

PROCEEDINGS OF THE INTERNATIONAL WORKSHOP ON

KASHIN-BECK DISEASE

AND

NON-COMMUNICABLE DISEASES

Sponsored and organized by:

UNEP, ILO, WHO

International Programme on Chemical Safety (IPCS)

People's Republic of China

Ministry of Public Health (MPH)

WORLD HEALTH ORGANIZATION, 1990

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**OPENING ADDRESS BY DR GUO ZIHENG, VICE-MINISTRER
OF THE MINISTRY OF PUBLIC HEALTH OF
THE PEOPLE'S REPUBLIC OF CHINA**

The International Workshop on Kashin-Beck Disease and Non-communicable Diseases sponsored and organized by the International Programme on Chemical Safety, the World Health Organization and the Ministry of Public Health of China has started today at the Institute of Health of the China National Centre for Preventive Medicine. On behalf of the Ministry of Public Health, I would like to warmly welcome our colleagues from World Health Organization and the International Programme on Chemical Safety and all the participants from different countries.

Specific disease with chemical etiology are hazardous to human health to a varying extent in different parts of the world. The causes of some of these diseases are very complicated. With the development of science and technology, scientists in different countries have made great progress in studying their etiology and measures of control. Our scientists have also made great efforts in this field and obtained certain achievements, such as the prevention of Keshan disease by selenium intervention, studies on the prevention and control of Kashin-Beck disease, the prevention of endemic goiter by iodized salt, the control of endemic flourosis, the prevention of human selenosis and so forth. All these results have greatly promoted a better health of our people. I believe these experiences will also be helpful to other nations of the world.

As from tomorrow, our scientists engaged in the study and control of Kashin-Beck disease and Keshan disease will present their research findings, including the epidemiology, clinical features, geographical characteristics, pathology and biochemistry of Kashin-Beck disease. Next week, the Workshop will discuss the criterion for establishing chemical etiology of specific disease, according to the arrangements made by the International Programme on Chemical Safety and the World Health Organization.

I believe at this Workshop, our scientists and those from other countries will take this unique opportunity to exchange information and experiences to their mutual benefit.

Undoubtedly, this Workshop will promote studies on the chemical etiology of specific disease in China and make a greater contribution to people's health.

**SUMMARY REPORT ON THE MEETING ON KASHIN-BECK DISEASE
ORGANIZED BY THE INTERNATIONAL PROGRAMME ON CHEMICAL
SAFETY (IPCS), THE WHO REGIONAL OFFICE
FOR THE WESTERN PACIFIC, AND THE MINISTRY OF
PUBLIC HEALTH OF THE PEOPLE'S REPUBLIC OF CHINA**

BEIJING, 28 OCTOBER - 1 NOVEMBER, 1985

Background

Kashin-Beck disease was first described by Kashin and later by Beck in the Urov River area eastern Siberia in the last century. Further research and practical measures in the USSR resulted in the successful control of the endemic disease in this area.

In China, this disease, which has probably existed in the endemic regions for centuries, was first studied during the first half of this century. However, it is only during the last decades that this health problem, prevalent in 14 out of 29 provinces, municipalities, and autonomous regions of mainland China, has received appropriate attention. The basic pathological change in this disease, commencing mainly in children, is the degeneration and necrosis of articular cartilage and the growth plate, which can result in permanent disability. Present estimates indicate that about 2 million people are affected by this disease and that more than 30 million people living in the endemic areas of China are at risk of acquiring it. The disease seems to be linked with the consumption of food produced in the endemic areas and is possibly related to the presence of certain specific chemicals in food and / or water. The People's Republic of China, expressing interest in the WHO / ILO / UNEP International Programme on Chemical Safety (IPCS), underlined the importance of this disease and, for this reason, a joint meeting was convened by the Ministry of Public Health of the People's Republic of China, the International Programme on Chemical Safety, and the Western Pacific Regional Office of the World Health Organization to review the status of present knowledge on this disease and its possible etiology.

Scope of the Meeting

The meeting was held at the Institute of Health of the China National Centre for Preventive Medicine, in Beijing, from 28 October to 1 November, 1985. After the opening by Professor Chen Chunming, Director of the China National Centre for Preventive Medicine, the meeting was addressed by Dr. Guo Ziheng, Vice-Minister of the Ministry of Public Health of the People's Republic of China,

who explained the importance of the meeting for the promotion of studies on the chemical etiology of specific diseases. The Vice-Minister stressed the importance of the fact that the meeting on Kashin-Beck disease would be followed by a second IPCS meeting on criteria for establishing the chemical etiology of specific diseases as a basis for their prevention, which would be hosted by the same Institute, during the following week. Dr E. Goon (WHO Representative, Beijing) and Dr J. Parizek (IPCS/WHO) addressed the meeting on behalf of WHO. Professor Chen Chunming was elected Chairperson, Professor V.A. Nasonova and Dr Niu Shiru, Vice-Chairmen, and Dr Chen Junshi and Dr O.A. Levander, Rapporteurs.

The papers on Kashin-Beck disease presented at the meeting by Chinese scientists reviewed:

- (a) the epidemiology, geographical distribution, and clinical features;
- (b) pathological and biochemical features; and
- (c) prevention of the disease

In addition, a paper expressing the present views of a leading scientist from the region in the USSR where Kashin-Beck disease is endemic was presented, at the request of organizers of the meeting from the People's Republic of China.

During the last day, related papers were presented on Keshan disease and on endemic human selenosis in China.

Summary of the Results Presented at the Meeting: the Current Situation of Kashin-Beck Disease

I. Main epidemiological characteristics

(a) Geographical distribution

The disease mainly occurs in the mountainous and hilly areas of temperate forest and forest-steppe zones and is very rarely observed in the plains. The climate in endemic areas usually includes a long period of frost and big differences between the daily minimum and maximum temperatures. Affected villages are distributed in small foci within the endemic area. The prevalence in neighbouring villages can vary significantly and can change with time. New patients can be found in villages where patients have not been reported before. In rare cases, the prevalence in certain severely affected villages can decrease "spontaneously"; so that, after about 10 years, no new cases occur.

(b) Age of patients

The disease mainly develops in 5 to 13-year-old children, very few new cases are seen among adolescents and adults. In heavily affected areas, some new cases might be only 2-3 years of age, whereas, in lightly affected areas, some new cases may not occur until 10 years of age.

(c) Family characteristics and etiology

Within endemic areas, patients mainly occur in farming families. Children of farm (government-owned) employees are also vulnerable. Only very few cases occur in "professional" families. However, patients may occur in professional families, if they consume a large amount of the staple grains (corn, highland barley, wheat) produced in endemic areas. The incidence of new and severe cases of the disease may decrease after changing the staple grains.

In contrast with other endemic diseases, such as Keshan disease, it is important to note that, in some sites where Kashin-Beck disease is endemic, in recent years, the number of recognized cases is increasing. The etiology of the disease remains obscure, but has been linked with certain chemical constituents of food and possibly water. In China, Kashin-Beck disease mainly occurs in low-selenium areas, but selenium deficiency alone is not sufficient to explain the disease. The possible role of fungal (*Fusarium oxysporum*) or bacterial toxins was considered in some of the papers presented at the meeting.

2. Clinical signs and symptoms

In the clinical development of the disease, weakness is followed by joint stiffness. Limitation of flexion of the index, middle, and ring fingers towards the palm can be detected followed by limitation of elbow joint movement and joint enlargement and deformity. Signs are similar to those of primary osteoarthritis. In China, the disease has been clinically classified as follows:

-Early stage: flexion of distal joint part of fingers, bow-like fingers, and pain in knee and angle joints;

-First degree: enlargement and crepitus of small joints;

-Second degree: short fingers, enlargement and dysfunction of medium-sized joints;

-Third degree: enlargement and dysfunction of large joints (stunted growth).

"Muscular dystrophy" can be seen during the course of the disease.

X-ray examination reveals:

(a) the calcification line becoming blurred, thin, interrupted, and disappearing as is characteristic for chondronecrosis;

(b) defects of the metaphyseal plate, the end of distal bone, and carpal and metacarpal bones following chondronecrosis;

(c) deformity of the epiphysis, synostosis of the epiphyseal plate resulting from necrosis of the whole layer of the epiphyseal plate; and

(d) enlargement of joints and stubby fingers, the effects of secondary osteoarthritis.

Anatomical pathological examination reveals that lesions mainly involve hyaline cartilage. The epiphyseal cartilage, articular cartilage, and epiphyseal growth plate are the most affected sites.

Changes are "dystrophic" in nature. The most important pathological feature is multiple localized chondronecrosis in the deep portion of cartilage tissue. Chondronecrosis of the growth plate may result in disturbance of endochondral ossification and may even induce the early closure of the epiphyseal growth plate. The growth of long bones ceases and causes short fingers and toes, short limbs, and even stunted growth. The chondronecrosis in articular cartilage may induce scar formation and result in bony enlargement, osteophyte formation, and disfiguration of the joints affected ("endemic osteoarthritis deformans"). Disturbances of endochondral ossification of the growth plate and advanced secondary osteoarthritis are the two cardinal manifestations of the disease.

Biochemical research has indicated a series of metabolic disorders including:

- (a) changes in cartilage metabolism, mainly affecting chondroitin sulfate and proteoglycan;
- (b) changes in clinical chemistry, including the plasma enzymes (e.g., alkaline phosphatase, glutamate-oxalacetate transaminase, hydroxybutyrate dehydrogenase) and the urinary excretion of creatinine and hydroxyproline;
- (c) changes in the composition of certain lipids and selenium in the red blood cell membranes; and
- (d) characteristics of low-selenium status, including changes in glutathione peroxidase activity, tocopherol content, and lipid peroxides in plasma.

3. Preventive measures

The following techniques for the prevention of Kashin-Beck disease have been studied by Chinese scientists and were discussed at the meeting. All these approaches have been used in studies on subpopulations of varying sizes, and positive results from individual studies were reported at the meeting.

(a) Comprehensive prevention

Encourage the children to diversify their foods (variety and source), offer two servings / week of soup containing soybeans, sea weed, and multiple vitamins. Purify drinking-water (use deep well water with precipitation) and improve personal hygiene. It has been reported, that with this scheme, the prevalence of X-ray changes and of changes in the metaphysis decreased.

(b) Selenium intervention

Three different measures have been used for selenium supplementation, including:

- (i) oral administration of sodium selenite at 1 mg / week or 2 mg / week to children of 7 - 10 years and 11 - 13 years, respectively;
- (ii) selenized table salt containing a sodium selenite concentration of 16.7 mg / kg; and

(iii) spraying sodium selenite solution on wheat crops at the rate of 15 g/ha (1 g/mu)^① Using these measures, incidence rates dropped within one year, and recovery from changes in the metaphysis was noted.

(c) Water quality

Water can be purified by precipitation, filtration and chlorination. This technique and / or the use of deep groundwater have both been associated with an improvement in clinical symptoms and a reduction in incidence rate.

(d) Change of grains

Changing the locally produced grain for grains produced in other areas led to the prevention of new cases, a decreased incidence of X-ray changes, and better recovery from metaphyseal changes.

In endemic areas of the USSR, significant reductions in the prevalence rates and the severity of Kashin-Beck disease were connected with special governmental and public health measures. The most effective measures were the organization of population migration from endemic to non-endemic regions, the importation of food from non-endemic areas, thermal treatment of local products and water, annual checkups on children and adult populations in endemic regions by public health personnel, and the development of appropriate health education programmes. On the basis of the theory of biogeochemical provinces linking certain endemic diseases with the geochemical characteristics and quality of the soil and water in certain areas, studies in the USSR related very high phosphate and manganese contents in the soil, food, and drinking-water in the endemic area with Kashin-Beck disease. Reducing the contents of these chemicals in food and water is part of the present preventive measures in the USSR. As mentioned in the discussion, differences in selenium levels were not detected between the affected and non-affected areas studied in the USSR.

4. Conclusions and recommendations

(a) Kashin-Beck disease is a highly disabling disease of permanent character that mainly affects children. In China alone, about 2 million people are affected at present, and more than 30 million people living in the endemic areas at direct risk of acquiring the disease. All the evidence indicates that the disease is caused by a certain quality of the environment specific for the endemic regions, which encompass a large part of China and parts of neighbouring countries. Several studies indicate the involvement of certain chemicals in food and water (manganese, phosphorus, mycotoxins, microbial toxins, humic acid, etc.), as well as nutritional imbalance, in particular, selenium deficiency, as factors contributing to the etiology of this disease.

^①a. 1 mu = 0.067 ha. From: Beijing Language Institute (1979), Chinese English Dictionary, Commercial Press, Beijing

A coordinated effort is needed to elucidate the etiology of this disease, which is endemic in a very specific area of Asia. Cooperation among countries affected by the disease should be supported by international organizations with the aim of developing uniform diagnostic criteria and protocols for epidemiological studies to test the above hypotheses and elucidate more precisely the cause of the disease and contributing factors. Exchange of information on effective measures for the prevention of the disease should be another component of such intercountry cooperation, as well as the training of personnel needed for preventive, diagnostic, and therapeutic activities. As a first step in this direction, exchange of scientists between the countries with endemic regions should be supported as well as the development of methods and their use in the above-mentioned cooperative efforts.

Such activities would significantly contribute towards chemical safety and the solution of a problem affecting populations in several neighbouring member states.

(b) Kashin-Beck disease primarily affects the joint. However, the relation with other osteoarthroses is not clear, and the possible etiological factors involved are not known.

Thus, in cooperation with non-governmental and other organizations, a meeting should be convened to define specific characteristics, identify risk factors, and consider possibilities for the prevention and control of this disease and its possible relationship with other osteoarthroses.

(c) In spite of the public health significance of Kashin-Beck disease, the disease does not seem to be sufficiently recognized in the medical literature. In this respect, the data presented at this meeting was considered highly informative.

It was recommended that the co-sponsoring organizations should explore the possibility of publishing the full proceedings of this meeting as a monograph.

GEOGRAPHIC DISTRIBUTION OF KASHIN-BECK DISEASE IN CHINA AND THE RELATION OF ECOLOGICAL CHEMICO-GEOGRAPHY TO ITS OCCURRENCE

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Introduction

This paper reports the geographical distribution and epidemiology of Kashin-Beck disease in China. The disease is found in a belt running from the northeast to the southwest, with temperate, warm-temperate forest, and forest-steppe environment passing through the centre. The geographical distributions of Kashin-Beck disease and Keshan disease are somewhat similar, but not the same. The natural environments of Kashin-Beck disease affected areas could be divided into three types and eight groups. A low-selenium environment was a common feature of areas affected by the disease. Nevertheless, there must be another factor acting together with selenium-deficiency to cause the disease.

Kashin-Beck disease is regarded as an endemic and multiple osteoarthropathy. Although the disease was first recorded in 1664, its pathogeny is still not clear.

Since the distribution of this disease shows marked regional variation, and its occurrence appears to be related to abnormal environmental factors, the mechanism of pathogenesis was explored from the point of view of disease ecology. Extensive investigations and chemical analyses were conducted, with an emphasis on chemical geographical environment, with comparisons between the main affected areas and non-affected areas in the country. The results reinforced the belief that the external environment holds the clue to the pathogenicity of Kashin-Beck disease.

Geographical Distribution of Kashin-Beck Disease

Even though some data on the geographical distribution of Kashin-Beck disease was accumulated before the founding of the People's Republic of China, by the end of 1960's no systematic research had been carried out. Now it is known that this disease is distributed mainly in 303 counties in 15 provinces or autonomous regions, such as Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Beijing, Shandong, Shaanxi, Henan, Shanxi, Gansu, Qinghai, Sichuan, Xizang and Taiwan. The geographical distribution is shown in Fig. 1. It shows that Kashin-Beck disease occurs mainly in a wide belt running from the northeast to the southwest of China (The Group of Endemic Disease and Environment,

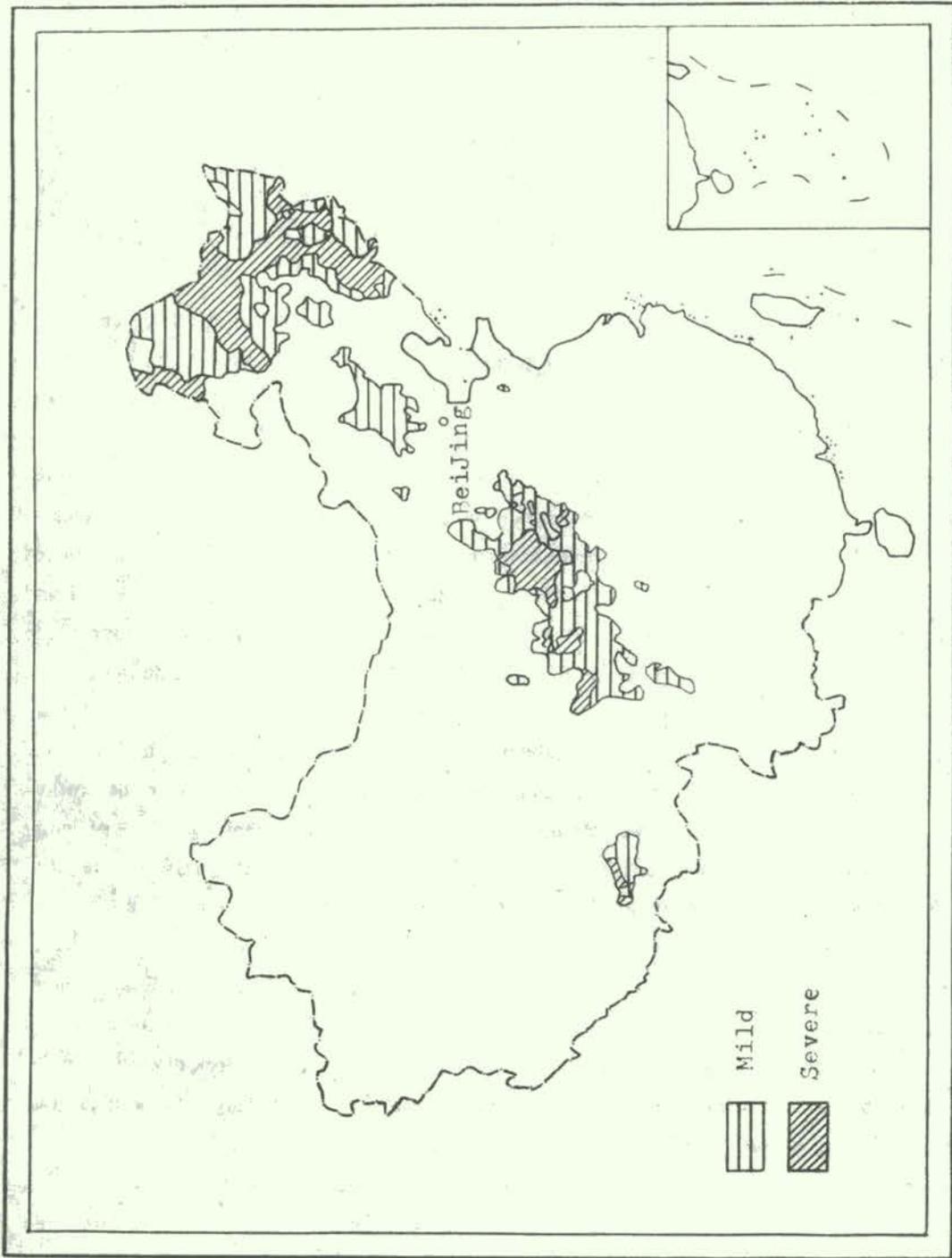


Fig.1. Geographical distribution of Kashin-Beck disease in China

1979). The areas with largest numbers of cases are located in the regions of Changbai mountain, Da Xingan mountain, Xiao Xingan mountain, Loess plateau, and the mountains in west Sichuan. This regular geographical distribution pattern suggests that the disease is closely associated with certain characteristics of the environment (The Group of Endemic Disease and Environment, 1979; Tan, 1982)

Geographical Epidemiology of Kashin-Beck Disease

Soon after the disease was first reported, research workers became aware of its close relation to the geographical environment. Eroded land forms, cold climate, moist soils, certain drinking-water sources, and some vegetation types were at first thought to be related to the occurrence of this disease. These findings led further studies of the cause of Kashin-Beck disease.

It is clear that the geographical distribution of the disease could not be the result of any one of the macroscopic factors, such as land forms, soil or vegetation. So it was necessary to study the interaction between the geographical zones and the complex features of the natural environment in the affected and unaffected areas. In this way, it was hoped that the essential differences between the affected and unaffected environments could be distinguished, and the specific characteristics of the environment in affected areas discovered. This type of study should then provide clues to guide studies of pathogenicity.

By means of comprehensive geographical analyses, we first established the general epidemiological pattern, namely that Kashin-Beck disease occurred in a belt running from the northeast to the southwest in areas with mainly temperate (or warm temperate) forest and forest-steppe soil, in particular where the dark brown forest soil (or dark brown earth), black earth, brown earth, drab soil, hei lu tu (black loess soil), and some forest-steppe soil, are found. The belt is a wide transitional zone situated between the south-east tropical and subtropical, red-yellow earths environment and north-west arid and semi-arid desert-steppe soils environment (The Group of Endemic disease and Environment, 1979, 1981, 1985). The typical steppe and desert to the northeast and the typical red-yellow earth belt to the southeast are free of the disease. The geographical distribution of Kashin-Beck disease is similar to that of Keshan disease, but not identical, because while in most cases the distributions of the two diseases are coincident in northern China, this is not so in the southwest of China. In some places only one of these diseases exists, and usually it is the Keshan disease that occurs in isolation. In comparison with Keshan disease, Kashin-Beck disease tends to occur more frequently in the temperate forest and forest-steppe soils environments. For example, in some parts of the Sichuan and Yunnan provinces soils similar to brown-drab earth (brown earth-like purplish earth, red drab earth and so on) are

found that have some properties similar to the real brown-drab earth found under the temperate zone conditions. In these areas Kashin-Beck disease is not found, but only Keshan disease.

