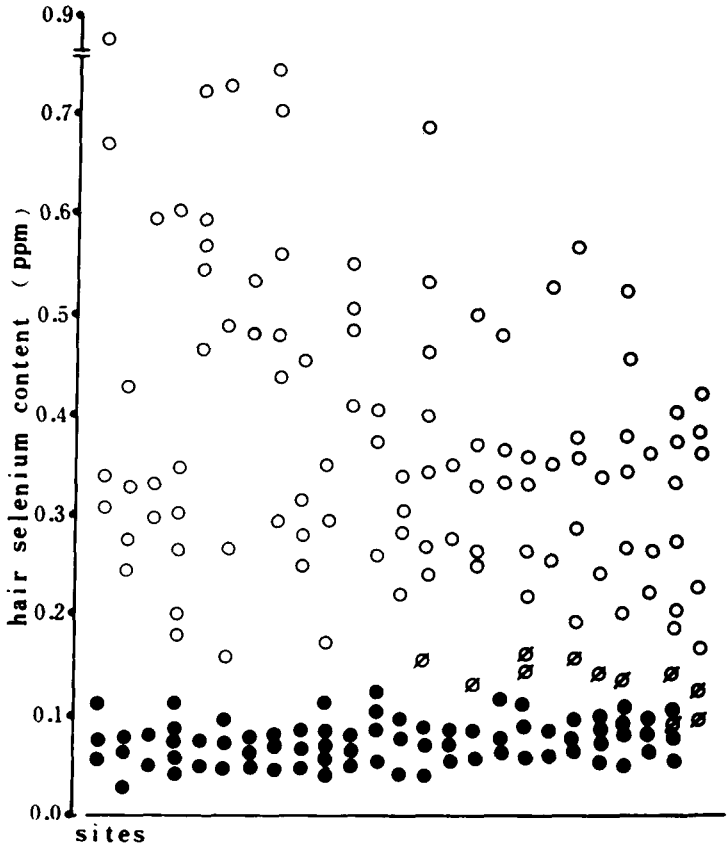

FIG. 5. Distribution of hair Se concentration in KD-affected (●) and KD-nonaffected (○) areas and∅ nonaffected area close to affected area.

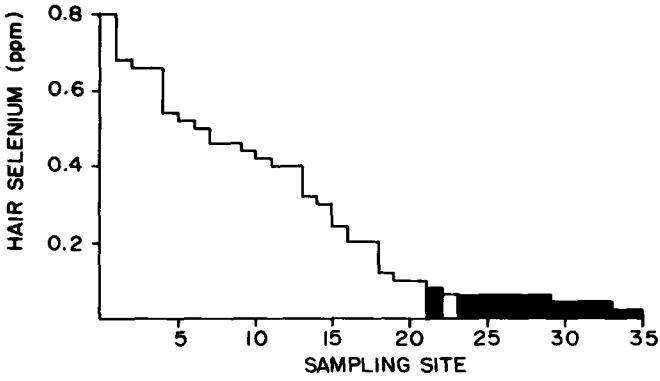

FIG. 6. Distribution of hair Se concentration in KD-affected (■) and KD-nonaffected (□) areas.

activities have been observed between healthy children and children affected with the disease living in the same area.

1. *Vitamin E.* No significant differences were found between patients affected with Keshan disease and healthy individuals (G. Y. Wang and G. Q. Yang, *unpublished data*); their plasma vitamin E concentrations were marginally deficient (Table 9). Because a low vitamin E intake may influence the bioavailability of Se or result in a greater requirement for Se, it does not seem appropriate to exclude the possibility that marginal vitamin E might sometimes act as a cofactor to Se deficiency.

TABLE 3. *Se concentration in hair and Se content of staple foods*

Sampling site	Se conc (ppm ± SE)		
	Hair	Corn	Rice
Enshi, Hubei	23.34 ± 2.56 (15)[a]	6.330 (5)	1.483 (4)
Fushu, Xinjiang	0.860 ± 0.031 (10)	0.286 (1)	0.145 (1)
Tiandeng, Guangxi	0.482 ± 0.016 (20)	0.146 (2)	0.095 (2)
Lingan, Zhejiang	0.405 ± 0.011 (18)	—	0.048 (2)
Longhai, Fujian	0.233 ± 0.030 (20)	—	0.047 (2)
Huanglong, Shanxi	0.076 ± 0.008 (22)	0.005 (2)	—
Shangzhi, Heilongjiang	0.075 ± 0.006 (20)	0.004 (4)	0.024 (25)
Miangning, Sichuan	0.071 ± 0.003 (20)	—	0.009 (2)
Nanhua, Yunan	0.068 ± 0.004 (20)	0.004 (3)	0.004 (3)

[a]Numbers in parentheses are the numbers of samples analyzed.