Within the affected belt, the disease does not occur everywhere; "healthy islands" may be present in some affected areas, and in unaffected areas, some cases of disease may occur. This patchy distribution of the diseases is due mainly to local differences in the natural environment. For example, large alluvial plains, such as the Weihe, Liaohe and North China Plains, are the catchment areas of large rivers, with many tributaries and are mostly free of the disease, and in the west of Sichuan and southeast of Xizang, Kashin-Beck disease occurs only at a certain altitude where the mountain brown earths or drab earths are usually present. In addition, the history of the region and the regional economic development influence the distribution, especially the existence of "healthy islands" within affected areas.

The prevalence of the disease appears to show regional variations. For example, in the loess plateau, in the affected areas the disease prevalence is relatively moderate and stable in the "yuans", a particular kind of land form in the loess plateau, whereas areas with much higher prevalence are mainly located in the loess hilly regions with "Liang", "Mao"; in northeastern China, the areas with high prevalence are usually found on both sides of the mountain ridges.

Factors of human geography, such as standard of living, the local customs of the inhabitants, and the types of crops grown, may also affect the prevalence of Kashin-Beck disease and should not be ignored. In general, the more wheat, rice or beans that the residents consume the lower the prevalence of the disease (The Scientific Investigation Group of KBD in Yongshou County, 1984).

The Types of Natural Environment in Kashin-Beck Disease Areas

On the basis of the soil map of China and the geographical epidemiological features mentioned above, the occurrence of Kashin-Beck disease in China can be divided into three belts as regards to the natural environment: two unaffected belts and one affected belt. The affected belt comprises three types of environment and eight subtypes (Fig.2) as follows:

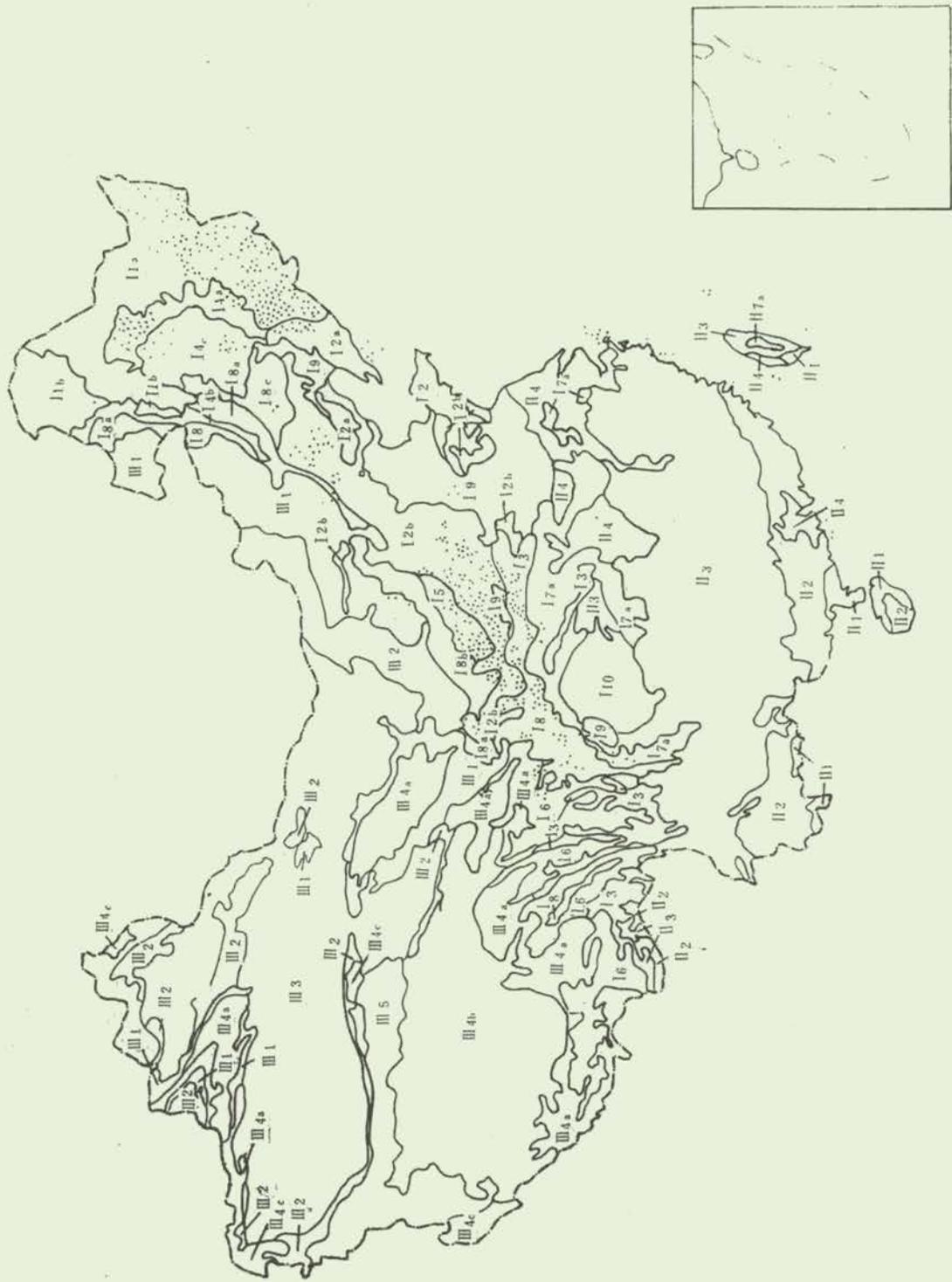


Fig.2. The environment types of Kashin-Beck disease in China

Legend:

Affected Belt

A. Affected Environment

Temperate Forest Soil Environment

I₁ Dark Brown Earth Environment

a. Dark Brown Earth; b. Brown Taiga Soil

I₂ Brown-Drab Earth Environment

a. Brown Earth; b. Drab Soil

I₃ Mountain Vertical Zone Brown - Drab Earth Environment

Temperate Forest - Steppe, Meadow Steppe Soil Environment

I₄ Black Soil Environment

a. Black Soil; b. Grey Black Soil; c. Meadow Chernozem

I₅ Hei Lu Tu (Black Loess Soil) Environment

I₆ Subalpine Vertical Zone Meadow Steppe Environment

B. Transition Environment

I₇ Yellow-Brown Earth Transition Environment

a. Yellow-Brown Earth; b. Yellow-Drab Soil

I₈ Humid Steppe Soil Transition Environment

a. Chernozems; b. Putong Hei Lu Tu; c. Hei Ma Tu; d. Chestnut

C. Unaffected Environment

I₉ Alluvial Plain Chao-tu Environment

I₁₀ Purplish Soil Environment

South-East Unaffected Belt

II₁ Tropic Laterate Environment

II₂ South Subtropic Laterate Red Soil Environment

II₃ Middle Subtropic Red-Yellow Earth Environment

II₄ Subtropic Alluvial Plan Paddy Soil Environment

North-West Unaffected Belt

III₁ Temperate Arid Steppe Soil Environment

III₂ Temperate Desert-Steppe Soil Environment

III₃ Temperate Desert Soil Environment

III₄ Alpine-Subalpine Meadow Steppe Soil Environment

a. Cao Zhan Tu; b. Sha Ga Tu; c. Ba Ga Tu

III₅ Alpine Desert Soil

I. Temperate forest soil environment

There are three subtypes:

(a) Dark brown earth environment

Most of the affected areas in northeastern China are of this subtype. The soils are mainly dark brown earth, Baijiang Tu (bleached earth), or brown Taijia soil, and broad-leaved forest with secondary oak forests.

(b) Brown earth - drab soil environment

This subtype is found in the east of the loess plateau and some parts of Liaoning, Hebei, Henan, and Shandong provinces, situated to the south of subtype (a).

The temperature is higher than that found in areas of subtype (a). The humidity gradually decreases from east to west in this area, the soils change from brown earth to drab soil, and the vegetation from deciduous broad-leaf forest to semi-arid forest.

(c) Mountain vertical zone brown-cinnamon soil environment

Affected areas with this environment subtype are found mostly in the Qinling mountain, the west of Sichuan, and the southeast of Xizang. The occurrence of the diseases in these areas is related to the presence of mountain brown-cinnamon soils.

II. Temperate forest-steppe, meadow-steppe soil environment

Within this environment type, three subtypes are found, situated to the west of type I. The precipitation is lower than in areas with type I environment.

(a) Black soil environment

Affected areas with this environment subtype are found in the west of both Heilongjiang and Jilin provinces. The major land forms are mountains and slopes. Soils here range from black soil, grey-black soil, to some meadow chernozems, and some of the humid steppe soils.

(b) Hei lu-tu (black loess soil) environment

The affected areas with this subtype are in north Shanxi, mainly located in clayey Hei lu-tu. The areas of high prevalence are mostly found where the land form is strongly eroded and the subsoil paleosol (drab soil) is exposed because of erosion.

(c) Subalpine, vertical zone, meadow-steppe environment

The southern of Xizang has this subtype environment with a high altitude and a cold and humid climate; and upland barley is the main crop.

III. The transition environment

The type is transitional between the affected belt and two unaffected belts situated on both sides of it. The prevalence of Kashin-Beck disease is very light here, and it occurs only in a few places.

(a) Yellow-brown earth transition environment



Fig. 3. Sampling regions in China

1. Desert Soil Series Environments
2. Desert-Teppe Soil Series Environments
3. Mean of the Middle Belt
4. Temperate Dark Brown Earth, Black Earth, Grey Forest Soil
5. Warm Temperate Brown Earth, Drab Soil, Hei Lu Tu
6. Purplish Red brown Soil, Red Drab Soil
7. Cinhai-Xizhang SW Mountain Brown-Drab, Meadow Steppe
8. North Subtropic Yellow-Brown Earth Environments
9. Middle Subtropic Red-Yellow Soil Environments
10. South Subtropic Laterization Red Soil Environments
11. Tropic Zone Laterite Soil Environments

This environment subtype is found in some places in the north of Sichuan.

(b) Humid steppe soil transition environment

This subtype occurs on the northwestern border of the black soil-grey-black soil, Hei lu-tu, and the disease occurs only in limited pockets.

Among the environment types and subtypes described above, the disease occurs mainly in areas with types I and II, and areas with environment subtypes I(a), I(b), II(a), and II(b) contain most of the affected localities. So the overwhelming majority of Kashin-Beck patients were found in areas with these environment subtypes (The Group of Endemic Disease and Environment, 1985).

The Features of Ecological Chemo-Geography in the Affected Areas

Ecological chemo-geography deals mainly with the geographical distribution, transportation, and transformation of chemical elements, especially elements related to living organisms, in the geographical environment and their effects on these organisms, especially their effects on the health of human subjects (Tan, 1982; Tan, et al., 1984, 1987). For several years we have studied the chemo-geographical environment types and gathered samples in relation to the different natural environment types and different prevalence levels discussed in the previous section (Fig. 3).

At each site, we collected samples of soil, grains, and human hair, as these materials reflect the ecological transfer pathway of chemical elements from the environment to the human body. The concentrations of the major and trace elements of biological significance in the various samples were determined and the sieving election study of multi-elements in relation to the disease was conducted. These studies showed that a low concentration of selenium in the geographical environment was a common feature of areas where Kashin-Beck disease occurred, as well as in areas where Keshan disease was prevalent. In fact, the distribution of areas of low environmental concentrations of selenium forms a belt in China, which coincides with the belt of high Kashin-Beck prevalence (The Group of Endemic Disease and Environment, 1981; 1985). The regions with a high environmental concentration of selenium coincide with the regions that are unaffected by the disease (Table 1, Table 2 and Fig. 3).

Within the affected belt, either the selenium concentration in the soil or the concentration in various food grains were lower than those in the two unaffected regions. The selenium content in the hair of the population in low selenium belt was also low (Table 1). In general, the total selenium content in

①The Group of Endemic Disease and Environment, Institute of Geography, Academia Sinica. The Investigation Reports on "Water-Land" Pathogeny of Keshan Disease, unpublished. 1976.

soils within low selenium belt was less than 0.150 mg/kg, in food grains less than 0.025 mg/kg, and in hair less than 0.200 mg/kg. The correlations between the concentrations of other elements and the prevalence of Kashin-Beck disease were not as clear as in the case of selenium (The Group of Endemic Disease and Environment, 1981, 1985).

Table 1. Comparison between the selenium concentrations (mg/kg) in soil, grains, and human hair from different parts of China^a

Belt	Region ^b	Soil	Maize	Rice	Wheat	Hair
NW belt		0.19(29)	0.049(69)	0.087(25)	0.106(157)	0.379(371)
Middle belt		0.13(80)	0.016(230)	0.022(120)	0.021(272)	0.129(1412)
	KS / KBD mixed area	0.17(5)	0.014(8)	0.012(5)	0.014(56)	
	KS area	0.09(16)	0.013(55)	0.011(51)	0.018(72)	0.085(851)
	KBD area	0.09(15)	0.009(3)		0.011(27)	
	No disease	0.09(23)	0.019(91)	0.031(64)	0.026(117)	0.187(597)
SE belt		0.23(77)	0.053(16)	0.063(256)	0.052(71)	0.378(245)

^a Figures for the soil selenium concentration are geometric means; the other figures are arithmetic mean. The figures in brackets indicate the sample sizes.

^b KS = Keshan disease; KBD = Kashin-Beck disease.

Further studies were made on variations in selenium concentrations in soils, food grains, and hair in various natural environment in the low selenium belt and the two relatively high selenium belts. In addition, variations in selenium concentrations were investigated in affected and unaffected areas of low selenium belt. These studies showed that the selenium concentrations in soils, food grains, and hair increases from low selenium belt southeastwards to the tropical lateratic environment, and also from the low selenium belt northwestwards to the desert environment (Table 2). It should be noted that within the low selenium belt, the selenium concentrations in the soil, wheat, maize, rice, and hair varied in different environments and in areas with different prevalence of Kashin-Beck disease. It should also be pointed out that within the low selenium belt there are some places where no Kashin-Beck disease occurs, even though the selenium concentrations in the soil, food grains, and hair are low (Table 2).

Statistical examination of the selenium concentrations in the soil, food grains, and hair (Tan, et al., 1987) showed that most of the correlations tested were significant (Table 3).

Table 2. Selenium concentrations (mg / kg \pm S.D.) in food grains and human hair in different parts of China

Belt	Region ^a	Maize	Wheat	Rice	Hair
NW belt (Unaffected)	1	0.052 \pm 0.031 (66)	0.128 \pm 0.094 (135)	0.091 \pm 0.046 (19)	0.470 \pm 0.091 (387)
	2	0.029 \pm 0.014 (3)	0.057 \pm 0.020 (15)	0.040 (5)	0.462 \pm 0.122 (23)
Middle belt (Affected)	3	0.016 \pm 0.001 (58)	0.017 \pm 0.001 (15)	0.021 \pm 0.005 (6)	0.10 \pm 0.054 (223)
	4	0.016 \pm 0.004 (119)	0.017 \pm 0.005 (98)	—	0.151 \pm 0.082 (697)
	5	0.016 \pm 0.004 (44)	0.023 \pm 0.006 (50)	0.018 \pm 0.007 (67)	0.129 \pm 0.059 (492)
	6	0.008 \pm 0.001 (11)	0.010 \pm 0.002 (20)	0.016 \pm 0.012 (8)	0.158 \pm 0.104 (101)
SE belt (Unaffected)	7	0.039 (7)	0.039 \pm 0.018 (13)	0.040 \pm 0.019 (25)	0.383 \pm 0.130 (31)
	8	0.044 \pm 0.024 (8)	0.055 \pm 0.029 (36)	0.060 \pm 0.030 (105)	0.333 \pm 0.079 (143)
	9	0.070 \pm 0.021 (4)	0.061 \pm 0.028 (22)	0.077 \pm 0.025 (106)	0.493 \pm 0.062 (30)
	10	0.090 (1)	—	0.101 \pm 0.025 (16)	0.491 \pm 0.085 (41)
	11	—	—	—	—

^a The numbers 1–11 refer to the areas shown in Fig. 3.

Thus it is clear that environmental levels of selenium have a close relationship with levels in human hair.

The factors affecting the levels of selenium found in the geographical environment in China have also been studied. The selenium concentration in the soil and the nature of the deposits are clearly important. The level of selenium in environment mainly depends on the selenium sources and their resistance to leaching, erosion, etc. In the relatively high selenium belt in north-west China, selenium is retained in soil because of the dry climate and the low level of leaching. In the red-yellow soil in the relatively high selenium belt in southeast China, the soils retain selenium because they contain high levels of kaolin and colloidal ferro-manganese oxides with a strong absorption capacity.

Table 3. Correlation coefficients between the selenium concentrations in the soil, food grains and human hair^a

	Soil, water soluble Se	Rice	Wheat	Maize	Hair
Soil, total Se	0.341 ^c (328)	0.208 ^b (85)	0.503 ^c (113)	0.191 (99)	—
Soil, water soluble Se		0.276 ^b (84)	0.538 ^c (118)	0.202 ^b (102)	
Rice					0.698 ^c (66)
Wheat					0.658 ^c (93)
Maize					0.654 ^c (81)

^a The figures in brackets indicate the sample sizes.

^b $p < 0.05$.

^c $p < 0.01$.

The unaffected belt in the south-east of China is adjacent to the sea and the soils there get more selenium from the atmosphere than in the affected belt. Soils that have developed as a result of silic weathering retain the crops absorb little selenium because of the soil conditions (pH 7.0, etc.) and other environmental variables and as a result the ecological environment is low in selenium (Nielson and Bisbjerg, 1970, 1971).

The Environmental Pathogenesis of Kashin-Beck Disease

Many hypotheses have been proposed about the pathogenesis of Kashin-Beck disease. In the field of eco-chemico-geography, there are several hypotheses which involve calcium deficiency, sulphur deficiency, and excess strontium in combination deficient with calcium, etc. These hypotheses fit the facts only in some cases. In the present study, the only correlation found to be significant is that between a low selenium environment and the prevalence of Kashin-Beck disease. Thus these studies have shown that a low selenium environment seems to be an important factor causing Kashin-Beck disease. This conclusion is supported by the following facts: (1) Kashin-Beck disease occurs only in areas with a low selenium environment. The disease has not yet occurred in areas with a relatively high selenium environment. (2) The concentration of selenium in human hair was low in the areas with a low selenium environment. This indicated that the level of selenium metabolism was low. (3) A study of typical areas showed that the frequency of positive X-ray examinations in children affected by Kashin-Beck disease

in Yongshou county, Shanxi province, was significantly negatively correlated with the selenium concentration in materials from the ecosystem (Table 4). (4) It was reported that selenium supplementation of the diet is an effective measure for prevention and treatment of the disease (The Prevention and Treatment Cooperation Group of KBD of Xinzhuang, 1979; The Scientific Investigation Group of KBD, 1982; The Scientific Investigation Group of KBD in Yongshou County, 1984).

Table 4. The correlation coefficients between the rate of positive X-ray results and the selenium concentration in soils, food grains and human hair in Yongshou ^a

	Soil Total Se	Soil Soluble Se	Water Se	Maize Se	Wheat Se	Hair Se
positive rate	-0.266 ^c	-0.388 ^c	-0.187 ^b	0.336 ^c	-0.563 ^c	-0.487 ^c

^a Data reproduced from reference Tan et al., 1987.

^b $p < 0.05$.

^c $p < 0.01$.

Although the above evidence is clear, it remains open to question whether a low selenium environment is the only factor or even the dominant factor causing Kashin-Beck disease. The following questions remain to be answered: (1) Why does Kashin-Beck disease not occur in certain low selenium areas, especially in the east of Sichuan and north of Yunnan in southwest China, where Keshan disease has been reported? (2) What is the environment explanation of the fact that although both Keshan disease and Kashin-Beck disease are related to a low selenium environment, the areas affected by the two diseases do not always coincide? It appears that Kashin-Beck disease generally occurs in places with a colder climate, and a slightly lower selenium level in the environment and in human hair than are found in the Keshan disease areas, but these differences are not statistically significant. Therefore, it is possible that in addition to low selenium levels as a basic factor, there may be additional factors which combine with selenium deficiency to cause Kashin-Beck disease. We are studying these possible additional factors in low selenium environments, and are investigating the following ideas: (1) A deficiency or unbalance of some dietary vitamins or amino acids. There may also be differences in the non-staple foods (vegetables, etc.) between the low selenium areas that are unaffected by Kashin-Beck disease in south-west China and affected areas in northern China. The epidemiological investigation showed that Kashin-Beck disease often occurs under poor dietary conditions. (2) Abnormal concentrations of other biologically important elements in drinking-water or foods. It is possible that the level of some other elements could be deficient or out of balance, e.g., the concentration of a selenium antagonists might be

so high that selenium metabolism and function would be curtailed. During the investigations of Kashin-Beck disease in Yongshou county, progressive regression analysis demonstrated that apart from the significant correlation between the rate of positive X-ray examinations and selenium, some other elements such as molybdenum, barium, cadmium, lead, and iron could be considered as related elements. (3) Some toxins or other organic compounds in the environment that enter human body through the food chain should be considered as a third kind of compounding factor.

Therefore, in order to make further progress in studying the pathogenesis of Kashin-Beck disease, it will be necessary to investigate both the external environment and human metabolism. As regards the external environment it will be important to study mechanisms by which a low selenium environment may be set up in China. Another important task will be to investigate the influence of different combinations of elements in various low selenium environments on the prevalence of Kashin-Beck disease. Thirdly, it would be valuable to test different ways of increasing the selenium level in the food chain in order to prevent selenium deficiency and also perhaps Kashin-Beck disease.