TABLE 4. *Concentrations of Se in hair and staple food in various areas*

Area	Se (ppm)	
	Hair	Staple food
Hair versus sweet potato		
Hilly region (A)[a]	0.097–0.123 (79)[b]	0.0029–0.0046 (15)
Coastal region (N)[c]	0.234–0.331 (40)	0.075–0.089 (4)
Hair versus oats		
Nonsalted soil (A)	0.056 (20)	0.0053 (5)
Salted soil (N)	0.170 (20)	0.0395 (4)
Hair versus sweet potato flour pancake		
Mountainous area (A)	0.084 (20)	0.027 (4)
Plain (N)	0.204–0.228 (40)	—
Hair versus rice		
Surrounding area (A)	0.060 (18)	0.0078 (5)
"Safety island" (N)	0.144 (20)	0.0202 (6)

[a]A; affected areas.
[b]Numbers in parentheses are the numbers of samples analyzed.
[c]N; nonaffected areas.

2. *Methionine.* After addition of 0.13% DL-methionine to a soy–corn diet, rat liver GSH-Px activities were significantly increased (9). Insufficiency of methionine in the diet in certain endemic areas may contribute to the occurrence of Keshan disease (Table 10). This, however, needs further confirmation.

3. *Virus.* The virus used in experiments on suckling mice (10) was isolated from the blood of a child who suffered from subacute Keshan disease. It was identified to be Coxackie B_4. The experimental diet was made of cereals and soybeans. A

TABLE 5. *Hair Se levels and blood GSH-Px activities in various population groups*

Populations of children	Hair Se (ppm) (mean ± SE)	No. of specimens analyzed	GSH-Px activity (units) (mean ± SE)	No. of specimens analyzed
Heilongjiang				
City	0.390 ± 0.018	20		
Commune	0.151 ± 0.011	20		
Sichuan				
City	0.131 ± 0.014	16		
Commune	0.069 ± 0.0005	10		
Commune staff	0.238 ± 0.011	7	90.0 ± 1.2	8
Commune peasant	0.128 ± 0.009	20	61.9 ± 2.9	20
Mine staff and miners'	0.161 ± 0.007	26	80.2 ± 1.2	23
Commune peasant	0.058 ± 0.003	22	60.5 ± 0.7	63

[a]All populations are living in areas affected with Keshan disease.

TABLE 6. *Seasonal changes in the hair Se concentration of children in an endemic area (Southwest China)*[a]

Year and month	Xianfeng commune		Fuxin commune	
	No. of samples	Hair Se content (ppm)	No. of samples	Hair Se content (ppm)
1973, March	21	0.085 ± 0.017	—	—
1973, June	22	0.088 ± 0.006	—	—
1973, Oct.	10	0.069 ± 0.005	—	—
1975, Jan.	18	0.064 ± 0.006	16	0.048 ± 0.004
1975, April	20	0.057 ± 0.004	20	0.069 ± 0.007
1975, July	22	0.058 ± 0.003	19	0.064 ± 0.004
1975, Oct.	20	0.053 ± 0.003	19	0.044 ± 0.004

[a]Results are given as the mean ± SE.

supplement of vitamins and minerals was also given. Of the animals in the Se-deficient group, 38% had a lesion of the myocardium: This was a significantly higher incidence than in the Se-supplemented group, where only 14% had a cardiac lesion. It shows that hearts of suckling mice fed a low-Se diet may be more sensitive to the virus and that supplementation with Se would protect them from heart necrosis (Table 11).

TABLE 7. *Seasonal changes in hair Se concentration of children in an endemic area (Fanrong Commune, Northeast China)*[a]

(1975) Month	No. 43 village		Xianfa brigade	
	No. of samples	Hair Se content (ppm)	No. of samples	Hair Se content (ppm)
April	12	0.054 ± 0.006	20	0.093 ± 0.005
Aug.	22	0.050 ± 0.008	22	0.086 ± 0.004
Dec.	21	0.052 ± 0.004	20	0.083 ± 0.005

[a]Results are given as the mean ± SE.

TABLE 8. *Incidence and prognosis of Keshan disease in Se-treated and control children during 1974–1977*

Year	No. of subjects	No. of cases of Keshan disease	Alive	Dead
Controls				
1974	3,985	54	27	27
1975	5,455	52	26	26
Treated				
1974	4,510	10	10	0
1975	6,767	7	6	1
1976	12,579	4	2	2
1977	12,747	0	0	0

TABLE 9. *Comparison of plasma vitamin E content in children affected with Keshan disease and normal children in Mianning County*[a]

Group	No. of subjects	Plasma vitamin E content (μg/ml)	Whole-blood Se content (ppm)
Keshan disease	16	5.5 ± 0.9	0.019 ± 0.003
Normal children	19	4.8 ± 0.6	0.026 ± 0.005

[a]Results are given as the mean ± SE.

DAILY SELENIUM INTAKE OF HUMAN BEINGS

The occurrence of both Se toxicity and deficiency indicates that there is a wide range of Se intakes in China. Data concerning intakes and tissue levels of Se (11) in different districts are given in Table 12. Daily Se intakes between 1.08 and 38 mg appear to be toxic for adults. Both dosage and duration of intake should be taken into consideration; the highest daily intake of 38 mg caused signs of toxicity after 3 to 4 days, whereas the lower daily Se intake of 1.08 mg (92% from sodium selenite) needed a long time to cause intoxication and caused lesions of the nails; the latter dose is only 1/56 of the 1.0 mg/kg daily intake suggested by Smith and

TABLE 10. *Se content and GSH-Px activities of rat liver tissue (wet) and whole blood when* DL-*methionine is added to a diet containing Se 0.03 ppm at the rate of 0.13%*[a]

Diet (low Se)	Se content (ppm)		GSH-Px activity (unit)[d]	
	Whole blood	Liver	Whole blood	Liver
No supplementation	0.020 ± 0.03 (10)[b]	0.17 ± 0.005 (10)	20.8 ± 1.6 (10)	6.0 ± 0.4 (10)
Supplemented with DL-methionine	0.22 ± 0.01 (10)	0.21 ± 0.01[c] (10)	17.2 ± 0.8 (10)	9.4 ± 0.9[c] (10)

[a]Results are given as the mean ± SE.
[b]Numbers in parentheses are numbers of rats used for assay.
[c]Significant.
[d]One unit is defined as the activity of 1 ml whole blood which causes after incubation for 1 min at 37°C a decrease of log(GSH) for 1 after correction for nonenzymatic value.

TABLE 11. *Heart lesion and blood Se levels of 2-week-old suckling mice 1 week after virus injection*

Diet	No. of animals	No. of positive cases	% of positive cases	Blood Se content (ppm)
Low Se	44	17	38	0.033
Se supplemented[a]	58	8	14	0.070
Stock (normal)	72	8	11	0.321

[a]By stomach tube: Na_2SeO_3, 1.0 mg/kg body weight/week.

TABLE 12. *Daily Se intake of human adults*

Daily Se intake	Intake adequacy[a]	Diet	Method of estimation
38 mg	Toxic	Vegetable	Inferred from Se concentration of staple maize, which caused three newcomers to lose their hair 3–4 days after arrival in a heavy-prevalence village
15 mg	Toxic	Vegetable	Inferred from average Se concentration of staple maize
4.99 mg	Chronically toxic	Vegetable	Dietary survey in villages where individuals presented diseased nails and low hemoglobin values
1.08 mg	Chronically toxic	92% from Na_2SeO_3	Disease of the nail after 2 years of supplementation with Na_2SeO_3
0.75 mg	No pronounced signs of toxicity	Vegetable	Dietary survey in a high-Se area with no signs of toxicity (one case with diseased nails was, however, found recently; his hair Se concentration was 8 ppm, comparable with the top level of normality in that village)
92 μg	Adequate	Mixed	Dietary survey by the Institute of Health, Beijing
60 μg	Protected from KD	90% from Na_2SeO_3	After Na_2SeO_3 tablet supplementation and table-salt fortification with Na_2SeO_3
17 μg	Smallest amount needed to protect from KD	Vegetable	Dietary survey in nearby nonaffected area
7–11 μg	KD and KBD areas	Vegetable	Dietary survey in affected areas

[a]KD: Keshan disease. KBD: Kaschin Beck disease.