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REFERENCES

- Antiepidemic Station of Henan Province, and Institute of Geography, Academia Sinica. Experiment of KBD treatment with Na_2SeO_3 and vitamin E and relation with pathogeny. *Henan Medicine*, 5: 4-6, 1980.
- The Group of Endemic Disease and Environment, Institute of Geography, Academia Sinica. The Keshan disease in China: A study of the geographic epidemiology. *Acta Geographica Sinica*, 34: 85-103, 1979.
- The Group of Endemic Disease and Environment, Institute of Geography Academia Sinica. Relationship between the distribution of Keshan disease and selenium content of food grains as a factor of chemical geographic environment. *Acta Geographica Sinica*, 36: 370-376, 1981.
- The Group of Endemic Disease and Environment, Institute of Geography, Academia Sinica. The relation of Keshan disease to the natural environment and the background of selenium nutrition. *Acta Nutrimenta*, 4: 175-181, 1982.
- The Group of Endemic Disease and Environment, Institute of Geography, Academia Sinica. Geographic

- distribution of selenium content in human hair in Keshan disease and nondisease zones in China. *Acta Geographica Sinica*, 37(2): 136-143, 1982.
- The Group of Endemic Disease and Environment**, Institute of Geography, Academia Sinica. The characteristics of geographic epidemiology for Kashin-Beck disease in China and its pathogenicity. *Scientia Geographica Sinica*, 5: 30-36, 1985.
- Nielson, G.G. and Bisbjerg, B.** Influence of pH and texture of the soil on plant uptake of added selenium. *J. Agri. Food Chem.*, 19: 1165-1167, 1971.
- Nielson, G.G. and Bisbjerg, B.** 1. The uptake of applied selenium by agriculture plants. 2. The utilization of various selenium compounds. *Plant and Soil*, 32: 382-396, 1970.
- The Prevention and Treatment Cooperation Group of KBD of Xinzhuang**, Ningxian county, Gansu Province. The observation of effects on selenium and vitamin E treatment for 224 cases of KBD as well as probation of pathogeny. *National Medical Journal of China*, 59: 169-171, 1979.
- The scientific Investigation Group of KBD.** Effect and mechanism of selenium in the prevention and treatment of 424 KBD patients. *Chinese Journal of Endemiology*, 1: 145-149, 1982.
- The Scientific Investigation Group of KBD in Yongshou county.** Beijing, People's Medical Publishing House, 1984, pp 55-103.
- Tan J.**, The Keshan disease in China: A study of ecological chemico-geography. *The National Geographic Journal of India*, 28: 15-18, 1982.
- Tan J., et al.** Selenium ecological chemico-geography endemic Keshan disease and Kashin-Beck disease in China. In: *Proceedings of the Third International Symposium of Selenium in Biology and Medicine (1984)* pp 895-876, 1987, AVI, New York

THE GEOGRAPHICAL DISTRIBUTION CHARACTERISTICS OF KASHIN-BECK DISEASE IN SICHUAN PROVINCE

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The Kashin-Beck disease has an obvious endemicity. Its occurrence and prevalence are related to the natural geographical environments. The endemic areas in China are located in a belt zone running from the northeast to the southwest of China, and they are mainly located in the transitional zone between the moist and dry zones (Research Group of Ecological Environment in Yongshou Investigation, 1984; Lin, 1983). The endemic areas of Kashin-Beck disease in Sichuan province are situated in the south-west section of the endemic zone in China. The disease is distributed not only in the medium and high hilly areas and in the high plateaus of northwest parts of Sichuan with temperate and cold climate, but it is also found in the subtropical areas in the east part of Sichuan in a form of focal distribution. Therefore, it is very important to investigate the geographical epidemiology of the disease.

Current Situation of Kashin-Beck Disease in Sichuan and Its Distribution

Sichuan province is located in the southwest of China, between 26-35 degrees of North latitude and 97-111 degrees of East longitude. It has an area of about 560 000 square kilometers, with a population of about 100 million, including 14 nationalities. It consists of 20 cities, prefectures, minority autonomous regions and 215 counties. Until now, Kashin-Beck disease has been found in 141 villages of 23 counties. These endemic areas are in Aba, Ganzhi and Liangshan Minority Autonomous Regions and Yaan, Daxian, Mianyang and Guanyuan prefectures. The prevalence of the disease varies in each area. Using the village as a unit, the lowest prevalence rate was 7.8 %, while the highest one was 96 % in the whole population. The endemic areas presented themselves in the form of pieces and foci which formed clear lines between endemic and non-endemic areas. We also found some cases of Keshan disease in some endemic areas of Kashin-Beck disease.

The existence of Kashin-Beck disease in Sichuan could be traced back to more than one hundred years ago. With the improving of living standard, the prevalence of the disease has been stable or even decreased in some degree. However, it is still in an active form in some endemic areas. Recently, several new endemic areas have been found in three counties, but with a low prevalence.

Relationship between Kashin-Beck Disease and Natural Geographical Environment

The endemic areas of Kashin-Beck disease in Sichuan are situated in 101-107 degrees of East longitude and 28-34 degrees of North latitude. The natural geographical environments of the endemic areas can be divided into four types, i.e. (1) hilly temperate zone with brown soil; (2) high mountains and high plateau with meadow soil in cold zone; (3) subtropical xerariumic zone with mountainous red soil; and (4) hilly subtropical zone with purple soil. The natural geographical environment of the endemic areas in Sichuan are complicated, which includes both the characteristics of endemic areas of north China and that of south China.

1. The relationship between Kashin-Beck disease and petrography

Kashin-Beck disease is mainly located in the western glosyncline area and its nearby places where the crust of earth moves relatively active, and only part of the eastern plain area proved to be endemic. On the whole, the disease is mainly distributed in the epimetamorphic rock's zone, while the quaternary sedimentary horizontal Chengdu plain is a non-endemic area.

2. The relationship between Kashin-Beck disease and the terrain and landform

Most of the endemic areas are located at over 1000 meters above sea level. Among them, the highest is at 3400 meters, which is much higher than the endemic areas in the northeast and northwest of China (Institute of Geography et al., 1972; Shanghai First Medical College, 1981).

The endemic areas are usually located in the areas of erosive low and middle hills. Serious endemics appears in the transition zone between the plateau and valley of high mountains; there are also some endemic foci in zorory hills and hilly-zone between mountain ridges in east part, but there is no case either in denudation steplike hill in the middle part or in the west plain. However, there are some disease-free "healthy islands" in the endemic areas and some "disease islands" in the non-endemic areas.

3. The relationship between Kashin-Beck disease and soil

In Sichuan, Kashin-Beck disease is mainly distributed in areas with brown or drab soil. It is in accordance with the distribution of the endemic areas in North China (Institute of Geography et al., 1972; Reseach Group of Ecological Environment in Yongshou Investigation, 1984). However, we have found this disease in Dazhu county with purple soil in the east part of Sichuan. It is a particular soil type which is quite different from that of the north endemic areas. The disease was never found either in the loess area, or in the red soil or the meadow soil areas (all belonging to the acid soil). Even in the Da Ba Shan mountains of Chengkou county (a brown soil area), no case of the disease was found.

4. The relationship between Kashin-Beck disease and climate

Most of the endemic areas are in the western mountains, valleys and plateaus that are in the tem-

perate and cold zones. The endemic areas in Aba and Ganzhi Minority Autonomous Regions and the neighbouring areas belong to this kind of geographical area. In subtropical and temperate zones, only Mianning and Dazhu counties are endemic areas. No cases of the disease were found in Yaan and Leshan, the two rainy centers of Sichuan, with an annual rainfall over 13000 mm, while the endemic disease was found in Hanyuan and Maowen where it did not rain much, with an annual rainfall of 473 mm. The disease prevalence was rather high in some part of these two counties.

5. The relationship between Kashin-Beck disease and vegetation

The endemic areas of this disease were found in the ropencedrymion, broadleaf mixed forests and dark coniferous forests which are similar to the north endemic areas of China (Institute of Geography et al., 1972). Meanwhile, the disease appeared in some part of meadow land and subtropical vegetation areas, which are different from the north endemic areas. In terms of the food crops in the endemic areas, in the cold zone of Aba and Ganzhi Minority Autonomous Regions, the main crops are Qingke (a kind of highland barley) and wheat. In Dazhu, Mianning and Hanyuan counties, the main crops grown are rice, with other crops, such as wheat, corn and sweet potatoes. In other endemic areas, the main food crops are corn and wheat.

The Relationship between Kashin-Beck Disease and Nutrition

In Sichuan, the endemic areas of the disease are found not only in agricultural areas, semi-agricultural and semi-pastoral areas, but also in pastoral areas. Their dietary patterns can be divided into 3 types: (1) the Qingke type; (2) the corn and wheat type; and (3) the rice type. The residents in pastoral areas take much meat, butter, tea and milk. But in the agricultural areas, the residents eat very little meat. All in all, the nutrition status of the residents in the endemic areas is in the medium level.

The analyses of water and grains show that the endemic areas are low selenium areas. The hair Se content of the children aged from 5 to 13 years was below 137.5 ppb (Deng, et al., 1983, unpublished; Deng and Yi, 1987). In the endemic areas of Aba county in Sichuan, there is a significant negative correlation between hair Se and the indices of activity and severity of the disease (Deng and Yi, 1987). However, we found no cases of Kashin-Beck disease in other similar low-Se areas, such as Nanbu and Dechang counties, the children's hair Se content being below 100 ppb.

Conclusion

The occurrence and prevalence of the disease are related to the natural geographical environments of the endemic areas. The relationship between Kashin-Beck disease and low-Se level needs further

investigation.

REFERENCES

- Institute of Geography, Chinese Academy of Sciences and Sichuan Provincial Sanitary and Anti-epidemic Station**, Investigation Report of Natural Geographical Environments and Etiology from Water and Soil in the Areas of Kashin-Beck Disease and Keshan Disease in Sichuan Province. p. 38-47, 1972 published by the Chinese Academy of Sciences, Beijing. (in Chinese)
- Lin, Y.F.**, A fifteen year (from 1968 to 1982) study on the advances of organic water and soil etiology on Kashin-Beck disease. p.2, published by Changchun Geological College, 1983, Changchun. (in Chinese)
- Research Group of Ecological Environment in Yongshou Investigation.** In: Monograph of Scientific Research on Kashin-Beck Disease in Yongshou (1979—1982). p. 60, 1084. People's Medical Publishing House, Beijing. 1984.
- Shanghai First Medical College**, Epidemiology. p.514, 1981, People's Medical Publishing House, Beijing. (in Chinese)

STUDIES ON THE PREVALENCE OF KASHIN-BECK DISEASE IN HEILONGJIANG PROVINCE

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Heilongjiang province is located in the northeast of China. In order to find out the distribution and severity of Kashin-Beck disease (KBD) in this province, a study was carried out in 1981.

Methods of Investigation

1. Scope and subjects

All the 78 cities and counties including state farms were surveyed. In the well-known endemic cities and counties, every household was investigated. In places which were considered as non-endemic areas, pupils in the primary school were sampled and investigated.

2. Criteria for endemic areas

A natural village is the basic unit of endemic area. The natural village was considered as endemic, if the clinical detection rate of Kashin-Beck disease was above 5%. The commune (formed by a number of natural villages) was considered as endemic, if there were more than one endemic natural villages in its area. A city or county could be designated as endemic, if there were more than one endemic commune or town.

3. Diagnostic criteria for KBD cases

The clinical diagnostic criteria in the book "Kashin-Beck Disease" (Institute of Kashin-Beck Disease of Heilongjiang province, 1980) were used in diagnosis of Kashin-Beck disease.

4. Data statistics

A city or county was used as a unit to calculate the prevalence rate and the standardized prevalence rate.

$$\text{Standardized prevalence rate (\%)} = B / A \times P$$

A—Population of the city or county

B—Population of the endemic city or county

P—Prevalence rate of Kashin-Beck disease

$$P (\%) = D / C \times 100\%$$

C—Total number of subjects examined in the city or county

D—Number of cases diagnosed as Kashin-Beck disease

The standardized rate was calculated by the direct standardization method described in the text book "Hygienic Statistics" (Sichuan Medical College, 1979). The population data used was obtained from the provincial census carried out in 1980.

Results

1. The general prevalence rate of Kashin-Beck disease in Heilongjiang Province

The population of endemic areas was 11,871,522, which accounted for 37.1% of the total population in this province. The subjects investigated were 12,871,619, which accounted for 38.4% of the total population in the province. The investigation showed that 692,117 cases of Kashin-Beck disease were diagnosed and the general prevalence rate was 2.08%, with a 95% confidence limit of 2.00 to 2.16%.

2. The distribution of the disease

The disease occurred in 66 cities and counties out of 78 cities and counties in Heilongjiang. Twelve cities and counties were free of the disease. There were 578 endemic communes, which accounted for 50.9% of total number of communes. There were 9918 endemic natural villages which accounted for 28.1% of total number of natural villages. In 13 cities and counties, more than 80% of the natural villages were endemic.

3. The prevalence rate of Kashin-Beck disease in endemic cities and counties

Of the 66 endemic cities and counties, 4 had prevalence rates more than 10%; 20 more than 1% and less than 5%; and 23 less than 1%.

The Characteristics of Geographical Distribution of Kashin-Beck Disease

According to the investigations carried out by the Institute of Kashin-Beck Disease of Heilongjiang Province in 57 cities and counties from 1963 to 1984 (Wang, et al., 1974), the Comprehensive Work Team on Endemic Disease in 8 counties, towns and 56 natural villages in 1971 (Comprehensive Work Team on Endemic Diseases, 1971), the Collaborative Epidemiological Study Team of Heilongjiang, Kirin and Liaoning Provinces in 4 cities, counties and 8 natural villages in 1979 (Li, et al., 1982), as well as the work by Wang and his colleagues in 1981 (Wang, et al., 1982), the characteristics of geographical distribution of Kashin-Beck disease in this province were as follows:

1. Focal and intercalated distribution of the disease

Despite of its wide distribution in Heilongjiang, the disease does not occur in all cities and counties. Even in an endemic city or county, there are natural villages free of the disease. The endemic

areas are focally distributed and intercalated into the non-endemic ones. For example, there are 45 endemic villages out of a total of 46 in Wuhu Commune. The only non-endemic village is Yin Sun, which is referred to as the "Healthy Island". Another example is the Xing Long and Xing Xian villages of Long Gua Commune, which are separated by a road of less than 5 meters wide. However, the prevalence rate of the former village diagnosed by X-ray was 63.0% and that of the latter was 11.1%. This information shows that the distribution of the disease has obvious endemicity.

2. Relative stability of distribution

Except the 35 state farms in the north and east of the province, where the disease occurred after 1968 or 1970 due to late cultivation, all the other areas have an endemic history of more than 30 years and some even over 70 years.

Although there are obvious seasonal and yearly variations in prevalence rate, the endemic areas can not easily be converted into non-endemic ones. This shows that the spatial distribution of the endemic areas is relatively stable.

3. Most endemic areas are mountainous and hilly lands

Although the distribution of the disease spread all over the mountains, hilly lands and plains, it occurs mainly in the mountainous or hilly land areas and rarely in the plains. In Heilongjiang province, the highest prevalence rate (5.16%) is in the north of Daxingan and Xiaoxingan mountains, and hilly lands. The second (2.81%) is in the east mountainous and hilly land. The lowest (0.71–0.20%) is in the Song-Nen and Sang Jian plains; and the prevalence rate of alluvial plain of Xingan mountain chain is between that of hilly land and plains (2.68%).

In the mountainous or hilly land, the endemic areas are usually located in valley between two mountains and in tributary valleys, and in plains; 90% of the endemic areas are in the waterlogged lowland.

4. Moist weather in endemic areas

The average rainfall is about 600–700 mm per year in the endemic areas and higher than in the non-endemic ones, which is about 400–500 mm. Conversely, the evaporation is lower in the endemic areas than in the non-endemic ones. In the autumn of 1969, the rainfall was more than 50% of the total rainfall of the whole year, which caused waterlogging and early frost. In 1970, the occurrence of the disease was significantly increased. For instance, in Guang Hui Village of Sang Zhi County, the morbidity rate of children with normal X-ray examination in 1969 had reached 94.4% of abnormal X-ray appearance in 1970. This reveals that climate has great influence on the disease.

5. Sticking and poor water permeability of soil in endemic areas

In the east and the north hilly lands, the soil of the endemic areas is mainly dark brown forest soil and grassy bog soil. In the west and the south endemic areas, the soil is mainly carbonate chernozem

soil. In these places, the organic matters in such soil are not fully decomposed, so the soil texture is sticky with poor water permeability.

6. Norelationship between the distributin of the disease and sources of drinking water

The sources of drinking water for the inhabitants of endemic areas are deep well, shallow well, spring, river, reservoirs etc. Up to now, the disease has not been found to be associated with any specific kind of drinking water source.

7. Most endemic areas are located in low selenium zone

Concerning the topography of most endemic areas, chemical elements are easy to be eroded and washed away. This results in the lack of some elements in local environment. Through the analysis of chemical elements in soil, water and grains, the conclusion can be made that on the whole, the content of chemical elements of the endemic areas is less than that in the non-endemic areas. This can be clearly seen in the analytical results of drinking water (Table 1).

Table 1. Water element contents of endemic areas and non-endemic areas of Heilong-jiang province

	Endemic areas	Non-endemic sites in endemic areas	Non-endemic areas
Total hardness (Germanic Deg.)	9.21	12.28	31.70
Total ion (mg / L)	284.62	593.30	1190.56
Na (mg / L)	19.87	45.22	51.26
Ca (mg / L)	36.86	85.61	183.43
Mg (mg / L)	17.84	22.92	23.73
Bicarbonate (mg / L)	72.71	255.54	377.96
Chlorate (mg / L)	52.54	73.41	137.92
Sulphate (mg / L)	12.86	42.46	103.79
Sr (mg / L)	0.306	0.336	1.182

But there is hardly any significant difference among the above three areas in Fe, nitrite, nitrate, carbon dioxide, F, I, Mn, Cu, B, and Ba.

However, not all the endemic areas have these characteristics. Chemical elements also tend to accumulate in plains, which are endemic.

A survey of the selenium contents in children's hair, cereals, and drinking water was carried out at 587 villages in 78 cities and counties from 1982 to 1984. The samples include 478 from water, 4550 from cereals, and 2680 from children's hair. The results show that the average concentration of selenium in

drinking water is 0.07 ppb; corn, 9.4 ppb; wheat, 14.0 ppb; rice, 24.0 ppb and hair less than 150.0 pp. Because the concentration of selenium in drinking water and grain is associated with the quantity of selenium in environmental soil, it is clear that the whole province is in the low selenium zone. In addition, selenium is lower in the endemic areas than that in the non-endemic ones (Li, et al., 1984), but the concentration of selenium in drinking water and grain in endemic areas is not always lower than that in non-endemic areas. In 70% of the non-endemic villages, the concentrations of selenium are the same as those in endemic villages. These results suggest that the importance of selenium in the cause of the disease should be further studied.

8. The influence of social economic status on the distribution of the disease

The inhabitants in the endemic areas live mainly on locally produced corn and wheat. If their staple food were changed into rice or if they should eat cereals from the state depots or grown in non-endemic areas, the prevalence of the disease could be controlled in this area. This also shows that the geographical distribution patterns of the disease are associated with social conditions and lifestyles.

REFERENCES

- Comprehensive Work Team on Endemic Disease in Heilongjiang Province:** The investigation data of Kashin-Beck disease, 1971, Heilongjiang.
- Li, C.,** A study on the mineral deficiency and imbalance and the cause of Kashin-Beck disease, in: Data Compilation of Research and Control of Kashin-Beck Disease of Heilongjiang Province, 1979, p. 18, Heilongjiang.
- Institute of Kashin-Beck Disease of Heilongjiang Province,** Kashin-Beck Disease, 1980, p.120, Heilongjiang.
- Sichuan Medical College,** Health Statistics, 1979, p. 54-57, People's Medical Publishing House, Beijing.
- Wang Z. et al.,** The investigation of prevalence rate of Kashin-Beck disease in Heilongjiang province. In: Proceedings of the Conference on Research and Control of Kashin-Beck Disease in Heilongjiang Province, 1982, p. 30, Harbin.
- Wang, Z., Li, L. and Li, C.,** The survey of selenium contents in children's hair, water and grain in endemic areas and non-endemic areas of Kashin-Beck disease in Heilongjiang province, China Endemiology Journal 4:330, 1985.
- Wang, Z.,** Some epidemiological characteristics of Kashin-Beck disease, in: Data Compilation of Research and Control of Kashin-Beck disease of Heilongjiang Province, 1974, p.110 Heilongjiang.

CLINICAL INVESTIGATION OF KASHIN-BECK DISEASE AND ITS DIAGNOSIS

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Introduction

Kashin-Beck disease is an endemic disease that occurs most often in children and seldom in adults. The disease is endemic in remote hilly and mountainous areas. No difference in incidence has been noted between males and females. The cartilages are commonly affected; developmental deformities are followed by epiphyseal cartilage impairment. According to its clinical development, this disease could be divided into four stages - early stage, 1st degree, 2nd degree, and 3rd degree. Diagnosis involves a special physical examination, X-ray studies and laboratory tests.

Cartilages (such as epiphyseal plate and articular cartilage) are commonly affected. Normally, the epiphysis is a centre of bone growth and therefore impairment of epiphysial cartilage is followed by developmental deformities, such as the shortening of fingers or toes or the humerus, Madelung's deformity, and reduced stature. The reduction in growth is determined by the severity of the disease and the age at onset. The most severe deformities are therefore seen in those who developed a severe form of the disease at an early age.