Lillie (12) as being toxic to man. A daily Se intake of 4.99 mg caused a decrease of hemoglobin in addition to lesions of the nails. Until the mechanism of Se toxicity has been well understood, it seems wise to avoid using too large a dose in long-term trials. Seventy percent of the daily Se intake in KD areas originates from staple cereals. Because the average Se content of maize is only 0.0045 ppm, the daily Se intake of adults in affected areas is usually below 10 μg daily. The daily Se intake of 3- to 6-year-old children is usually below 3 μg, or less than 1/10 the RDA suggested in the United States (13). This level of Se intake cannot even cover the minimum requirement of children susceptible to Keshan disease. Table 13 shows that tissue levels of Se are dependent not only on the dose of Se but also on its form. The whole blood Se concentration of a man who is chronically intoxicated by a daily intake of 1.08 mg Se (mainly from selenite) is comparable to individuals having an adequate daily Se intake of 0.09 mg/day from a natural diet. It appears therefore that the determination of tissue Se alone is not adequate for the diagnosis of selenosis without distinguishing between the forms of Se ingested.

CONCLUSION

Evidence has been presented that the primary factor responsible for Keshan disease is Se deficiency. Keshan disease is a biogeochemical disease; it is similar

TABLE 13. *Daily Se intake of human adults and tissue Se levels*

Daily Se intake		Hair (ppm)	Whole blood (ppm)	Urine (μg/ml)
Amount	μg/kg BW[a]			
38 mg	633	—	—	—
15 mg	250	59.3 (7)[b,c] (22.0–100.6)[d]	—	—
4.99 mg	83	23.3 (15)[b] (10.6–31.8)	4.39 (18)[b] (2.60–5.92)	2.68 (17)[b] (0.855–6.63)
1.08 mg	18	0.828 (1)[b]	0.179 (1)[b]	—
0.75 mg	13	3.69 (14)[b] (1.90–8.20)	0.444 (14)[b] (0.346–0.584)	0.144 (14)[b] (0.043–0.333)
92 μg	1.5	0.581 ± 0.050 (11)[b]	0.161 ± 0.027 (11)[b]	—
60 μg	1.0	0.280 ± 0.050 (13)[b]	0.056 ± 0.016 (12)[b]	—
17 μg	0.28	0.160 ± 0.040 (40)[b]	0.027 ± 0.009 (40)[b]	—
7–11 μg	0.12–0.18	0.074 ± 0.050 (1,478)[e]	0.018 ± 0.010 (325)[e]	0.007 ± 0.001 (43)[e] 0.005 ± 0.003 (43)[b]

[a]On the basis of 60 kg body weight (BW).
[b]Adults.
[c]Numbers in parentheses are the numbers of subjects.
[d]Range.
[e]Most are children 8 to 14 years of age.

to the white muscle disease of lambs observed previously by Chinese veterinarians. When Se is supplemented, the primary factor responsible for the development of the cardiomyopathy disappears. Selenium supplementation therefore constitutes the basis for the prevention of Keshan disease.

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DISCUSSION[1]

Dr. Mertz: I would like to make two comments. First, Dr. Diplock showed beautifully the very central role that selenium plays in metabolism. He has indicated the greatly increased risk for damage to the genetic material in selenium deficiency, which fits very well with increasing evidence from animal experiments that selenium at physiological levels is in some way negatively correlated with cancer risk. We are not talking here about any therapeutic aspects, but we may have to accept selenium deficiency as one risk factor. Second, Dr. Diplock clearly showed the many interactions of selenium, and that selenium in glutathione peroxidase works in concert with many other trace elements in many other enzymes. I have been interested in selenium for almost 40 years, since I first met Klaus Schwarz. Selenium has always impressed me as a very peculiar trace element; it is different from all the other elements in that a pure selenium deficiency has mainly subtle biochemical consequences. When you want a clinical picture of selenium deficiency in animals or humans, you have to have some other interacting factor. This is, I think, quite unique for selenium. Superimpose vitamin E deficiency on a deficient animal, and it will die. If you want to kill the animal immediately, give it some polyunsaturated fat. If you are exposed to certain heavy metals, your selenium status can determine the outcome. Parizek has shown very important interactions. The selenium requirement is very difficult to define because of these interactions; it may be very low, provided we live in an ideal world, provided our intake of polyunsaturated fat is not excessive, and provided our exposure to heavy metals and organic pollutants, including carcinogens, in the environment is low. As to myself, I am living in the real world, and I am exposed to many of these substances. I personally feel better if I have a reasonable margin of safety over the minimal selenium requirement. As we have no conclusive dates yet to show long-term benefits of what we could call an RDA selenium intake, I present these comments as my personal opinion.

Dr. Delange: Prof. Yang, I noticed that the prevalence of Keshan disease in the longitudinal study you showed from 1974 to 1977 markedly decreased irrespective of the fact that infants might be treated. Is there any possibility that there was a silent prophylaxis by selenium in the area under investigation? This has been documented for iodine.

Dr. Diplock: I have been told by some of the Chinese scientists that at the beginning there was some resistance to actually taking sodium selenite; and although it appeared that according to the records selenite was being taken, it may not in fact have been taken at all. I think that during the crucial period before it became apparent that selenite was a viable prophylactic agent the figures perhaps are slightly distorted by the resistance of the people to taking the selenite.

Dr. Gebre Mehdin: May I give an example of the silent prophylaxis that may occur in a population with marginal selenium intake. We recently did a study of serum selenium in diabetic children, and much to our surprise we found that our patients had higher levels than the healthy controls. We do not know how to interpret our findings. Our data probably

[1]This discussion also includes material concerning the chapter by A. T. Diplock *(this volume)*.

indicate that the diabetic children have a well-controlled diet with a good representation of animal food, particularly protein. We are not sure about this because the preliminary dietary survey results did not show very impressive differences, but we need to come back to this question.

Dr. Lombeck: Glutathione peroxidase has been mentioned in regard to patients with Down's syndrome. There are some publications from The Netherlands and France which indicate that not only is glutathione peroxidase activity altered but the superoxide dismutase activity is increased.

Dr. Diplock: Down's syndrome is very interesting. The gene for superoxide dismutase is of course carried on chromosome 21, and Down's patients have considerably elevated levels of superoxide dismutase. I believe that this results in an increase within these patients in what you might regard as the titer of hydrogen peroxide. The results you showed for glutathione peroxidase may simply be a homeostatic response to that increased hydrogen peroxide titer. The only other point I would like to make concerns your diabetic children: I wonder if what you are seeing is simply a dietary difference; presumably the diabetic children are eating a somewhat different diet from even the siblings who are not diabetic, and this might give rise to the small change in selenium that you have seen.

Dr. Chandra: The proportion of individuals who are affected with Keshan's disease, even in the deficiency region, is very small. Thus the majority of those who are ingesting low selenium diets are free of the disease. I personally believe that there must be other factors, e.g., virus infection, which precipitate Keshan's disease.

Dr. Diplock: I think the conclusion is inescapable that the people in these areas are poised on a knife edge, and there may be a number of factors which just push them over the edge, with Keshan disease resulting. These factors, which we have not yet been able to identify fully, may be several, but I think that the underlying selenium deficiency is the particular thing that we need to consider.

Dr. Lombeck: The investigation of methionine supplementation of mice leading to increased liver glutathione peroxidase activity was of special interest to me. Considering the Se intake, we always must know the protein intake, especially the amount of methionine ingested. The methionine intake can be one of the critical factors. I think research has to be done in this field.

Dr. Janghorbani: Selenium supplementation in relation to animal husbandry, of course, is widely practiced all over the world. The most efficacious form, from a biological point of view as well as cost-effectiveness, of selenium that has been applied is selenite. This is the form that is currently employed in TPN, and this potentially poses an important problem. Because of the multivalent nature of selenium, in contrast to other trace metals, e.g., copper and zinc, and the fact that many selenium compounds are labile in biological systems, addition of selenite to TPN, especially when practiced in relation to relatively high levels of reducing agents such as vitamin C, might not only be an efficacious way, but it could also have potentially adverse effects. The reason for this is the very well known property of selenite that it reacts with many sulfhydryl-containing as well as other reducing agents such as ascorbic acid resulting in the production of many intermediate compounds and forms, one of which might very well end up being colloidal selenium.