Striking early features are fatigue and flexion of the distal parts of the index, middle or third fingers. The onset may be insidious, with initially doubtful enlargement of the distal and proximal interphalangeal joints; the overlying soft tissue appears normal. Another sign is limited flexion of the fingers so that the tips of the fingers are unable to touch the distal ends of the heads of the 2nd, 3rd, and 4th metacarpals. Morning stiffness may be present in the joints of the lower limbs, elbows, and fingers. Friction within the joints is common, as is limitation of flexion and extension at the elbow.

Pain is present in the weight-bearing joints, and is aggravated by exposure to cold and exercise. In the late stages, there is enlargement of the joints and fingers, and the development of flat feet. Elbows, knees, and ankles are usually affected symmetrically. The patients's capacity to perform physical work is reduced. Involvement of the hip leads to a flexion deformity of the hip causes a compensatory lumbar lordosis. The patient may find it impossible to squat for field work. The signs and symptoms are more marked in the right hand than in the left.

Clinical Classification of Kashin-Beck Disease

Clinically, the disease is classified into four stages (Table 1). The early stage of Kashin-Beck disease is diagnosed if two of the following symptoms and signs are present:

- flexion of the terminal parts of fingers. or bow-like fingers;
- doubtful enlargement of the distal and proximal interphalangeal joints;
- painful ankles and / or knees.

Table 1. Symptoms and signs of Kashin-Beck disease at different stages of the disease

Symptoms and signs	Early stage	1st degree	2nd degree	3rd degree
Fatigue	+	+	+	+
Arthritic pain	?	+	++	+++
Morning stiffness of joints	?	+	++	+++
Friction in joint	?	+	++	+++
Loss of weight	-	-	++	++
Muscular atrophy	-	+	++	+++
Joint enlargement	?	+	++	+++
Flexion of fingers tip or bow-like finger	+	+	+	+
Short fingers (or toes)	-	-	+	+
Developmental deformity	-	-	+	++
Limitation of flexion and extension of the elbow	-	+	++	+++
Limited movement in ankle and wrist	-	+	++	+++
Limited movement in knee	-	-	+	++
Limited movement in hip joint	-	-	?	+
Limited movement in shoulder and spine	-	-	-	?

In addition, the patient should have been living in an endemic area for more than six months. Most cases at this stage are under 10 years of age. X-ray films should be taken to confirm the diagnosis.

Most patients with 1st degree disease have stiffness, especially marked in the morning. Ankle joints

are affected most frequently, knee next, and then elbow and wrist joints. Pain is often relieved by rest and exacerbated by exercise. Adults develop painful ankles and knees almost as often as children. The signs of disease are present most commonly in the hand, but 11.6% of all cases have no signs at all. Reduced growth is 26.4% in all patients, but 79.3% in patients between the ages of 11 and 15 years.

Some clinical investigations

Somatometric studies. Detailed measurements were carried out on 496 patients in the disease affected regions and in 1615 normal control patients in the same regions. The results revealed that there were no significant differences between the two groups in the trunk and leg indices, i.e., length of hand and width of palm, length of hand and body height. However, the patients with 2nd and 3rd degree Kashin-Beck disease (especially the patients with 3rd degree), had shorter body stature than the patients with less severe disease; they had shorter legs and a shorter trunk. Their body weight was below normal, but foot length and width was nearly the same as in healthy people.

In 1981, we investigated 46 of our previous patients who had been affected with Kashin-Beck disease 20 years previously. We traced their history and reexamined them. Some patients who were previously at an early stage of the disease had recovered, but some had become worse. However, no improvement had occurred in any of those patients who had 1st, 2nd, or 3rd degree disease 20 years before. Some healthy adults immigrated from the non-endemic regions had also been affected by Kashin-Beck disease.

Clinical Diagnosis Procedures

1. Physical examination

A quick routine method has been developed by Xi'an Medical University and is detailed in Table

2.

2. X-ray studies

Hand. In the early stage of the disease, changes in the epiphyseal cartilages of the hand can not be seen, except when thinning of the epiphyseal plate becomes visible. The subcartilaginous tissue appears uneven and wavy with sclerotic changes at the adjacent edge. Some trabeculae in the metaphysis become interrupted. In some cases, funnel-shaped defects in the metaphysis appear with a sclerotic edge and cystic changes near the adjacent spongiosa. Local osteoporosis may also occur. If the lesion perforates the whole epiphyseal plate, synostosis follows and extension of the long axis of the bone will be permanently arrested. The head of the phalanx becomes flattened and irregular, and there may be sclerotic changes and fragment-like shadows over both sides of it. In other cases, fragmentation may

be observed. This abnormal feature is seen over a long period. Some X-ray films show local resorption of the edge of the epiphysis and, later, spontaneous repair. Proliferation at the margin of the diseased bone causes enlargement.

Feet and ankle joints. X-ray changes in the toes are similar to those seen in the fingers. In the ankle joints, flattening of the talus is particularly conspicuous; its articular surface is rough; the upper convex side is depressed; the joint space is narrowed; and osteophytes are seen on both sides of the talus and on its head.

Carpal bones. These may be compressed and fused together; in other cases, only peripheral defects are seen. Erosion of the capitalum often occurs.

Elbows. In the late stages of the disease, the radial head is enlarged and irregular, forming a characteristic mushroom-shaped deformity. The olecranon and coracoid processes develop osteophytes. The trochlear incisura is shallow and the joint space is narrowed.

Knees. The articular surface is rough and irregular; the joint space is narrowed; the ends of the fibula and the tibia are slightly enlarged.

Hip joints. Involvement of the acetabulum and of the femoral head may mimic the appearance of osteoarthritis.

3. Laboratory Findings

At the early stage of the disease, laboratory findings reveals low serum concentrations of selenium, glutathione peroxidase, hydroxyproline and low urinary concentrations of selenium, while thiobarbital acid reaction, free fatty acid, creatine phosphokinase and creatinine are elevated. In addition, the chondroitin sulfate in the cartilage matrix shows low sulfation. Cyclic guanosine monophosphate levels appear to be inversely proportional to the severity of Kashin-Beck disease. Up to the late stages, all laboratory findings tend to be normal, resembling those found in osteoarthritis.

4. Summary of diagnostic features

This disease is much like a chronic, systemic osteoarthropathy and its characteristic features can be summarized as follows:

(a) Its cause is unknown. The disease occurs in limited areas in the agricultural regions. The onset is insidious, and the sufferers are usually farmers and their families.

(b) The incidence in children is high, involving the metaphysis, epiphysial plate, epiphysis, and articular surfaces. X-ray films show a special irregular defectes with a sclerotic edge at the early stage.

(c) Early ossification of the epiphysial plate leads to arrest in the development of the longitudinal axis of the bone, resulting in shortening of the bones (including the fingers) and reduced stature.

Table 2. Routine method of physical examination

Doctor's instruction	Points to be noted
Raise the upper limb with the dorsal surface of hand upward	Is there flexion of terminal parts in fingers or any deviation? Prominence of the lower end of ulna at the back of wrist
Turn the palm upward	Inspect soft tissue, palm contours claw hands and flexion of fingers
Flex the 2nd, 3rd, and 4th fingers to touch the distal metacarpal head with the finger tips	Note whether the fingers touch the distal place beyond the transverse skin fold of hand?
Clench the fists	Is the fist tightly clenched?
Hold right and left palms together vertically, with fingers straight	Compare the range of extension and flexion of wrist
Hold the hands back to back, with the fingers directed vertically downwards	Note the range of flexion
Pronate forearms	Keep neutral position of forearm before test
Supinate forearms	The medial sides of each humerus touch the thoracic wall closely
Standing at attention	Measure the distance between knees and ankles
Squat down – stand up	Note whether there is any space between the heels and the floor

(d) Generally, there is no exudation in the enlarged joint, except after excessive exercise and in the presence of the complicated inflammatory process.

(e) Initially, flexion of distal interphalangeal joint or crooked fingers are seen, but systemic symptoms are not apparent.

(f) Typically, joint involvement is bilateral (knees, ankles, elbows, wrists, hands, and toes). Spine and shoulder involvement is rarely seen, except in patients who have had severe disease since childhood.

5. Differential diagnosis

(1) Other arthroses can be differentiated as follows:

(a) Osteoarthritis: occurs usually after middle age and is scattered in distribution, whereas Kashin-Beck disease is prevalent in children and occurs endemically.

(b) Rheumatoid arthritis: the peak age for the incidence of rheumatoid arthritis is 35–40 years, and the pathological process starts in the synovial membrane of the joints, with a fusiform shape; in this way it can be differentiated from the bony enlargement of the joints seen in Kashin–Beck disease. In juvenile rheumatoid arthritis, high intermittent fever, rheumatoid rashes, and hepatosplenomegaly or generalized lymphadenopathy are often noted, but these systemic manifestations are not seen in Kashin–Beck disease.

(c) Osteochondritis diseases of the knees: the knee is the most commonly involved joint; but there may be no physical signs except wasting of the quadriceps. The range of movement at the knee is usually complete. A diagnostic sign is seen when the medially rotated knee is gradually extended; at 30–40 degrees of flexion, pain is experienced at the anterior aspect of the medial condyle. X-ray films show a defect over the femoral condyle, and sometimes loose bodies are present. Osteochondritis diseases can be differentiated from Kashin–Beck disease since it is found only in the knees.

(2) Diseases that result in reduced stature or other deformity should be differentiated from Kashin–Beck disease. Young children with more severe Kashin–Beck disease are often of reduced stature and developmental deformities without other stigmata, such as mental retardation, sexual infantilism, or corneal clouding. These are different from children affected by genetic or chromosomal abnormalities such as, cretinism, Down's syndrome, Turner's syndrome and mucopolysaccharidoses.

Other groups of patients with normal sexual and mental development have to be distinguished carefully. Usually congenital diseases are apparent at birth or by 1–2 years of age. Most of them have no joint pain and no epiphyseal enlargement.

REFERENCES

- Yin, P., The physical examination of Kashin–Beck disease. In: Monograph of Scientific Research on Kashin–Beck Disease in Yongshou (1979–1982). People's Health Publishing House, 1984, pp. 130–134. Beijing.
- Wang, H., The studies of clinical early signs and diagnosis in patients with Kashin–Beck disease. In: Monograph of Scientific Research on Kashin–Beck Disease in Yongshou (1979–1982). People's Health Publishing House, 1984, pp. 127–129. Beijing.
- Shanghai Institute of Anthropology, Anthropometry. Fu Dan University. 1979.

ADVANCES IN THE PATHOLOGY OF KASHIN-BECK DISEASE AND ITS RELATIONSHIP WITH SELENIUM AND OTHER ELEMENTS

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Aspects of Pathologic Research

The main pathological lesions of Kashin-Beck disease (KBD) involve the hyalin cartilage in the bones of the type of endochondral ossification. Epiphyseal cartilage, articular cartilage and epiphyseal growth plate at the two ends of tubular bone are the most common sites affected. Thus, the corresponding joints are often involved in the pathological process. The disturbance of endochondral ossification and deformity of affected joints are the two cardinal manifestations of this endemic osteoarthropathy of unknown etiology.

I. Studies of osteochondral tissue in patients

The cartilage tissue involved shows dystrophic changes, such as atrophy, degeneration and necrosis, with reparative and adaptive changes. In the presence of atrophic process, the chondrocytes in all layers, including the superficial layer, reduce in size and number. The chondrocytic columns become shortened and scanty. The intercolumnar spaces look widened. The affected articular cartilage and epiphyseal plate appear thinner than normal. The degenerative changes observed in cartilaginous matrix include weak or red staining, appearance of fibrils, asbestos degeneration, fissure formation mucoid degeneration, etc. But these changes also occur in physiological aging process and are not specific to the disease. The early characteristic Kashin-Beck lesion is chondrocytic necrosis beginning at the deep layer of articular cartilage and the hypertrophic chondrocytes of epiphyseal plate (Chen, et al., 1978).

We have studied the process of chondronecrosis histologically (Mo, 1982) and ultrastructurally (Mo, 1979, 1983). In the necrotic areas, chondrocytes die, lose their stainable nuclei and persist in their cytoplasmic staining (so-called cell ghosts). Then, the cell ghosts disappear and the necrotic areas gradually become homogenized. We believe that this sort of chondronecrosis, in fact, belongs to the category of coagulation necrosis in pathology. It has been shown that chondronecrosis in KBD has the following six characteristics (Mo, 1984): 1) in each of the patients, cartilage tissues of many bones were involved and the necrotic lesions in articular or epiphyseal cartilage were multiple and localized; 2) chondronecrotic lesions affected different bones in different cases, with the bones of extremities involved most often, but the affected sites did not strictly show bilateral symmetry; 3) in their distribution, those chondronecrotic areas situated in the deep layer of cartilage almost always approxi-

mated to bone tissue and bone marrow tissue; in severe cases, however, the chondronecrotic process might expand to the upper zone (or zones); 4) it was the maturing chondrocytes that were mostly affected; 5) chondronecrotic lesions might be punctate, patchy or zonal, and different in size; 6) chondrocytes, died repeatedly and in batches. The disintegrating processes of necrotic chondrocyte and cartilaginous matrix assumed distinctly the form of phasic changes. In histochemical preparations and electron-micrographs, the degradation and disappearance of proteoglycan and the subsequent decomposition of collagen fibrils were easily confirmed. Newly-formed lesions could be seen at the same time in the same patient.

After the death of chondrocytes, secondary changes happen. The surviving chondrocytes adjacent to these necrotic areas soon proliferate and even form chondrocyte clusters. Safranin O staining demonstrates clearly plenty of glucosaminoglycan in the matrix inside the clusters. By electron-microscopy, it is found that the chondrocytes inside a cluster have many secretory vesicles and rough surface endoplasmic reticulum, showing the pattern of secretory hyperfunction.

As a result of the reactions from the primordial bone marrow, the processes of the absorption, removal, organization, dystrophic calcification and ossification gradually take place in the cartilaginous masses. Eventually, the necrotic areas heal up with the repaired scarbone tissue, which, in turn, undergoes absorption and adaptation remodelling thereafter.

As the normal epiphyseal plate is the growing center of bone, epiphyseal impairment is often followed by developmental disturbance leading to short fingers (toes), short limbs and even dwarfism. The pathological changes in the articular cartilage may last for a rather long time and progress slowly. Secondary osteoarthritis with bone enlargement and disfiguration of joints would become prominent.

Since the Yongshou Investigation from 1979 to 1982, pathological research has made some progress (Mo, 1984):

1. The locations of chondronecrosis other than extremities has been documented. Extending the sampling in patients' skeleton during autopsy, we found that chondronecrotic lesions may occur at the sternal extreme of clavicle, near the clavical incisure of the manubrium sterni, inside the vertebrae and their articular processes as well as the iliac crest including spina iliaca anterior, superior, under the facies symphyseos of pubic bone, and in the cartilage tissue between the clivus of occipital bone and the body of sphenoidal bone. The lesions in certain places mentioned above, however, are not necessarily found in every patient. Therefore, biopsy of iliac crest may not be very helpful for the diagnosis of early-stage patients.

2. It is noted that chondronecrotic lesions in different phases, i.e., early-, intermediate-, and late-stage lesions, could be found in the same individual even in the same affected site. It would be important to bear this in mind in interpreting X-ray film or analyzing results involving the biochemical

components of patient's cartilage tissue.

3. It is suspected that the causal agent of the disease may operate once in a way and inferred that the cartilage tissue at the two ends of a phalanx must be affected synchronously. According to our observation in histologic sections, this is not necessarily the case. The most common finding is that the cartilage lesions at the metaphyseal end are older than those at the bone end.

4. A new source of organization concerning chondronecrotic focus was discovered. Besides coming from bone marrow tissue, connective tissue invading chondronecrotic foci can also have its source at the mesenchyme inside a cartilage canal.

5. The softening in a chondronecrotic lesion occurs sooner or later. Because of pressure stress, the necrotic mass in the deep portion of articular cartilage can be pressed through the gaps of the subchondral bone plate. The mechanical force transmitted via these weak points into the spongiosa can destroy the local trabecular bone and cause cyst formation. This is one of the mechanisms of cyst formation in this disease.

6. The retardation of ossifying-nucleus development in some children with KBD has been demonstrated histologically. This is a phenomenon contrary to the premature aging which was possibly related to this disease.

In addition, it has been recognized that some autopsy cases of infants with the disorder have quite severe chondronecrosis but very slight roentgenologic changes. Besides zonal necrosis adjacent to bone tissue, there are multiple punctiform or / and extensive necrotic foci in the immature cartilage around blood vessels and in the mid zone of the cartilaginous epiphysis. Occasionally, some "naked nuclear cells" inside a necrotic area can be seen. It is considered that the necrosis of immature cartilage of KBD infant has notable differences with the necrosis in mature cartilage. As the necrotic lesions are on intimate terms with blood vessels, some authors tend to believe the intoxication theory of this disease.

II. Ultrastructural studies

The author and co-workers (Mo and Li, 1979, 1983) used the transmission electronic microscope to examine the articular and epiphyseal cartilages in 6 patients. The ultrastructural changes predominantly in maturing chondrocytes were described in two types, namely, condensation and vacuolization of cytoplasm. In the latter type of cellular lesion, calcium granules were found to be deposited on plasma membrane. Chondrocytic degeneration changes also include the increased amount of intracytoplasmic filaments and myelin figures, the decrease of membrane organelles, swelling and distorsion of mitochondria as well as distension of endoplasmic reticulum, etc. The changes mentioned were interpreted as an indication of membrane injury in the early-stage lesions. Besides, changes in the cartilaginous matrix were described as well.

Ultrastructures of the red blood cell and skeletal muscle of KBD patients were reported by Ren

and associates (Ren, et al., 1984).

III. Studies on pathological basis of X-ray signs

The occurrence of roentgenologic signs in the affected bones of this disease principally results from the dystrophic calcification and ossification, i.e., scar bone formation, following chondronecrosis in the deep zone of articular or / and epiphyseal cartilage including growth plate as well as results from the destruction and deformity of original bony structures (Mo, 1984). More than 20 X-ray shadows were verified by histological sections (Mo, 1978; Wang, et al., 1984; Xi, et al., 1984 and Zhu, et al., 1980). The skeletal changes of the disease were summed up into 20 most common and basic X-ray signs and pathological interpretation was given for each of them (Mo, et al., 1985). The evolution of osteochondral changes and their X-ray signs in various bones was investigated (Xi, et al., 1984). All these works have provided a pathologic foundation for the reontgenology of the disease.

IV. Studies on the osteochondral changes of fetuses in endemic areas

Autopsy materials of 140 fetuses obtained from endemic areas were compared histologically with those of 155 fetuses obtained from non-endemic areas. No typical KBD has yet been found in them (Liu, 1984; Mo et al., 1982; Ren, 1984; Wang et al., 1984). In 2 of 12 fetuses and newborn autopsies, however, necrosis of hypertrophic cells in the cartilaginous epiphysis of a few tubular bones was found (Feng, 1981). Neither typical process of chondronecrosis nor articular deformity was described in the 2 cases. This problem still needs further investigation.

The above mentioned 4 aspects of pathologic studies have been carried out in the recent years. As KBD is non-fatal, whether the pathologic changes in endocrine glands and viscera suggested by Japanese authors (Takamori, 1968) are specific to this disease or result from other accompanying diseases also need to be clarified.

Selenium Studies Related to KBD

The low selenium (Se) content in cereals and drinking-water of KBD-affected areas in China was accidentally discovered in the experiments of feeding rats (Xi'an Medical College, 1973) (Mo, et al., 1975). As a number of rats (39.71%) died of acute massive liver necrosis in those experiments, a fluorimetric analysis of the Se content of the feed, cereals and drinking-water was performed. It was found that the Se contents in the feed and drinking-water from the KBD-affected areas was lower than those obtained from the disease-free area, so the cereal and drinking-water of KBD-affected area are low in selenium and responsible for the Se-deficiency in experimental animals.

In 1974, Dr. Li C. Z. in Gansu Province began to use sodium selenite to treat the early-stage patients with this disease and reported that selenite therapy was rather effective (Li, 1979). In recent years,

repeated checks at selected spots as well as in the population in quite a number of endemic areas have demonstrated that selenite is effective both in treatment and prevention of this disease.

As researchers in geography have made a great effort, an ecological chemo-geographic investigation in 26 provinces (autonomous regions) has been completed. It is found that there is a Se-deficient belt in our country. The areas affected by both Keshan disease and KBD are located mostly in this belt. The selenium contents in soil, water and grain of endemic areas are lower than those of non-endemic areas. The inhabitants in endemic areas are in a low Se nutritional status: the Se contents reported in whole blood (Hou and Zhu, 1984), urine and hair (Li, et al., 1981) are significantly lower than those of the inhabitants in non-endemic areas. There is a positive correlation between the total Se content in water and grain and the Se content in human hair (Geography Research Group in Yongshou Investigation, 1983,) as well as between the Se content in soil and water and grain (Hou, et al., Acta Scientiae Circumstantiae (in Chinese with English abstract,) in press) are highly significant.

The effectiveness of selenite in the prevention and cure of the disease and the low Se level in the milieu interne / externe of the population including KBD patients in endemic areas have laid an important foundation for the further study on the selenium hypothesis.