Dr. Lombeck: What is the amount used?

Dr. Janghorbani: I think, and Dr. Mertz might want to correct me on this, that the general practice in the United States is around 100 μg of selenium per day for a 70-kg reference man.

Dr. Bergmann: Dr. Yang, how often do you find cardiomyopathy in newborn infants in the Keshan disease area?

Dr. Yang: This is a very interesting problem. The fetus occasionally suffers from Keshan disease (KD) just as his mother does. Breast-fed infants less than 1 year of age (but not infants depending on milk substitutes made of local products) do not suffer from KD. Samples of human milk in KD-affected areas, nearby nonaffected areas, in the city of Beijing, and in seleniferous areas of chronic toxicity were obtained for Se determination. The average Se content (micrograms per gram) was found to be 0.0026, 0.0038, 0.02, and 0.28, respectively. The daily Se intakes of a 1-year-old infant could be calculated to be 2.0, 3.0, 16, and 220 μg, respectively, and it appears that 2 μg/day could not meet the requirement. Milk Se concentration appears to be very sensitive to Se intake and is a good indicator of the regional selenium status. It is interesting to note that the Se intake from mother's milk in seleniferous areas is 100-fold higher than the intake in KD-affected areas, although no signs of toxicity have ever been observed. The Se intake of children 3 to 6 years old in affected areas is 3.0 μg/day and in the nearby nonaffected areas 5.0 μg/day. 3 μg Se intakes cannot meet the daily requirements at that age.

Dr. Lombeck: The selenium intake can be very low but still adequate. The selenium intake in dietetically treated patients with inborn errors of amino acid metabolism is between 3 and 12 μg Se/day with a median value of 7 μg Se/day. There are no clinical signs of selenium deficiency. If you extrapolate these data to adults, they would have a daily selenium intake of 15 μg without adverse effects.

Dr. Golden: I was fascinated by the methionine data as well. In relation to that, I was wondering about the whole question of the other substrate for glutathione peroxidase, glutathione itself, and the problems that one can run into in glutathione production or regeneration in these biological systems. I am particularly thinking about conditions such as glucose-6-PD deficiency, nicotinic acid, riboflavin, or thiamine deficiency, where one has difficulty generating any NADPH for glutathione production; also in the production of glutathione-S transferases and whether there is a rebound in patients who have a low glutathione-S transferase. Maybe the provision of methionine can lead to more glutathione or glutathione-S transferase.

Dr. Lombeck: You know that nearly all the experiments with glutathione transferase were done in animal models. Glutathione transferase activities exhibit great species and tissue differences. Glutathione-S transferase can be measured in human platelets, but for this we need a lot of material. Therefore few data about human glutathione transferase activities have been published so far.

Dr. Diplock: We have done some experiments on an animal model using liver glutathione-S transferase; I think the important point here is that in selenium deficiency we see a substantial increase in the activity of glutathione-S transferase. The function, or a major function, of the transferase is dealing with carcinogens, and I think that it is particularly important to appreciate that when the level of selenium intake increases the level of glutathione-S-transferase may be depressed. This of course means that the very enzymes you need in order to protect yourself from environmental toxins and carcinogens have a lower activity.

Dr. Lombeck: That is what I meant. We know little about the interaction of selenium with other metabolic pathways. If we disturb an unknown balance by a normal or high selenium intake given orally or parenterally, we could run into more troubles than with a so-called marginal selenium deficiency state.

Dr. Gebre Mehdin: Is it always necessary to give vitamin E along with selenium supplementation?

Dr. Diplock: The level of vitamin E intake appears to be adequate in most human populations in the West.

Dr. Zlotkin: Just one further complicating factor, especially for the American audience, is that unlike the parenteral formulations available in Europe and Canada which contain cystine few of the American formulations contain cystine. Glutathione certainly is a major cystine reservoir; therefore the small preterm infant whose ability to endogenously produce cystine is limited may also be limited in his ability to produce glutathione.

NOTE ADDED IN PROOF

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