In the recent years, evidences supporting a close relationship between Se-deficiency and the disease have been increasing. For example, in 1984 Hou and Zhu reported the relation between the whole blood Se content and this disease as follows: (i) The whole blood Se content of our country of the inhabitants living in the low Se belt ($0.009-0.020 \mu\text{g} / \text{ml}$, $X = 0.020 \mu\text{g} / \text{ml}$) is the lowest one seen in the world literature. Of these inhabitants in the low Se areas, 66.61% have their whole blood selenium level less than $0.020 \mu\text{g} / \text{ml}$. Again, this percentage is by chance in accordance with the incidence of the disease in severely endemic areas. (ii) The negative correlation between the whole blood Se content and the incidence of the disease is highly significant in statistics. (iii) The whole blood Se level in the children from 7 to 14 years of age is the lowest and the incidence of the disease in this age group is the highest one. The whole blood Se content in population is low in spring and high in summer, and the incidence of the disease is higher in winter / spring, too. Moreover, the whole blood Se content of the population who consume imported grain is higher than that of the rural population consuming local grain, while the former does not suffer from the disease. It is considered that all of these facts concerning the Se level in population can well explain most of the epidemiological characteristics of the disease.

In addition, the elevation of the natural recovery rate of the KBD in the population of an endemic area without Se administration also has a good agreement with the increment of the average hair Se-content of inhabitants as well as with the increment of percentage of the individuals who have high Se content in hair (Hou, et al., Acta Sci. Circumst. (in Chinese) , in press). After Se treatment, the elevation of the roentgenologic recovery rate and the increment of the Se content in patients' hair also

happen at the same time (Niu et al., 1984).

Thus, the consideration regarding the disease to be caused by the low selenium in environment appears to be reasonable.

However, there are facts and points of view which do not support the causality between Se deficiency and the disease. They are listed as follows.

1. Using the data of Yongshou Investigation, Jiang et al., (1984) analyzed the relation between the urinary Se content and the X-ray detection rate of children living in endemic and adjacent non-endemic areas. As a result, a dose-response relation between them was suggested. But, it is also noticed that the incidence of KBD in Duma Village was found to be increased in 1982 while the hair Se concentration of the villagers in that year did not decrease. Hence, no dose-response relations was found. (Lu, 1982).

2. One report showed that the X-ray detection rate of the disease and the hair Se content in the population had a highly significant negative correlation ($r = -0.9083$, $p < 0.001$) (Hou et al., Acta Sci. Circum. [in Chinese], in press). On the contrary, there is another report (Deng and Ge, 1984) involving three affected spots, indicating that where the hair Se content of the population is the highest, there is the highest incidence of the disease. The result of a correlation analysis revealed that there is a positive correlation between the incidence rate and the Se content in human hair as well as in drinking water.

3. A certain number of elements including Se were determined in the samples of children's hair, wheat and corn obtained from Yongshou endemic areas, and no significant correlation between the content of each element and the prevalence of the disease could be found (Research Group of Epidemiology in Yongshou Investigation, A research report [in Chinese], 1983).

4. It has been found that the population of some endemic areas had rather high Se contents in hair, for example, 139 ppb (Mo, 1984), 159.97 ppb (Jiang et al., 1984), and 192-450 ppb (Niu and Jia, manuscript submitted). All of these have exceeded the critical value of hair Se contents of population in most endemic areas 100 ppb—(Hou and Zhu, 1982); 110 ppb—(Li et al., 1981).

5. It was reported that the incidence and hair selenium content in the population of 2 adjacent endemic spots were not always negatively correlated. For example, in one spot, the X-ray detection rate was 90.9% and the selenium content in hair was 32 ± 6.6 ppb; in the adjacent spot, the X-ray detection rate was 11.5%–13% and the selenium content in hair 126 ± 16 ppb (Li et al., 1985). But, there were reverse instances. For example, the selenium content of children's hair of one endemic spot (159.99 ± 35.96 ppb) and that of an adjacent non-endemic spot (155.18 ± 41.68 ppb) could be almost the same level (Jiang et al., 1984). You (1983) also reported such an example (You, A research report [in Chinese], Institute of Prevention and Cure of Endemic Diseases, Shandong, 1983).

6. There are 4 different types of areas low in selenium, namely, areas with KBD, areas with Keshan disease, areas with the two diseases and areas without the two disease. For this reason, it is difficult to accept the idea that low Se is the cause of the two different human diseases. It is suggested that the two diseases might be separately resulted from Se deficiency + X and Se deficiency + Y (Han et al., 1983), here X and Y may be considered as different etiological factors and Se deficiency as a common conditional factor (Deng and Ge, 1984), or the conditions may be reversed. However, all these hypotheses need further proof.

7. In the Yongshou KBD-endemic areas, the activities of glutathione peroxidase (GSHpx) as well as the contents of vitamin E (VE), lipid peroxide (TBA) and free fatty acid (FFA) in children's blood were determined (Han et al. 1983). It was noted that the activities of blood GSHpx and the level of VE were lower while the levels of blood TBA and FFA were higher than those of the children in adjacent non-endemic areas. Moreover, no difference of these biochemical markers was found in the blood between the normal and the sick children in the same endemic areas. It seems that the difference of environmental factor (Se) in the endemic and non-endemic area accounts for the blood biochemical characteristics of the children living in different areas while the same environment factor in the same affected area accounts for the same biochemical characteristics of the children whether suffering from KBD or not.

8. The urinary hydroxyproline concentration of patients with KBD was found to be lower than that of normal person (Li, F.S., Annual Report of Liaoning Basic Medical Science (in Chinese), 1978). The same result was observed in the KBD patients from 15 to 66 years of age by a Soviet Laboratory. Rats fed on the diet containing *Fusarium oxysporum* toxin for 15 days were found to have the same urinary change (Yang and He, 1982). Analyzing the data of Yongshou Investigation, (Jiang et al., 1984) reported that the content of urinary Se was positively correlate with the content of urinary hydroxyproline. He considered low Se to be able to reduce the urinary hydroxyproline excretion. However, the levels of urinary hydroxyproline excretion in KBD patients with higher Se content in hair is still unknown.

9. The activities of 6 serum enzymes, i.e., glutamate-oxalacetate transaminase (GOT), lactic dehydrogenase (LDH), beta-hydroxybutyric dehydrogenase (HBDH), creatine phosphokinase (CPK), gamma-transpeptidase (GT) and alkaline phosphatase (ALP) in KBD patients were found to be elevated (Sun et al., 1983; Xi et al., 1984). Feeding rats on a basic diet (33ppb Se) mainly composed of grain from low Se endemic area of KBD (Mo and Zhang, unpublished data), we found that the activities of serum GOT, LDH and GPT in these rats were higher than those in the rats fed on non-endemic diet (89ppb Se), while the endemic basic diet with sodium selenite supplementation (109ppb Se) was able to reduce the activities of serum GOT, LDH, and GPT. Another feeding experiment with the grain

obtained from 3 different low Se areas (including KBD-, Keshan disease- and non-endemic area) was performed. The concentrations of Se in the diets were 13.9ppb (KBD-endemic area), 26.5ppb (Keshan disease-endemic area) and 14.1ppb (non-endemic area) respectively. All of these 3 diets was able to elevate the activities of serum GOT, LDH, and GPT. When rats recieved these 3 diets with sodium selenite supplementation (200ppb Se), the serum enzyme activities reduced. Though the responses to low Se factor may be quite different between human and rats, from the above mentioned feeding trials, one can raise such a question: is the serum enzyme change of KBD patients with higher Se content different from the KBD patients with low Se content in hair?

10. Semi-synthesized selenium-deficient diet (Ren, et al., 1982) and selenium deficient diets mainly composed of cereals from low Se area with KBD (Mo, Zhang and Bai, unpublished data) could cause cartilaginous atrophy in the tibial growth plate of rats. No chondronecrosis in these rats was seen. Moreover, no change was found in monolayer culture of rabbit chondrocytes in a low Se medium (Wei, personal communication).

From the above results, it is hypothesized that Kashin-Beck disease in low Se areas is closely related to Se-deficiency. But, the causality between Kashin-Beck disease and selenium deficiency has not been fully elucidated yet.

Studies on the Relation of Other Elements to KBD

Studies on elements other than Se, which were related to KBD, have not been carried out thoroughly. Hence, the data available are rather controversial.

An etiological theory about high Sr and low Ca in the drinking-water of endemic areas was first put forward by the Soviet authors (Vinogradov and co-workers, 1935-1938) (A.P. Vinogradov, Tr. Biogeoхим. Labor. (in Russian), IX, 1949). But this hypothesis has not been confirmed by the researchers in China (Wang and Li, 1959).

In Yongshou Investigation, 10 elements in cereals from endemic areas were determined. It was found that the content of Sr and Ba in the cereals of endemic areas were lower and the contents of molybdenum (Mo) were higher than those in the cereals of non-endemic areas. The concentration of Fe and Mn in the wheat of endemic area was higher, too. Meanwhile, by means of a stepwise regression analysis, it was shown that the contents of Mo and Se in corn as well as Ba, Fe and Se in wheat were correlated with the disease (Ju et al., 1984).

But, in the corn from other endemic areas, the content of Mo is lower and the contents of P, Fe, and K are higher than those of non-endemic areas (Difangbing Ziliao Huibian (in Chinese), Institute of Prevention and Cure of Endemic Diseases, Shanxi, 1975, No.4, P.51).

A group of Soviet researchers (Rosin et al., 1983) reported the contents of 8 trace elements in the 25 femurs of adults with KBD (II° — III°) as well as in the femurs of 128 normal adults. The level of Ca in bone ash was found to be reduced significantly while the relative contents of Sr, Pb, Zn, Mn, Fe and Ag were elevated and there was no difference in Cu content between the two groups.

Recently, Zaiko and co-workers (1981) has determined the contents of 52 elements in soil, water, plants and food obtained from KBD-endemic areas in Soviet Union as well as in the serum and urine of KBD patients (L.V. Zaiko, A.V. Voshenko, and E.E. Ustinova, Tezisi Dokladov k Predstoiachei Konferentsii "Problemi Revmatologii i Urovskoj — Kashina-Becka Bolezni" (in Russian), Chita, 1981, p. 42). The most noticeable finding was the level of phosphate to be significantly higher in the external environment of endemic areas as well as in the patients' body when compared with the controls. As the results, a new theory regarding chronic phosphate intoxication as the cause of KBD was suggested. The authors also developed an animal model. They found that rats fed on a diet and water with phosphate supplementation (500–1000 mg phosphate / kg body weight) had high phosphorus level in the blood accompanied by marked osteoporosis and retardation of growth. They believed that these changes in rats were clinically, morphologically as well as roentgenologically similar to those in KBD patients (A.V. Voshenko, N.E. Shieber, V.N. Chugaev, A.M. Urieu, T.B. Cheresova, S.V. Nechepaev, Tezisi Dokladov k Predstoiachei Konferentsii "Problemi Revmatologii i Urovskoj—Kashina-Becka Bolezni" (in Russian), Chita, 1981, p. 43).

In China, an endemic area of KBD nearby a phosphate mine was documented. Because of the deposit on the same level of the water source, the pollution of drinking water by phosphate is quite possible. As it happened in the years (1977–1978) of mining, the incidence of KBD increased abruptly (Jiang et al., 1984).

In addition, it is noted that, in certain endemic areas the ratio of PO_4 / Ca in drinking water as well as the ratio of P / Ca in diet are higher than those in non-endemic areas; in particular, the ratio of P / Ca in corn can be as high as 6.76 (Difangbing Ziliao Huibian (in Chinese), Shanxi Institute of Prevention and Cure of Endemic Diseases, 1975, No.4, p.51). We have used a diet of cereals from KBD-endemic areas ($Ca / P = 0.7—0.8$) and only caused a growth retardation and osteoporosis in rats just like the findings of the Soviet authors, but no KBD lesions were found (unpublished data).

It is well known that feeding rats on a high P diet ($Ca / P < 1$) can cause nutritional secondary hyperparathyroidism and then osteoporosis (Draper and associates, 1972).

However, osteoporotic changes are also seen in KBD patient. The relation between the dietary high phosphorus and the osteoporosis in patients' bone needs to be further studied.

But, the population in Yongshou endemic areas has a low P level in blood, which is correlated with the disease in a regression analysis (Mo unpublished data).

Besides P / Ca, high ratios between elements in endemic areas reported are:

(Sr+Ba) / (Mg+SO₄) / Total amount of ions in drinking water (Difangbin Ziliao Huibian (in Chinese), Institute of Prevention and Cure of Endemic Diseases, Shanxi, 1975, No.4, p.51), Sr / Ca • SO₄ in cereals and water, P / S in water and corn (A research report (in Chinese), Institute of Geochemistry, Guiyang, 1971), Si / S as well as Si / Se in cereals and water (Ma, 1984). Could these disbalances of elements be found in other endemic areas and how do they cause the disease? These problems are still obscure.

To sum up briefly, the KBD-endemic areas both with high P and low P are in existence. The same is Se: most endemic areas in China are located in low Se areas; still, in a few endemic areas, the hair Se levels of local residents are not very low. The effect of selenite in cure and prevention of the disease in the low Se areas is affirmative; however, the causality between a certain element (or elements) and the disease has not been fully proved.

REFERENCES

- Chen, T.Z., He, M.C. Liang, C.W. Song, J.Y. Lin, H.Z. Hu, M.H. and Zhang, G.Q. 1978. A pathomorphologic study on the bone lesion in early stage of Kashin-Beck disease (in Chinese). Zhongguo Yike Daxue Xuebao (1): 1-7.
- Deng, J.Y. and Ge, S.Z. 1984. The role of Se in the etiology of Kashin-Beck disease, in the view of the relationship between the incidence and the Se content in water, cereals and human hair (in Chinese). Difangbing Tongxun (2):64.
- Department of Pathology, Shenyang Medical College, Kexue Yanjiu Ziliao Huibian (in Chinese), 1959, No. 4, p. 37.
- Draper, H.H., Sie, Ten-Lin and Bergan, J.G. 1972. Osteoporosis in aging rats induced by high phosphorous diets. J.Nutr. 102:1133 - 1142.
- Feng, H.S. 1981. A morphologic observation on cartilage tissue of fetuses and neonates in Kashin-Beck disease-affected area (in Chinese). Difangbing Tongxun (3):30 -32. Geography Research Group in Yongshou Investigation (in Chinese), 1983, P.24.
- Han, Z., Li, G.Y., Chao, S.G., Yang, J.G., Wei, Y.Z. and Ren, Y.Y. 1983. An analysis of determination results of blood GSHpx, VE, TBA, and FFA in the children of Kashin-Beck disease-affected area (in Chinese with English abstract). Chinese J. Endemiol. 2 :65-71.
- Hou, S.F. and Zhu, Z.Y. 1982. A study of selenium nutrition status of the population in the Kashin-Beck disease region in China (in Chinese with English abstract). Chinese J. Endemiol. 1 :84 -89.

- Hou, S.F., Zhu, Z.Y. 1984. A study of the relation between the whole blood selenium of the population and Kashin-Beck disease in the low selenium regions in China (in Chinese with English abstract) . *Geograph. Res.* 3:14-23.
- Hou, S.F., Zhu, Z.Y. and Tan, J.A. 1984. The relationship between the selenium dynamic in the course of human body growth and the Kashin-Beck disease epidemiology (in Chinese with English abstract) . *Acta Geographica Sinica.* 39:75-85.
- Jiang, Y.F., Bai, C., Zhang, S.Y., Xu, Y.C. and Yu, Z.D. 1984. A study on the urinary creatinine content of children in Kashin-Beck disease-endemic area and nearly non-endemic area—A concurrent discussion on the relationship between Se deficiency and the incidence of the disease (in Chinese) . *Xi'an Yixueyuan Xuebao* 5:43-48.
- Jiang, Y.F., Xu, Y.C., Bai, C., Zhang, S.Y., Yu, Z.D., Chao, J.L., Li, Y.L., Lu, X.Z. and Xue, S.Y. 1984. A study on the urinary hydroxyproline content of children with different urinary Se level in the KBD-affected and non-affected areas (in Chinese) . *Difangbing Tongxun* (2):62.
- Jiang, Z.S., Chen, Y.X., Ling, G.D. 1984. An epidemiological investigation of Kashin-Beck disease in Guangling County (in Chinese) . *Difangbing Ziliao Huibian* (6):1-9.
- Ju, S.J., Jun, Z.H. and Yue, S.Y. 1984. A study of correlation between incidence and some trace elements in the cereals of KBD-affected area in Yongshou County (In Chinese) . *Difangbing Tongxun* (2): 35-36.
- Li, C. F., *Keyan Fangzhi Ziliao Huibian* (in Chinese) , Provincial Institute of Kashin-Beck Disease, Heilongjiang, 1979, No.2, p.76).
- Li, C.Z., 1979. A roentgenological observation of the effect of Se and VE treatment on children with early-stage Kashin-Beck disease (in Chinese) . *Chinese Med. J.* 59:169-171.
- Li, J.Y., Ren, S.X. and Chen, D.Z. 1981. A study on relationship between Kashin-Beck disease and selenium from Se contents in human hair of various natural environments in Shaanxi province (in Chinese with English abstract) . *J. Environment Sci.* 2:18-24.
- Li, J.Y., Ren, S.X., Chen, D.Z., Wan, H.J., Liang, S.T., Zhang, F.J. and Gao, F.M. 1985. Distribution of selenium in micro-environment related to incidence of Kashin-Beck disease (in Chinese with English abstract) . *Acta Sci. Circumstantiae* 5:140-148.
- Liu, Y.T., Yang, T.X., Li, J.C., Yang, Y.S. and Gao, F.M., 1984. Histopathologic study on the finger bones of fetuses in KBD-affected area, p.261-163. In: CPC Leading Group Office for the Prevention and Cure of Endemic Diseases (ed.), *Monograph on Scientific Research on Kashin-Beck Disease in Yongshou (1979-1982)*. People's Health Publishing House, Beijing.
- Lu, M.J. 1982. A study on the relation between elements and Kashin-beck disease—4th report (in Chinese) . *J. Harbin Medical University* (4): Suppl., 30-34.

- Ma, J.J.** 1984. Experimental observation of effects of sodium silicate on monkey—A study on the pathogenesis of Kashin-Beck disease (in Chinese with English abstract) *Chinese J. Endemiol* 3:83-87.
- Mo, D.X.** 1978. The problems of morphology and pathogenesis of chondronecrosis in Kashin-Beck disease (in Chinese) . *Difangbing Fangzhi Keyan Dongtai* (13):1-10.
- Mo, D.X.** 1982. Histopathology of chondronecrosis in Kashin-Beck disease and its clinical significance (in Chinese with English abstract) . *Chinese J. Endemiol.* 1:116-120.
- Mo, D.X.** 1984. Pathologic basis for the roentgenological changes of Kashin-Beck disease, p.221-214. In: CPC Leading Group Office for the Prevention Cure of Endemic Diseases (ed.), *Monograph on Scientific Research on Kashin-Beck Disease in Yongshou (1979-1982)*. People's Health Publishing House, Beijing.
- Mo, D.X. and Li, W.Z.** 1979. Electron-microscopic observation on chondrocytes of articular and epiphyseal cartilage of Kashin-Beck disease (in Chinese). *Xi'an Yixueyuan Xuebao Congkan* (a pamphlet of KBD). p.34-40.
- Mo, D.X. and Li, W.Z.** 1983. Ultrastructural changes in the matrix of articular and epiphyseal cartilage of KBD patients (in Chinese) . *Xi'an Yixueyuanbao Fukan* (2):117-124.
- Mo, D.X., Wang, Y.Z., Ren, H.Z., Zhu, C.R., Ying, M.X., Xing, S.Z. and Liu, Y.T.** 1984. Advances of pathologic research in Kashin-Beck Disease (in Chinese with English abstract) . *Chinese J. Radiol.* 18:90-94.
- Mo, D.X., Wang, Y.Z., Zhu, C.R., Ren, H.Z., Xing, S.Z., Liu, Y.T. and Ying, M.X.,** 1985. Pathologic interpretation of basic X-ray signs in Kashin-Beck disease (in Chinese with English abstract) *Chinese J. Radiol.* 19 :87-92.
- Mo, D.X., Du, Z.M., Zhang, Z.T. and Jin, J.** 1982. The patho-morphologic comparison of finger bone and cartilage between human fetuses obtained from endemic areas of Kashin-Beck disease and non-endemic areas (in Chinese with English abstract) . *Chinese J. Endemiol.* 1 :31-34.
- Mo, D.X., Guo, S.W., Liu, Z.H., Yu, Z.D. and Chen, F.S.** 1975. Dietary liver necrosis in rats fed on cereals and drinking water from Kashin-Beck disease-affected areas (in Chinese) . *Xi'an Yixueyuan Xuebao* (4):76-85.
- Niu, G.H., Zhang, B.Z., Liu, X.Z., Zhu, Z.Y. and Hou, S.F.** 1984. Results of study of two years on the preventing and controlling Kashin-Beck disease with selenium seen under X-rays and discussion of etiology (in Chinese with English abstract) . *Chinese J. Endemiol.* 3: 197-201.
- Ren, H.Z., Bai, X.C., Zhu, T.Y. and Liu, J.F.** 1984. A transmission electron-microscopic study on the skeletal muscle of a child with Kashin-Beck disease (in Chinese) . *Chinese J. Endemiol.* 3:282-283.

- Ren, H.Z., Wang, G.L. and Zao, Y.H.**1982. A histological study concerning the effects of trace element selenium on the development of metaphyseal cartilage of rat (in Chinese with English abstract) . Chinese J. Pathol. 11: 220-221.
- Ren, H.Z., Bai, X.C., Wang, G.L. Liu, G.F., Weng, Y.P., Wang, Y.Z. Zhu, C.R. and Xing, S.Z.**1984. Roentgenologic and pathologic observation of fetal cartilage (in Chinese) , p.255-260. In: CPC Leading Group Office for the Prevention and Cure of Endemic Diseases (ed.), Monograph on Scientific Research on Kashin-Beck disease in Yongshou (1979-1982). People's Health Publishing House, Beijing.
- Rosin, I.V., Butko, V.S. and Kalabukhov, E.P.**1982. Some biochemical and biophysical aspects of the pathogenesis of Kashin-Beck disease (in Russian with English summary) . Terapev. Arkhiv 54:80-82.
- Rosin, I.V. and Govorin, A.V.,**Tezisi Dokladov k Predstoiachei Konferentsii "Problemi Revmatologii i Urovskoj Kashin-Becka Bolezni" (in Russian) , Chita, 1981, p.58.
- Sun, K., Li, L.Q., Wang, Z.W., Yang, J.B., Wang, Z.Z., Li, Y.H. and Guan, S.Y.**1983. Analysis of seven enzymes in blood plasma of KBD patients (in Chinese) . Difangbing Tongxun (2):33-38.
- Takamori, T.**1968, Kaschin-Besk's Disease (Dysostosis Enchondralis Endemica) vol.1, p.188. Professor Tokio Takamori Foundation, Gifu, Japan.
- Wang, F. and Li, G.S.**1959. An experimental study on the effect of strontium on the skeleton of rats (in Chinese) . Jilin Yike Daxue Xuebao (3):11-21.
- Wang, Y.E., D.H.Xue, S.E.Xing and C.R.Zhu,** 1984. Intra-cartilaginous acellular areas of human fetus and young dog in Kashin-Beck disease-affected region, (in Chinese) , p.251-254. In: CPC Leading Group Office for the Prevention and Cure of Endemic Diseases (ed.), Monograph on Scientific Research on Kashin-Beck Disease in Yongshou 1979-1982). People's Health Publishing House, Beijing.
- Wang, Y.Z., Xing, S.Z., Zhao, Y.D. and Zhu, C.R.**1984. Pathologic changes of slight X-ray signs in infants with Kashin-Beck disease (in Chinese) , p.236-240. *ibid.*
- Wang, Y.Z., Zhu, C.R., Ying, M.X., Ren, H.Z., Mo, D.X., Xing, S.Z. and Liu, T.Y.**1984. The evolution of X-ray sign and pathologic changes in various sites of bones in Kashin-Beck disease (in Chinese) , p.228-232. *ibid.*
- Xi, G.Z., Li, Z.Q. and Wang, Y.L.**1984. The changes of some plasma enzyme activities and the changes of some chemical components in the blood, urine and hair of children with Kashin-Beck disease (in Chinese) , p.287-300. *ibid.*
- Yang, J.B. and He, F.L.**1982. The experimental biochemical study effects of Fusarium oxysporum toxin on Kashin-Beck disease (2) (in Chinese with English abstract) . Chinese J. Endemiol.1:90-94.

Zhu, C.R., and Cui, D.Y.1978. Pathological study of metacarpophalangeal joints in Kashin-Beck disease. Chinese Med. J. 4:309-318.

Zhu, Z.Y. and Hou, S.F.1980. The relationship between Se-VE defeciency and Kashin-Beck disease—A discussion on the etiology of the disease (inChinese) , Difangbing Tongxun (2):18-26.

RADIOLOGY AND PATHOLOGY OF KASHIN-BECK DISEASE

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In recent years, the development of diagnosis on Kashin-Beck disease was based on the following achievements: (1) epidemiological investigation on Kashin-Beck disease (Ying, et al., 1989); (2) studies on the X-ray changes in 425 cases of Kashin-Beck disease (Ying, et al., 1984); (3) comparative studies on X-ray changes and pathological changes from autopsied children with Kashin-Beck disease (Wang et al., 1984; Mo et al., 1984).

This report focuses on the study of comparing X-ray examination with pathological changes in autopsied children. The follow-up observation on X-ray changes is also discussed.

Materials and Methods

1. Autopsy specimens

a. Male, 10 year-old, died of Keshan disease in Shuang Yashan area, Heilongjiang; X-ray diagnosis, irreversible stage of Kashin-Beck disease. b. Female, 13 year-old, died of Keshan disease in Dedu county, Heilongjiang, reversible plastic reconstruction stage of Kashin-Beck disease. c. Male, 10 years old, from Huma county, Heilongjiang, reversible early stage of Kashin-Beck disease. d. Female, 13 years old, from Da Xinganling area, Heilongjiang, reversible early stage of Kashin-Beck disease. e. Male, 5 years old, from Shangzhi county, Heilongjiang; a specimen of left knee joint was collected and X-ray examination showed unevenness, thinning, broken and partly disappearance of the early calcification zone. One hundred fresh bone specimens were obtained from the above 5 autopsied children with Kashin-Beck disease. X-ray films of the bone specimens were compared with their pathological findings.

2. Follow-up examination

X-ray films were taken from 295 children at 5-13 years old with Kashin-Beck disease at each year in Shuang Yashan area for 10 successive years. Besides, X-ray films of 130 children at 3-13 years old with Kashin-Beck disease from Yongshou county, Shaanxi were taken in 8 follow-up examinations within 2 years and 5 months.

Results and Discussion

The chief pathological changes in Kashin-Beck disease is cartilage necrosis. However, since the characteristic lesions of epiphysial plate, and epiphysial, distal bone end and carpal bone cartilage are different, the discussion here is made according to their different anatomy sites.

Necrosis of cartilage in the epiphysial plate

1. X-ray changes (Ying et al., 1982)

The basic changes include: a. blurring, thinning, interruption and disappearance of the provisional calcification zone; b. several kinds of depression, including small depression, tongue-like depression, big and deep oval-shaped depression. Once the depression is formed, the sclerosis, to varying degrees, of the bottom would soon appear, including a broader sclerotic zone or multilayer sclerotic lines. The sclerosis may have obvious bone absorption at short period (3 months), then the depression becomes shallower gradually. Or the sclerotic depression may not be absorbed for a long time (3-4 years) and present continuously; c. growing barrier line at metaphysis; d. early closure of epiphysial line, including needle-like bony connection at middle epiphysial line and complete closure.

2. Pathological changes

The early change after cartilage necrosis is the disappearance of physiological calcification zone or replacement by pathological thin layer calcification. At this moment, X-ray shows blurring, thinning, interruption or multiple zigzag small defect of provisional calcification zone. When the osteogenesis can not be continuous in the focus of necrotic cartilage and meanwhile the survival cartilage is going on osteogenesis, X-ray shows different forms of depression. The duration of the depression depends on the severity of the disease. Since the necrotic foci adjacent to bone edge forms irregular pathological calcification and/or below it rough and multilayer transverse trabeculae present, calcification at the bottom of the depression can be seen on X-ray film. Besides, necrosis of epiphysial plate often occurs at the middle part, so the middle metaphysis usually forms depression.

Necrotic cartilage itself can not be restored, and it becomes foreign body. The granulation tissue which is from marrow side eliminates the necrotic matter and pathological calcification. Then organization, calcification and osteogenesis are followed to fill the necrotic foci. The repairing process shows that the depression becomes shallow on X-ray (Zhu et al., 1977; Mo et al., 1984). When chondroclasts and granulation tissue pass by the transverse trabeculae and go into the necrotic foci to absorb and break it up, X-ray shows that the depression becomes further shallow or disappears. If there is no new necrotic tissue formed at above necrotic foci, a new provisional calcification zone will appear at the corresponding site of epiphysial plate. That is the reappearance of provisional calcification zone shown

on X-ray film. The transverse trabeculae formed at primitive necrotic cartilage foci exist in spongy bone of metaphysis to cause growing disturbance line. It will disappear after quite a long time.

After the necrosis of epiphysial plate cartilage, absorption, organization and osteogenesis will occur from the two sides up and down the epiphysial plate. The primitive epiphysial line is replaced by new bone. An early closure of epiphysial line can be seen on X-ray film.

There is a close relation between the above X-ray changes and the severeness of cartilage necrosis. The less the cell layers are involved in primary cartilage necrosis, the milder is the osteogenetic disturbance. The more the chondrocyte layers are involved in necrosis, the severer is the osteogenetic disturbance, and the deeper is the depression of the metaphysis, the longer it lasts. The more mature the chondrocytes are involved in the primary necrosis, the shorter the osteogenetic disturbance lasts and the shallower is the depression. The more primitive the cells are involved, the more are the chances of development of deformity in the metaphysis and epiphysis. The extent of necrosis near the ossified bone corresponds to the severity of osteogenetic disturbance and the size of the depression. Hence the degree of metaphysial depression represents the size of the necrotic foci near the ossified bone, and the depth of the depression represents the necrotic duration (Wang et al., 1984).

Necrosis of bone end cartilage

I. X-ray signs

Bone end cartilage is that in which there is no secondary ossification center. After necrosis, the main changes seen on X-ray film are (Ying, 1984): a. The bony articular surface becomes vague, thinner and disconnected. They are the early changes after cartilage necrosis. These changes can develop depression, sclerosis and unevenness in two and a half years, and aggravate gradually; b. Plain and straight sclerosis of bone end with small thornlike depression at the middle bone end and straight angle at the margins will form bony thorn at the margins and deep depression at middle part in two and a half years; c. Blurring, roughness with spotted bone grain at lateral bone end will evolve to big and sclerotic cutting-angle changes in some of the patients in two and a half years. In other cases, they could recover to normal bone end width, but the articular surface here is sclerotic and uneven; d. Half-moon shaped shallow depression in middle bone end could form big cystoid change in 8 months and disappear in two and a half years; e. the free bone mass at bone end originally exists in and out the bone end freely. Later, it gets bigger and finally connects with bone end resulting in big distal bone end and smooth articular surface.

The above X-ray signs indicate that X-ray signs of distal bone end appear 2 to 3 years later than that of metaphysis, the longer for 4 to 5 years. The pathological changes in distal bone end also develop

slowly. Some sclerotic depression are getting more obvious, others become smooth and even. But most cases can not restore to normal distal bone end condition. Some cases will keep the depression, unevenness and sclerosis for a long time and finally form a big and deformed joint.

2. Pathological changes (Wang et al., 1984)

The main pathological changes corresponding to X-ray signs are described as follows: a. Blurring and thinning of bony articular surface in X-ray film is due to the disappearance of physiological calcification zone after the deep layer cartilage necrosis. Interruption of bony articular surface results from the absorption and elimination of foreign body, pathological calcification and bony shell by granulation tissue to cause a gap; b. Plain and straight distal bone end reflects the complex signs, such as pathological calcification after the band-like necrosis at the deep layer cartilage, reactive bone proliferation of survived cartilage, incomplete bony plate and the absorption of survival cartilage, incomplete bony plate and the absorption of bony shell by granulation; c. The thornlike proliferation at the distal bone end is the new bone formed by the connective tissue in the process of absorption and organization of the peripheral cartilage following necrosis; d. Depression of the distal bone end is the dysosteogenesis resulting from extensive necrosis of the band-like necrosis of cartilage. Proliferation occurring at the bottom of the depression is due to pathological calcification and increased cicatricial bony tissue; e. The free bone mass at distal bone end results from the intramembranous ossification occurring in the process of organization of the necrotic cartilage foci; f. The cystoid change at the distal bone end is due to the cavity resulting from local destroyed bone below the articular surface and reactive bony proliferation around it to form a cystoid wall.

The X-ray signs, such as blurring, thinning and interruption of calcification zone, and their developed process in distal bone end appear slower than those in metaphysis. However, with the absorption, elimination, organization, ossification and survived cell proliferation, those changes could disappear gradually. Then the bony articular surface becomes smooth and only a small dent remains at the middle part of distal bone end. This result is quite common in the mildly affected areas of Kashin-Beck disease. Since the recovery from necrosis is faster in epiphyseal plate cartilage than in distal bone end, the X-ray signs also disappear earlier in epiphyseal plate cartilage. Therefore, in mildly affected areas, slight X-ray change of distal bone end often can be seen, without changes in metaphysis observed.

Necrosis of the epiphyseal cartilage

1. X-ray signs

a. Early change is thinning, blurring and interruption of the calcification zone surrounding the epiphyseal nucleus; b. epiphyseal development is not symmetrical, one side bigger, and another smaller;

c. depression and sclerosis in epiphyseal articular surface and lateral wall of epiphyseal nucleus; d. sclerosis of epiphyseal terminal plate; e. epiphyseal terminal plate embeds epiphyseal plate to make local epiphyseal line in higher density and blurring; f. deformation of epiphyseal nucleus including malformed epiphysis, deviated epiphyseal nucleus and broken epiphyseal nucleus which are late stage changes (Ying et al., 1984; Wang, et al., 1984).

2. Pathological changes

When necrosis occurs in epiphyseal cartilage, including the cartilage surrounding epiphyseal nucleus, articular cartilage of epiphysis and subterminal plate cartilage, the local physiological calcification zone is absorbed and disappeared, which show blurring and interruption of calcification zone around epiphyseal nucleus on X-ray film (Wang, et al., 1984). When deep layer necrosis of epiphyseal articular cartilage occurs, the physiological calcification zone covered on bone plate disappears. Then the uneven bone plate is exposed, which shows rough epiphyseal articular surface on X-ray film. After the necrosis of epiphyseal articular cartilage, as the enlarged epiphysis, dysosteogenesis in necrotic foci following pathological calcification and reactive bone proliferation happen. At this time, depression and sclerosis of epiphyseal articular surface can be seen on X-ray film. Once the fat layer cells below the terminal plate are involved, there is a reactive bone proliferation from epiphyseal marrow, which shows thickening and sclerosis of epiphyseal terminal plate on X-ray film. If the necrosis involves germinal cells below the epiphyseal terminal plate, the invasion of connective tissue from epiphysis side, absorption, organization and ossification of foreign body cause epiphyseal terminal plate closing to metaphysis gradually. At this moment, a narrow epiphyseal line and epiphyseal nucleus closing to metaphysis can be observed clearly on X-ray film. Since the source of multiple chondrocytes is cut off, an early closure of epiphysis and metaphysis will happen finally. Before the secondary ossification center appears or early large piece necrosis occurs, epiphyseal nucleus development and ossification will be involved. According to different severeness of the disease, the changes in X-ray film are deformation, deviation and unsymmetry of the epiphyseal nucleus (Wang et al., 1984).

X-ray changes of necrosis in epiphyseal cartilage appear much slower than in metaphysis. Once the sclerotic depression of the articular surface of epiphysis occurs, it will exist until adulthood. Some of the changes in epiphysis may become more smooth because of bone reconstruction. In other cases, the bony articular surface will never recover to normal condition.

Necrosis of the cartilage in carpal bones

1. X-ray changes

The earliest sign is thinning, blurring and interruption of calcification zone to various X-ray signs

of carpal bones. Consequently, local depression, sclerosis and defect of carpal ossific nucleus follow. The early signs and the time when they occur in carpal bones are similar to those in metaphysis to the children below 7 years old. Therefore, the change of carpal depression, sclerosis and defect in young children basically represents the severeness of necrosis. The depth of depression represents the disease duration. The notable sign is that there is a high incidence at the head of capitatum. Once the necrosis of the cartilage occurs, the early signs at the head of capitatum appear soon. So if there are depression, sclerosis, defect at the head of capitatum and its diameter is longer than its length, it indicates that the disease occurs when the child is quite young. In this case, the necrotic range is large (Ying, 1984). For an elder child, such as 11 years of age, his 8 carpal bones are complete. If there are more horns, his capitatum and thinning and interruption appear on more carpal bones, and only irregular sclerosis of articular surface can be seen without deformation.

2. Pathological changes

Cartilage around carpal ossific nucleus becomes necrosis. As the results of absorption, restoration and reconstruction of necrotic cartilage, the consequent changes are blurring, depression, sclerosis, defect, and deformity of the local ossific nucleus. That indicates the dysosteogenesis of cartilage necrosis, the recovery process from early to late stage and the remaining bone deformation (Wang et al., 1984).

Conclusion

Based on the continuous observation of 425 cases for 10 years, the X-ray signs of Kashin-Beck disease include thinning, blurring, interruption and disappearance of calcification zone for early stage; various depression and sclerosis for middle stage; and bone defect and deformity for late stage. They reflect the regulation of generation, development and result of clinical process. The width of depression in metaphysis represents the necrotic range of epiphysial plate cartilage adjacent to bone edge. The depth of depression represents the time when necrosis occurs. According to the standard in which phalanges grow 1 to 2 mm each year, one may estimate the duration of the disease. X-ray changes appear early and develop fast in metaphysis. Meanwhile, so long as the epiphysial terminal plate is smooth and complete, most of the cases can recover to normal state without any remaining abnormalities. In other sites, the changes will remain with different traces.

The comparative observation of X-ray signs with pathological changes from 5 autopsied cases indicates that from early necrosis to complete absorption, there are three stages, i.e. necrosis, restoration and reconstruction. It reflects the regulation of generation and development of cartilage changes.

X-ray changes and pathological lesions in Kashin-Beck disease are affected by age, site, severeness, quality and quantity of the causative factors, nutrition and the intensity of labour.

REFERENCES

- Mo, D., Wang, Y., Ren, H., Zhu, C., Ying, M., Xing, S. and Liu, Y.,** Advances in pathologic research in Kashin-Beck disease, Chinese Radiological Journal 18:90-94, 1984. (in Chinese)
- Wang, Y., Zhu, C., Ying, M., Ren H., Mo. D., Xing S. and Liu, Y.,** The pathology and evolution of X-ray signs of the bone and cartilage in Kashin-Beck disease. Chinese Radiological Journal 18:81-84, 1984. (in Chinese)
- Wang, Y., Zhu, C. and Ying, M.:** Comparison of X-ray sign with pathological changes in Kashin-Beck disease. Chinese Medical Journal, 64:357-359, 1984. (in Chinese)
- Ying, M. and Fu, G.,** The basic X-ray signs of phalanges and their pathological basis in Kashin-Beck disease. Journal of Harbin Medical University (supplement), 16:41-45, 1982. (in Chinese)
- Ying, M,** X-ray evolution in Kashin-Beck disease and its divided models. Chinese Radiological Journal, 18:85-89, 1984
- Zhu, C. and Cui, D.,** Pathological study on metacarpophalangeal joints in Kashin-Beck disease. Chinese Medical Journal 57; 244-248, 1977. (in Chinese)

EPIDEMIOLOGICAL STUDIES ON THE CAUSES OF KASHIN-BECK DISEASE

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Kashin-Beck disease is an endemic, multiple, and deforming osteoarthropathy of unknown cause. The basic pathological change is the degeneration and necrosis of hyaline cartilage in the joints of growing children; the deforming osteoarthropathy is secondary.

Geographical Distribution of Kashin-Beck Disease

Kashin-Beck disease exists in the northeast, northwest, and in the Qinghai province and Tibet autonomous region of the People's Republic of China. It has also been reported in Siberia in the USSR and in the northern mountainous region of Democratic People's Republic of Korea.

The disease was initially discovered and reported by a Russian research worker in east Siberia in 1849. Later on, two Russian military doctors, Kashin and Beck, reported the presence of the disease in the Urov river valley.

Kashin-Beck disease was first reported in China in the Changbai Mountain Annual of 1908.

The epidemiology of Kashin-Beck disease is very complex and there is much controversy about the causes of the disease, because of incomplete epidemiological studies. In China, Kashin-Beck disease occurs in mountainous and semi-mountainous regions of a long narrow zone from Heilongjiang province to Tibet autonomous region. However, it has also been found in the northern plains, e.g. in some villages in the Songnen and Songliao plains. All areas in which Kashin-Beck disease occurs have a continental climate. The summer season is short, the frosty season is long, and there is a considerable difference between day and night temperatures. Villages in which the disease occurs are focally distributed and there may be significant differences in morbidity rates between adjacent villages. Endemic areas are characterized by "natural" extension and reduction. For example, within 5-10 years, Kashin-Beck disease may emerge in villages in areas in which the disease has never been found before, while in some villages where there have been many cases of the disease, the incidence may gradually decrease until no new cases occur for several decades. However, there are still some villages where the prevalence, though showing some fluctuation, remains high for many years. The state of disease areas

can be classified, according to the number of new cases, into active areas, relatively stationary areas, and stationary areas. An active area is one in which there are many new cases of Kashin-Beck disease between the ages of 5 and 13 years with distinct symptoms. The morbidity rate using X-ray diagnosis is higher than the clinical morbidity rate. X-ray of the hands shows that changes in the metaphyses are greater than changes in the distal end of bones. In a relatively stationary area, the number of new cases between the ages of 5 and 13 years is lower than that in an active area and the morbidity rate diagnosed by means of X-ray is lower than the clinical morbidity. X-ray of the hands in this type of area shows more people with changes in the distal end of the bones than with changes in the metaphyses. In a stationary area, there are no new cases of the disease in the 5-13 year age group and, generally speaking, not more than 10% of cases show changes in the distal end of the bones or slight changes in the metaphyses when the hands are X-rayed (Yang, 1979, 1984).

How the Factors Causing Kashin-Beck Disease Enter the Human Body

This is an important question in epidemiological research on Kashin-Beck disease. The causes of the disease are unknown, and there is much controversy about the ways in which such causative agents might enter the body. As far as I know, nobody doubts that entry is by mouth and there is no possibility that the causative agents enter the body through the skin, respiratory tract, or sense organs. What are the vehicles of the agents? Are they cereals, drinking-water, vegetables, non-staple foods, or a combination of all these? Definite conclusions cannot be drawn yet. However, what we can do is to make certain deductions on the basis of epidemiological phenomena that have been verified and briefly tested using experimental epidemiology. Many examples of obvious differences in morbidity have been seen in populations in Kashin-Beck disease areas with the same water source but with different sources of cereals. In the Xinchun production brigade, Shuangyashan city, Heilongjiang province, workers' families and peasants' families lived in the same block; the living standards of both families were similar and they used the same source of running water. The peasants' families ate maize produced in the area in which they lived while workers' families ate the cereals (also mainly maize) provided by the government. Many members of peasants' families suffered from Kashin-Beck disease, but almost no one suffered from the disease in workers' families (Table 1) (Kashin-Beck Disease Study Section of Harbin Medical University. Report of collaborating investigation on Kashin-Beck disease in Xinchun production brigade in Shuangyashan city. In: *Compilation of Scientific and Research Studies of Harbin Medical University*, 1979). In Linyuan pump station, Daqing city, Heilongjiang province, workers' families and peasants' families lived in the same village and used the same running-water. Peasants' families ate maize produced in the area in which they lived while workers' families ate maize shipped

from other areas. A high incidence of the disease was found in the children in peasants' families but almost nobody in the workers' families was affected (Table 2)(Feng, 1981).

This great difference in morbidity can only be attributed to the fact that the sources of the cereals were different. From this, we can assume that cereals are the main, or the only vehicle through which the causative agents enter the human body. Another fact is that cereals from different sources can induce different rates of morbidity. No matter how active and serious the disease is in an area, as long as the people grow and eat rice, they do not suffer from the disease at all. In highly endemic areas, the people who suffer from the disease eat mainly wheat flour or maize. Although the living standards in some state farms, where mainly wheat is grown, is high and there is no shortage of non-staple foods, Kashin-Beck disease is still highly endemic. However, those who live in such areas and eat mainly rice do not suffer from the disease. For example, in Yuanbo village, Shangzhi county, Heilongjiang province, people of Han and Korea nationalities lived together under the same conditions, using the same water source, drinking the water in the same way. However, the people of Han nationality ate mainly maize and those of Korean nationality, mainly rice. As a result, many people of Han nationality suffered seriously from the disease, while almost no Koreans were affected (Kashin-Beck Disease Study Section of Harbin Medical University, 1973). Such examples can be frequently found in the northeastern disease areas, especially in the eastern areas of Heilongjiang province. After repeated investigations, we have concluded that the main difference in living conditions between the two nationalities is the different kind of cereals that they consume (Table 3).

Table 1. Children suffering from Kashin-Beck disease in the Xinchun production brigade, Shuangyashan city (October 1971)

	Workers families	Peasants families
Numbers examined	87	149
Changes seen in X-ray examination	4	113
Changes in distal end and epiphyses	0	45

Table 2. Children suffering from Kashin-Beck disease in Linyuan pump station (1980)

	Workers' families	Peasants' families
Numbers examined	65	86
Changes seen in X-ray examination	4	77
Changes in metaphyses	4	65
Changes in distal end	0	63

Table 3. Comparison of morbidity rates^a (%) of Han nationality and Korea nationality in Yuanbo village (September 1965)

	Han nationality	Korea nationality
Native	28.2	0.0
Residents for no more than 4 years	14.1	0.0
Residents for more than 5 years	50.0	3.6

a. Diagnosis made on the basis of clinical signs.

Even in active and severe Kashin-Beck disease areas, the growing and eating of rice instead of maize will control the occurrence of new cases. For example, in Shengli village in Wuchang county, Heilongjiang province, which was a highly endemic area, no cases of Kashin-Beck disease have been found since the early 1950s, when people started to grow and eat rice instead of maize. Now, there are almost no people between the ages of 14 and 40 years suffered from this disease (Table 4) (Kashin-Beck Disease Study Section of Harbin Medical University, 1973).

Table 4. Morbidity rates (%)^a of Kashin-Beck disease in Shengli village (July, 1970)

Age (years)	Number of subjects	Cases		
		I degree	II degree	III degree
0-9	83	0	0	0
10-19	98	0	0	0
20-29	52	3	2	0
30-59	30	4	6	2
>60	33	10	17	2

a. Diagnosis made on the basis of clinical signs.

The above information shows that rice probably does not produce or carry the causative agents of Kashin-Beck disease under natural conditions. The causative agents enter the human body by means of different kinds of cereals can cause different morbidity rates. Some more specific epidemiological phenomena prove that cereals from Kashin-Beck disease areas can carry the causative agents to non-disease areas resulting in the development of new cases. In Nenjiang town, Nenjiang county, Heilongjiang province, railway workers' families lived together with local army families and peasant families in the same geographical area, but only the railway workers' children suffered seriously from the disease. The main difference in living conditions was that each year, the railway workers' families were able to buy a lot of wheat flour from Kashin-Beck disease areas, because of their occupation. Peasant families bought less and the families of the local army did not buy any (Kashin-Beck Disease Study Section of Harbin Medical University, 1980). Children of staff members and workers living in disease areas usually do not (or only very few of them) suffer from this disease. On the contrary, a lot of children in railway workers' families suffer from Kashin-Beck disease. This shows that it is the wheat flour from Kashin-Beck disease areas that carries the causative agents of the disease to the families (Table 5).

Table 5. Kashin-Beck disease in Nenjiang town (August 1979)

	Railway workers' families	Peasants' families	Local army families
Age (years)	8-14	6-15	7-15
Numbers of children examined	98	42	43
Clinical morbidity rates (%) ^a	28.6	9.5	2.4
X-ray morbidity rates (%) ^b	57.7	26.2	9.5

a. Diagnosis made according to clinical signs.

b. Diagnosis made according to X-ray examination.

Similar phenomena can be found in large cities, such as Mudanjiang, Harbin, and so on. For example, we found 2 cases in Harbin, with the following histories.

X.X. Guo, a 16-year-old boy, born in Harbin, had never been to a Kashin-Beck disease area. His father was a staff member. The boy was diagnosed as 1st degree of the disease in our institute on 22 January, 1984. There was nothing exceptional in his life history, except for the fact that 100-150 kg of

wheat flour had been bought from a very seriously affected Kashin-Beck disease area (Zhaoguang Farm) during 1980-81.

X.X. Zhang, a 13-year-old girl, born in Harbin who also had never been to a disease area became ill in 1983. There was nothing abnormal in her life, except for the some hundred kg of wheat flour bought from Kashin-Beck disease areas during 1978-79.

These examples are quite similar to those in Nenjiang town. They show that cereals from disease areas can carry the causative agents to non-endemic areas and that, perhaps, even a small amount of cereal carried from an endemic area can cause the occurrence of this disease. Thus, on the basis of the above epidemiological data, I believe that the causative agents of the disease exist in maize and wheat from Kashin-Beck disease areas. The results of further experimental epidemiological studies have proved that the disease can be brought under control when cereals from disease are replaced by cereals from non-disease areas, with other fundamental conditions remaining unchanged. In Xinchun production brigade in Shuangyashan city, which was a highly endemic Kashin-Beck disease area, the disease has remained under control since the change in cereals began in 1972, though it still developed in adjacent villages (Kashin-Beck Disease Study Section of Harbin Medical University, 1979).

The Relationship between some Chemical Elements or Microbiological Toxins and Kashin-Beck Disease

The specific aim of further epidemiological studies on the cause of Kashin-Beck disease is to reveal the causative agents of the disease in cereals. This is very difficult, because there are countless numbers of normal and abnormal chemical substances in cereals. We are carrying out studies most of which focus on chemical elements or organic toxins in cereals. However, these studies are far from complete. There are many reports on relationships between chemical elements in cereals and Kashin-Beck disease in China. The data from investigations in Yongshou county, Shaanxi province, concerning the relationship between 8 elements in maize and wheat and Kashin-Beck disease are the most complete (Yang, 1984). The investigation was carried out in 4 types of disease areas. One was a non-disease area; the second was a slightly endemic area; the third was a medium endemic area; the last was a highly endemic area. The investigation involved 130 production brigades and 1950 samples. No significant differences in the contents of chemical elements were found in the hair of children from the different Kashin-Beck disease areas (Table 6).

These data were also studied by using correlation analysis. When the relationships between the contents of 8 elements in maize and wheat and the morbidity of Kashin-Beck disease were studied, we did not find any correlation between most of the elements and the prevalence of the disease. There was

no significant relationship for any of the 8 elements in maize and there was a negative correlation between selenium in wheat and the prevalence of the disease, which was not significant. The degree of correlation between the elements in these samples and the state of Kashin-Beck disease was weak and that there was no consistency between different samples from several areas (Table 7).

Table 6. Comparison of contents ($\mu\text{g/g}$) of 8 elements in the hair of children in 4 types of Kashin-Beck disease areas in Yongshou county (1982)

	I	II	III	IV	p Value
Cu	8.58	9.37	8.91	7.34	>0.05
Zn	142.95	144.30	149.88	137.95	>0.10
Mo	0.051	0.044	0.046	0.048	>0.10
Se	0.088	0.069	0.070	0.055	>0.10
Sr	11.67	7.64	7.73	6.98	>0.10
Mn	4.71	4.98	5.40	4.83	>0.10
Fe	15.03	17.00	18.07	12.93 ^a	>0.10
Ba	6.85	53.937	3.480	3.818 ^b	>0.05

Note: I - non-Kashin-Beck disease areas.

II - slightly endemic areas.

III - medium endemic areas.

IV - highly endemic areas.

a. $P < 0.01$.

b. $P < 0.05$.

In order to minimize the confounding factors, the results of investigations carried out in neighbourhood areas with significantly different prevalence rates are considered to be more valuable. In this paper, all 3 examples of investigations quoted involve comparison of a highly endemic Kashin-Beck disease village with an almost disease-free area or a slightly endemic area. No consistent trend was found among the elements studied (Table 8) (Bai, 1985). To sum up the relationship between some elements and Kashin-Beck disease, we suggest that:

1. No element has been found to be significantly correlated with the prevalence of the disease.
2. The correlation with some elements varies according to different areas and cereals.

Table 7. Partial correlation coefficients between the contents of 8 elements in maize and wheat and the morbidity rate of Kashin-Beck disease

	Cu	Zn	Se	Sr	Mn	Ca	Mg	S
				Maize				
Activeness ^a	0.055	-0.062	-0.117	0.097	-0.014	0.003	-0.129	0.102
Seriousness ^a	-0.076	0.022	0.113	-0.085	-0.022	-0.102	0.114	-0.038
				Wheat				
activeness ^a	-0.032	-0.120	0.065	—	0.133	0.193 ^b	0.078	-0.140
seriousness ^a	-0.020	0.131	-0.207 ^b	—	0.018	-0.140	0.098	0.170

a. Both activeness and seriousness degree are the indexes of morbidity of Kashin-Beck disease.

b. $p < 0.05$.

During studies on elements in cereals, we also noticed that biotic toxins resulted from biotic contamination. One simple way of measuring biotic contamination is to determine the total volatile basic nitrogen (TVBN) in cereals, which increases with biotic contamination. Recently, we investigated some Kashin-Beck disease areas in Heilongjiang and Shaanxi provinces and found a positive correlation between the amount of TVBN in maize and wheat from Kashin-Beck disease areas and the prevalence of Kashin-Beck disease. We tried to avoid comparisons over long distances. Thus, the samples were all collected in adjacent areas with different prevalence rates. A consistently high correlation between TVBN and disease prevalence was found (Table 9).

In order to verify this phenomenon, we also made comparisons between highly endemic and slightly endemic areas or non-disease areas which were within a short distance of each other. The investigation was carried out in the same way as the element study. The results were the same as those from previous reports (Table 10) (Yang and Xi, 1984).

Obviously there is still not enough evidence to prove causality between TVBN and Kashin-Beck disease, but we cannot ignore such consistent findings. The data do at least show that the cereals in the Kashin-Beck disease areas were seriously contaminated by biotic agents, because of their higher content of TVBN. What we are most interested is whether the causative agents of the disease are involved in TVBN or whether the amount of TVBN parallels the unknown causative agents of the disease. This needs further investigation.

Table 8. Comparison of the contents of elements from maize and wheat flour in villages with different prevalences of Kashin-Beck disease

Villages	S	Ca	Mg	Mn	Sr	Zn	Cu
Maize							
1.severely endemic	1.127	0.472	1.632	5.943	2.271	21.687	2.139
slightly or non-endemic	1.020	0.467	1.818	5.026	2.299	25.047	2.694
2.severe endemic village	0.927 ^a	0.344	1.219	5.877	1.183	24.603 ^a	2.981
slight or non-endemic village	1.089	0.304	1.254	5.687	1.204	30.062	2.988
Wheat flour							
1.severe endemic village	1.270	0.587	1.116	13.220	5.042 ^a	21.170	4.290
slight or non-village	1.256	1.364	0.719	14.547	4.257	21.700	3.924
2.severe endemic village	1.443 ^a	0.351	0.588	12.193 ^a	3.178 ^a	24.256	3.219
slight or non-endemic village	1.234	0.325	0.622	14.464	3.871	25.643	3.021

Note: S, Ca, Mg: in mg / g; Mn, Sr, Zn, Cu, Se: in $\mu\text{g} / \text{g}$.

a. $p < 0.01$.

Table 9. Correlation coefficients between the amount of total volatile basic nitrogen (TVBN) in cereals and Kashin-Beck disease morbidity

	Heilongjiang disease areas	Daqing disease areas	Yongshou disease areas	
	Wheat	Maize	Maize	Wheat flour
1. Number of natural villages	9	20	137	142
2. Index of activeness	7.9 - 120.2	7.1 - 81.8	0.00 - 100	0.00 - 100
3. Index of seriousness	7.9 - 136.5	7.1 - 112.1	0.00 - 100	0.00 - 100
4. Correlation coefficient between activeness and TVBN	0.71 ^a	0.59 ^b	0.29 ^a	0.39 ^a
5. Correlation coefficient between seriousness and TVBN	0.70 ^a	0.56 ^b	0.20 ^a	0.26 ^a
6. Mean of TVBN (mg / g of cereal)	0.034 ± 0.0017	0.031 ± 0.0026	0.026 ± 0.001	0.023 ± 0.0006

a. $p < 0.05$.

b. $p < 0.01$.

Table 10. Quantity of total volatile basic nitrogen (TVBN)^a in cereals from a highly endemic Kashin-Beck disease area and a non-disease or slightly endemic area (1983)

	Highly endemic area	Non-disease or slightly endemic area
1. Maize	0.040	0.028
Wheat flour	0.040	0.025
2. Maize	0.040	0.023
Wheat flour	0.057	0.038

a. Amounts of TVBN in mg / g of cereal.

The kinds of microorganism that cause contamination an cereal and whether the cause is biotic contamination have not yet been proved. On the basis of our own studies and reference to the Russian reports, we think that fungus contamination is the greatest possibility, especially *Fusarium*. The reasons for this are: 1) generally speaking, the amounts of *Fusarium oxysporum* and *Fusarium moliniform* present in the cereals in Kashin-Beck disease areas are higher than those in non-disease areas; 2) *Fusarium* is not found in rice; and 3) *Fusarium* culture can induce similar pathological changes in the cartilage of young dogs (Yang, 1980).

The Seroepidemiology in Kashin-Beck Disease

Recently, seroepidemiology has made some progress as a new branch of study on the etiology of Kashin-Beck disease. The activities of some serum enzymes were measured in children from Kashin-Beck disease areas and compared with those in normal children. Basically, cases of 3 types were seen. In the first type, the abnormality was progressive and there were clear increases in the activities of all four enzymes under study, i.e., alkaline phosphatase (ALP), glutamate-oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH), and γ -glutamyl transpeptidase (GGT). This mainly happens in children from the current highly endemic areas. In the second type, the abnormality was at an early stage or was slight and in these cases the activities of all of the serum enzymes except ALP increased, but to a lesser degree. In convalescent cases (the third type), only the activities of GGT and GOT increased while those of the others remain fundamentally normal. This type mainly occurs in areas in which the disease was formerly highly endemic (Table 11) (Yang and Xi, 1984).

Table 11. Mean values of serum enzyme activities (IU / L) in patients with different clinical types

Enzymes ^a	Normal	Early	Progressive	Convalescent
ALP	189.9	141.2	215.9	159.0
GOT	26.7	39.6	48.6	22.0
LDH	83.1	91.4	127.5	83.7
GGT	6.1	9.8	13.4	13.8

a. ALP - alkaline phosphatase;

GOT - glutamate-oxaloacetate transaminase;

LDH - lactate dehydrogenase;

GGT - γ -glutamyl transpeptidase.

From further studies, we found that the changes in the activity of serum enzymes preceded the changes seen in X-ray examination. In highly Kashin-Beck disease endemic areas, the changes in the activity of serum enzymes were the same in both children that did not show changes on X-ray examination and those who were typical patients (Table 12) (Yang, 1985). Further studies showed that the above changes in enzyme activity were sometimes obvious in the spring but had basically returned to normal in the fall. Seasonal changes were also observed in the changes observed by X-ray examination, but they were not as obvious as those seen in the biochemical metabolism (Table 13) (Yang, 1985).

The real significance of the changes in serum enzymes is not fully understood yet and further studies are needed. However, we note at least three points: 1) The changes in serum enzymes in Kashin-Beck disease differ from those in myocardial infarction and hepatic necrosis. The changes in Kashin-Beck disease are obvious, but slight, and are quite similar to the changes caused by toxins (Bruin, 1976); 2) the seasonal changes in the activity of serum enzymes can be explained on the basis that natural factors that cause such changes are changeable, the most probable being biotic factors; 3) Changes in serum enzymes are early indicators of Kashin-Beck disease.

The close relationship between the cereals, mainly maize and wheat from Kashin-Beck disease areas and the disease is supported by experimental pathological studies. When experimental diets, which mainly consisted of cereals from Kashin-Beck disease areas and some necessary nutrients, were given to baby dogs for 6 months to a year, we found degenerative and focal necrosis, which was quite similar to that of Kashin-Beck disease, in the articular cartilages of the toe joints of the dogs (Zhang, 1982). But such changes did not appear often and we cannot say it is an ideal animal model for Kashin-Beck disease. When rats were fed diets composed of cereals or extracts of cereals from Kashin-Beck disease areas for 3-4 weeks, the changes in serum enzyme activity were quite similar to those seen in children from Kashin-Beck disease areas (Table 14) (Ma, 1985).

Conclusions

Studies on the causes of Kashin-Beck disease are far from complete. However, the following concepts are proposed on the basis of our studies.

- (1) The causative agents of the disease enter the body from cereals produced in the affected areas.
- (2) The effects of different kinds of cereals in inducing Kashin-Beck disease differ. Maize and wheat can cause the disease, but rice cannot.

Table 12. Biochemical indicators of serum in children, not showing changes on X-ray examination, from various types of Kashin-Beck disease areas

Indicators	Non-disease areas	Non-disease village in disease areas	Medium endemic	Highly endemic	Levels of significance
AKP (IU / L)	171.3	154.9	163.3	223.9	* *
GOT (IU / L)	27.6	39.5	47.1	53.6	* *
GPT (IU / L)	19.4	20.2	19.8	30.0	-
LDH (IU / L)	88.8	110.4	97.7	146.6	* *
CPK (IU / L)	60.4	67.2	64.4	72.3	-
GGT (IU / L)	5.5	9.6	8.4	12.1	* *
X-HBDN (IU / L)	220.0	286.0	262.9	267.6	* *
ACP (IU / L)	3.6	6.2	6.9	3.2	* *
Glucose (mg / dl)	92.8	99.0	101.0	97.9	*
Uric acid (mg / dl)	6.5	8.1	6.9	6.8	* *
Urea (mg / dl)	15.0	15.8	11.8	14.0	-
Creatinine (mg / dl)	0.7	0.0	6.66	0.57	* *
Cholesterol (mg / dl)	130.8	138.1	145.8	126.8	-
Glyceryl-ester (mg / dl)	23.6	169.3	58.1	89.2	* *
Ca ⁺⁺ (mg / dl)	9.0	9.8	13.1	11.3	* *
P (mg / dl)	6.3	5.6	5.5	5.1	* *
K ⁺ (mEq / L)	4.1	4.0	4.3	4.1	-
Na ⁺ (mEq / L)	143.8	142.0	142.4	142.2	*

* P < 0.01.

* * P < 0.05.

-Not significant.

Table 13. Number of Kashin-Beck disease cases with different biochemical types in slightly and severely endemic areas in Yongshou county

Biochemical types	Slightly endemic areas			Severely endemic areas		
	1982.4	1982.9	1983.4	1982.4	1982.9	1983.4
Normal	41	23	24	1	9	10
Early stage	51	16	21	5	12	8
Progressive	13	0	8	60	4	6
Convelascent	1	22	5	0	38	35
Total	106	61	58	66	63	59

Table 14. Biochemical changes in the serum of rats fed maize and extract of maize from Kashin-Beck disease areas for 3 weeks

	Control group	Experimental group 1 ^a	Experimental group 2 ^b	Levels of significance
No. of rats	16	16	18	
Weight at beginning (g)	53.3	53.7	52.6	
Weight at end (g)	127.5	138.8	139.2	
ALK (IU / L)	364.0	449.7	264.7	**
LDH (IU / L)	436.0	632.6	940.7	**
CPK (IU / L)	700.3	772.7	976.6	*
GOT (IU / L)	154.3	181.4	160.4	*
GPT (IU / L)	49.8	46.4	42.2	-

* P < 0.01

** P < 0.05

- P > 0.05

a. Experimental group 1 fed maize from Kashin-Beck disease areas;

b. Experimental group 2 fed maize and extract of maize from Kashin-Beck disease areas.

(3) No consistent differences in the amounts of elements in cereals from Kashin-Beck disease areas and non-disease areas were found. In studies on TVBN, consistent differences in the amounts in cereals from Kashin-Beck disease areas and those from non-disease areas were found.

(4) There are specific changes in the activity of serum enzymes in the process of Kashin-Beck disease.

(5) Biochemical changes in serum enzymes precede changes seen on X-ray examination and have a distinct seasonal variation.

(6) Kashin-Beck disease can be controlled when the cereals from Kashin-Beck disease areas are changed to cereals from non-endemic areas.

(7) When baby dogs are fed with cereals from Kashin-Beck disease areas, pathological changes that are similar to those in Kashin-Beck disease are found. When rats are fed with cereals from cereals from Kashin-Beck disease for 20-30 days, changes in serum enzyme activities that are similar to those in Kashin-Beck disease are found.

(8) The causative agents of the disease are possibly some kinds of biotic toxic agents.

REFERENCES

- Bai, X. Epidemiological studies on the pathological significance of TVBN and 8 elements in serious Kashin-Beck disease villages and their adjacent non-Kashin-Beck disease or slight Kashin-Beck disease villages. Postgraduate Essay of Harbin Medical University. 1985.
- Bruin A. De. Biochemical Toxicology of Environmental agent. Elsevier / North-Holland Biomedical Press. 1976; p. 1-30.
- Feng, G. Report of an epidemiological investigation on Kashin-Beck disease in two adjacent zones in the suburbs of Daqing city. Journal of Harbin Medical University (suppl.) 3:105 (1981).
- Kashin-Beck Disease Study Section of Harbin Medical University. Report of collaborating investigation on Kashin-Beck disease in Xinchun production brigade in Shuangyashan city. In: Compilation of Scientific and Research Studies of Harbin Medical University. 1979.
- Kashin-Beck Disease Study Section of Harbin Medical University. Report of dynamic investigation on new Kashin-Beck disease areas in the valley of Mangniu River in Wuchang county. In: Compilation of Scientific and Research Studies of Harbin Medical University. 1973, p. 32.
- Kashin-Beck Disease Study Section of Harbin Medical University. Report of investigation on Kashin-Beck disease in children of railway workers' families of Qiqihar railway bureau in Shuangshan and Nenjiang town. Journal of Harbin Medical University (suppl.) 2:41 (1980).
- Kashin-Beck Disease Study Section of Harbin Medical University. Epidemiological studies on the

- method of changing cereals in Kashin-Beck disease area, Shuangyashan City. In: *Compilation of Thesis for the 35th Anniversary of the Founding of Harbin Medical University*. 1979, p. 95.
- Kashin-Beck Disease Study Section of Harbin Medical University.** The second summary report of epidemiological studies on Kashin-Beck disease. In: *Compilation of Scientific and Research Studies of Harbin Medical University*. 1973, p. 7.
- Ma, X.** Experimentally biochemical studies of the cause of Kashin-Beck disease. Unpublished. 1985. Heilongjiang Kashin-Beck Disease Research Institute.
- Wang, Z.** Report of two Kashin-Beck disease cases in Harbin. *Journal of Harbin Medical University (suppl.)* 18(4): 163, 1984.
- Yang, J.** Epidemiological studies on Kashin-Beck disease. In: *Annual of Chinese Medical Science*, Tianjin Scientific Press, 1984, pp. 141-144.
- Yang, J.** Concept of active Kashin-Beck disease areas and classification of types of Kashin-Beck disease. In: *Special Anniversary Edition of Journal of Harbin Medical University*, pp. 21, 1979.
- Yang, J.** Epidemiological description. *Compilation of Epidemiological Investigation in Kashin-Beck Disease Area, Yongshou county*. People's Health Press. Beijing, pp 15-21, 1984.
- Yang, J.** Correlation between TVBN in the grain from Kashin-Beck disease areas. In: *Compilation of Epidemiological Investigation in Kashin-Beck Disease Area, Yongshou county*. People's Health Press. Beijing. pp 43-45, 1984.
- Yang, J. and Xi, G.** Classification of the biochemical types of Kashin-Beck disease and the application for diagnosis and the nature of Kashin-Beck disease areas. In: *Compilation of Epidemiological Investigation in Kashin-Beck Disease Area, Yongshou county*. People's Health Press. Beijing. pp. 23-26, 1984.
- Yang, J.** Relationship between biochemical types of serum of Kashin-Beck disease and X-ray diagnosis. *Chinese Journal of Endemic Diseases*. 1:64, 1985.
- Yang, J.** Seasonal changes in biochemical types of serum of Kashin-Beck disease. *Chinese Journal of Endemic Diseases*. 3:303, 1985.
- Yang, J.** Experimental pathological studies on the toxin of *Fusarium oxysporum* which causes Kashin-Beck disease. *Chinese Journal of Medicine*, 60 (5):312, 1980.
- Zhang, G.** Experimental pathological research for Kashin-Beck disease animal model. *Heilongjiang Medicine*. 7:7-10, 1982.

SELENIUM IN ENVIRONMENT AND ITS RELATION WITH KASHIN-BECK DISEASE

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Introduction

Shaanxi province is one of the regions in China most severely affected by Kashin-Beck disease (KBD) which has been prevalent in some areas of 67 counties (cities) in this province. It has been found through extensive surveys and studies that there are some significant differences of selenium (Se) contents in soil, water, grains and children's hair in various regions of Shaanxi province because of the variation in geochemical conditions and the uneven distribution of selenium. Kashin-Beck disease is distributed all around the environment where the Se contents are very low and the Se contents in human body (children's hair) is less than those in disease-free areas. (Yongshou Scientific Survey Team on Kashin-Beck Disease, 1984).

Methods

For more than ten years, the authors have carried out environmental surveys in the wide areas covering more than 190,000 km² in Shaanxi province, and then proceeded with the surveys of regional environment in a highly endemic county with an area of 800 km², and finally selected two micro-environments with areas of 2.8 and 10 km² respectively to study selenium in environments and its possible relationship with KBD.

1. Macro-environment investigation in Shaanxi province (190,000 km²)

Seventy KBD affected and non-affected counties and cities were selected from the whole province. In each of these counties and cities, 2 rural sites were further selected for investigation. The selection of KBD affected and non-affected sites was based on the epidemiological data supplied by the local health authorities. In total, 130 affected and disease free sites were studied which represent the natural regions of the whole province. Samples of drinking water, soil, grains (wheat, corn) and hair (5-13-year old boys) were collected for selenium analysis. Selenium was analyzed by the fluorometric method of Wilkie (1970).

2. Investigation of Yongshou County (800 km²)

One hundred and four affected villages were selected from a total of 192 villages in Yongshou county (a severely affected county in Shaanxi province), based on the clinical and X-ray examination data supplied by the local health authority. Samples of drinking water, soil, grains and hair (5-13 year old boys) were collected and analyzed for elements (Se, Cu, Zn, Mn, Fe, Ca, Mg, S, Mo, Sr, Ba and Cr). Se was measured by the fluorometric method of Wilkie (1970) and S by the colorimetric method of Blancher et al. (1965) and Beaton et al. (1968). The other elements were measured by the atomic absorption spectrophotometric method (Armannsson, 1979; Berndt and Messerschmidt, 1981). For S analysis, hair samples after washing were subjected to wet digestion by $\text{HNO}_3\text{-HClO}_4$ (2:1). For other element analysis, hair samples were digested with $\text{HNO}_3\text{-HClO}_4\text{-HF}$ and then dissolved in 0.1N HNO_3 . Correlation analysis was carried out using the analytical results and KBD prevalence for each village.

3. The investigation of two micro-environments (208 and 10 km²) in Yongshou and Zhangwu county

Villages of different KBD prevalences in these two micro-environments were subjected to the same survey protocol as above. Disease information was supplied by the Shaanxi Provincial Institute for Endemic Disease Control.

Results

1. The investigation of macro-environment (190,000 km²) in Shaanxi province (Li et al., 1979, 1981, 1982)

KBD was distributed in the following three regions in this province with different types of natural environment.

(1) The region of loess plateau: This region belongs to semiarid and semihumid forest steppe belt in the warm temperature zone. The zonal soils in the whole region are heavy clay Heilutu soil. The KBD prevalence among the local residents detected by clinical diagnosis was 10 to 30% in most of the disease-affected areas, and more than 30% in some other areas. The local inhabitants in these regions live mostly on wheat, corn and millet. The sources of drinking water are mainly the dry well water, stream and spring water.

(2) The region of Qinling-Daba mountain type: KBD distributed in the high and mid-hill region and appeared to be in the scattered blocks in the regions. The zonal soils are the brownearth and mountain brown soil. The prevalence of this disease among local residents was less than 10% in most disease-affected areas, and in only a few sites the prevalence was 10 to 20%. The local inhabitants live mainly on corn, wheat, millet and potato, while their drinking water is mainly stream water and spring water.

(3) The region of peatabog type: This region consists of the east part of arid and semiarid Mao-Wu-Su desert belt where KBD is distributed in scattered villages. The geographical features of environment are the lower relief and wide distribution in the peaty soil. The subsoils show a slight saline in some areas of this region. The incidence of this disease among local residents was less than 10% in the disease-affected areas. The local inhabitants live mainly on millet, wheat, potato, etc. The drinking water consists of the latent water in the sand layers deposited by the wind and lake in the Quaternary.

In addition, no KBD has been found in the following regions:

(1) The Weihe River Plain in Guanzhong areas; (2) the arid and semiarid steppe region in northern Shaanxi; (3) most part of Qin-Ba mountains in the southern Shaanxi, and (4) the saline region in the west part of Mao-Wu-Su desert belt.

The sites under investigation included 130 affected and non-affected sites in 70 counties and cities. One sample of soil and one sample of drinking water were collected from each of the survey sites. At the same time, around 200 samples of various grains were collected from households in these areas and about one thousand samples of children's hair were collected. The analytical results of Se contents of soil, drinking water, grains and children's hair in various locations are described as follows.

There were significant differences in the Se contents of grains and children's hair between the affected and non-affected sites. The Se contents in wheat and corn in the affected sites ranged from 7.0 to 9.4 ppb and 4.7 to 8.7 ppb respectively. And in the non-affected sites, the Se contents in wheat and corn ranged from 19 to 1057 ppb and 10.2 to 128 ppb respectively. On the other hand, in the affected sites, 92% of the 478 children had Se less than 110 ppb in their hair, while in the non-affected sites, 93% of the 555 children had Se more than 110 ppb (Table 1).

There were also some significant differences in the Se contents of drinking water and soil between the affected and non-affected sites. Water Se contents in all affected and non affected sites were less than 0.3 ppb. At the same time, there were 58% of the non-affected sites where Se contents in drinking water were less than 0.3 ppb and in the rest of the non-affected sites, they were more less than 0.3 ppb.

With regard to the water soluble Se in soils, 75% of the affected sites had a content below 1.8 ppb, while 71% of the non-affected sites had a content above 1.8 ppb. And yet in one third of these locations, the concentrations of water soluble Se in soils were the same.

Generally, the amount of Se intake from grains was much greater than that from drinking water. The above data show that Se contents in grains were very low in the severely-affected areas.

Table 1. Difference in Se content of environment and human hair between KBD affected and non-affected sites

Se (ppb)	No. of samples							
	Water		Soil		Grains		Hair	
	I	II	I	II	I	II	I	II
0.01-0.10	34	14						
0.11-0.20	17	19						
0.21-0.30	2	7	1					
0.31-0.60		4	1	2				
0.61-0.90		5	7	7				
0.91-1.20		5	9	5				
1.21-1.50		2	14	5				
1.51-1.80		-	10	3				
1.81-2.10		2	10	8				
2.11-2.50		1	1	17				
2.51-3.00		-	3	11				
3.10-4.00		2		10				
4.10-5.00		2		1	11			
5.10- 10		4		4	32	4		
10.1 - 30		2		1	1	35	39	
30.1 - 50				1		6	147	
50.1 - 70						3	114	
71 - 110						4	140	40
111 - 200						2	38	216
201 - 300								152
301 - 500								64
501 -1000								31
1000 -5000								45
> 5000								7
No. of samples	53	69	75	44		54	478	555

a. I--KBD affected sites.

b. II--disease-free sites.

2. The investigation of Yongshou County (800 km²) of Shaanxi Province (Li et al., 1985)

The results of an investigation on 104 villages in the KBD severely affected county show that the

incidence of KBD is closely related to the change of topography and various kinds of water and soil. There is a significant negative correlation between the incidence rate of KBD children detected by X-rays examination and Se contents in water, soil, food grain and hair (Table 2).

Table 2. Correlations between the amount of Se in drinking water, soil, grains^a and children's hair and the incidence of KBD

Correlations	No. of samples	Correlation coefficient(r)
Soil Se vs. grain Se	93	0.112
Grain Se vs. hair Se	82	0.348 ^b
Drinking water Se vs. hair Se	94	0.639 ^c
Total amount of Se in grain and water vs. hair Se	77	0.375 ^c
Grain Se vs. incidence of KBD	95	-0.425 ^b
Total amount of Se in grain and water vs. incidence of KBD	87	-0.431 ^b
Hair Se vs. incidence of KBD	66	-0.478 ^c

a. Se in grains represents the amount of Se in wheat and corn.

b. $p < 0.01$.

c. $p < 0.001$.

3. The investigation of two micro-environments (2.8 and 10 km²) in Yongshou and Zhangwu county of Shaanxi province (Yongshou Scientific Survey Team of Kashin-Beck Disease, 1984)

The first survey was carried out in two adjacent villages with highly different KBD incidences (incidence rate being 11.5% and 92% respectively). The low incidence village Jiangjashan is located in the low-lying land where 30% of the soil have higher level of Se, i.e. 130-340 ppb of total Se and 3.2-8.1 ppb of water soluble Se. As a result, the average Se content of wheat flour was 35 ppb, while the average hair Se content of local children was 134 ppb. On the other hand, the high incidence village, Beimen, is located in a seriously eroded loess mound, where the soil contains only 64.8 ppb of total Se and 1.6-2.2 ppb of water soluble Se. The Se content of wheat flour averaged 5.2 ppb while the Se content of children's hair averaged 45 ppb.

The second study was carried out in another micro-environment in Zhangwu Yuan, a high plain with abruptly descending edges in Zhangwu county under which there is the Jinhe river valley. In this micro-environment, four villages, i.e., Penggong, Langtou, Xianggong and Dongzhui had descending rates of KBD incidence (Table 3). The incidence among residents living in the village located in the center of Yuan was

extremely low, but the incidence of KBD increased gradually from villages in the center to those at the edge of Yuan. The Se amount in drinking water, soil, grains and children's hair decreases gradually from the

Table 3. Se content in environment and children's hair in the 4 villages of Zhangwu Yuan

	Non -affected village (Dongzhui)	Slightly -affected village (Penggong)	Moderately -affected village (Langtou)	Highly -affected village (Xianggong)
Percentage detected by X-ray (%)		0.0	20.0	92.5
Altitude (M)	900	1185	1165	1080
Total Se in soil (ppb)	70	98 ± 7	97 ± 16	85 ± 5.9
Soluble Se in soil (ppb)	1.3	2.6 ± 0.2	2.2 ± 0.7	1.6 ± 0.6
Se content in wheat (ppb) ^a	4.4 ± 1.2	9.4 ± 0.4	7.6 ± 4.3	5.8 ± 1.3
Se content in corn (ppb) ^a	5.3 ± 2.5	4.0 ± 0.6	3.8 ± 0.7	2.8
Se content in drinking water (ppb) ^a	2.13	0.38	0.24	0.06
Daily Se intake (μg)	8.73	5.03	4.0	2.63
Se content in hair (ppb)	93 ± 8.4	91.5 ± 14	52 ± 13	49 ± 9.6

^aThe total Se amount provided by the drinking water and grains to every child in accordance with the proportion of grain (70% wheat and 30% corn) and 3 liter drinking water every day.

center to the edge of Yuan. But there was no incidence of KBD in the village located in Jinhe River Valley and the drinking water for the residents was the well water with a Se content up to 2.3 ppb.

Discussion

1. It has been found through extensive surveys and studies that there are significant differences in the Se content of soil, water, grains and children's hair in various regions of Shaanxi province, because of the variation in geochemical conditions and the uneven distribution of selenium.

2. The sites severely and slightly affected by KBD and the non-affected sites are interdistributed in the micro-environment of KBD affected area, where the different soil types were formed by the change of micro-geomorphology, and the Se contents in various soils are significantly different. In addition, hydrogeological and geographical factors can affect the amount of Se in drinking water. As a result, where Se contents both in grains and drinking water are low, the KBD is bound to occur, and where Se contents in grains or drinking water are high, there may be no or only low incidence of KBD.

REFERENCES

- Armansson, J., Dithizone extraction and flame atomic absorption of Cadmium, Zinc, Lead, Copper, Nickel, Cobalt, and Silver in sea water and biological tissues. *Anal. Chim. Acta.* 110:21, 1979.
- Beaton, J.D., Burns, G.R., and Platon, J. Determination of sulphur in soils and plant material. *Tech. Bull. Sulphur Institute.* No.14. p.39. 1968.
- Berndt, H. and Messerschmidt, J., Microanalytical method of flame atomic absorption and emission spectrometry. *Spectrochim. Acta.* 36B: 809, 1981.
- Blancher, R.W., Rehm, G., and Caldwell, A.C., Sulphur in plant materials by digestion with nitric and perchloric acid. *Soil. Sci. Soc. Am. Proc.*, 29:71-72, 1965.
- Li, J., Ren, S., and Chang, D., A study of selenium associated with Kashin-Beck disease in different environments in Shaanxi. *Acta Scientiae Circumstantiae*, 2:93-101, 1982. (in Chinese).
- Li, J., Ren, S., and Chang, D., A study on relationship between Kashin-Beck disease and selenium from Se contents in human hair of various natural environments in Shaanxi province. *Journal of Environment Science*, 5:18-21 (1981). (in Chinese).
- Li J., Chang, D., and Ren, S., Relationship between Kashin-Beck disease and the selenium contents in environment. *Endemic Disease Newsletter*, 2:22-28, 1979. (in Chinese).
- Li, J., Ren, S., Chang, D., and Wan, H., The distribution of selenium in micro-environment related to

Kashin-Beck disease. *Acta Scientiae Circumstantiae*, 2:140-148, 1985.(in Chinese).

Wilkie J.B., Improvement in the 2,3-diaminonaphthalene reagent for microfluorescent determination of selenium in biological materials. *J.Agric. Food Chem.* 18:944-945, 1970.

Yongshou Scientific Survey Team on Kashin-Beck Disease Ecological environmental research on Kashin-Beck disease in Yongshou county. *Acta Scientiae.*, 1:1-19, 1985. (in Chinese).

STUDIES ON THE RELATIONSHIP BETWEEN SELENIUM AND HUMIC ACID EXTRACTED FROM KASHIN-BECK DISEASE REGIONS

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Introduction

Three independent factors, low selenium (Se) level in the environment, high organic compounds content in the drinking water and fungi toxins in the local grains have been considered in the study on the cause of the endemic Kashin-Beck disease. Removing any one of the three causes reduces the incidence of the disease (Niu, 1984; Wang, 1984; Yang, et al., 1983), but the actual relationship among the three factors has not been reported in detail. Selenium (Se) has been found to be an essential element to life. Its biological functions have been noticed because of its effects on several diseases (Peng and Xu, 1987). Humic acid (HA) is the main constituent of organic substances in natural water. Its physical and chemical effects on other substances, except its toxicity, have been studied. This study focuses on the relationship between Se and HA in their chemical and biological functions, which are evaluated by computer program, animal tests, several instrumental analysis etc. The relationship between Se and mycotoxin has been also studied (Peng et al., 1987a). The observations suggest that Se, organic matter in water and fungal toxin in grain might act synergetically in the causation of Kashin-Beck disease.

Methods and Materials

1. Pattern recognition computer program

The study on relationship between the incidence of Kashin-Beck disease and both Se and HA level in the drinking water was carried out with the pattern recognition computer program (Bei, 1987).

2. Chemical analysis

Humic acid was extracted from the drinking water in Yongshou, Shaanxi province. Fulvic acid (FA), a fraction of humic acid, was obtained by extraction.

SeO₃ and Na₂SeO₃(A.R.) were produced by Beijing Chemical Plant. For determination of Se: RF-520 Spectrofluorophotometer, and 3-diaminonaphthalene were used. Free radical was determined

[Ⓐ]Renamed as Research Center for Eco-environmental Sciences

by E-109 ESR spectrometer (Wang, 1984) and bio-luminous photometer produced by Nanjing Soil Institute. Activity of GSHpx was determined with the improved Mills method (Mills, 1959).

3. Biological experiments

Animal test. Wistar rats and Kunming strain mice fed with laboratory diet were tested in three groups. In the first group, the toxicity of humic acid extracted from disease region water was tested; in the second, the inhibition of FA on the toxicity of Se was observed; in the third, the inhibition of Se on HA was observed. FA and Se (IV) were injected intraperitoneally to mice. The survival time of mice was recorded.

Luminous bacteria test. Photobacterium phosphoreum T₃ was isolated from Tripteryphycis intermedus (a fish from Australia), and provided by Nanjing Soil Institute, Academia Sinica. The method of culture and determination was referred to Peng et al., (1987b) and Bei, (1987).

Results and Discussion

1. The non-linear recognition computer program was applied to the study of pathology of Kashin-Beck disease (Bei, 1987). Among the parameters studied such as the state of illness, contents of elements (Mn, Fe, Cu, Mo, Zn, etc.) in drinking water, soil, grain and human hair from the disease regions, the selenium and humic acid contents in drinking water were the most significant parameters, and the correct classification coefficient could be as high as 0.87 between the disease and non-disease regions. When pattern recognition technique was applied to the data of elements contents in human hair from both Kashin-Beck disease and Keshan disease regions and non-disease regions, the following regression equation was obtained (Bei, 1987; Bei, 1986)

$$Y_1 = 0.9880X_{Se} - 0.1230X_{Mn} + 0.0710X_{Fe} - 0.0386X_{Cu} - 0.0525X_{Mo} - 0.0569X_{Zn} = 0.9880X_{Se}$$

The above data indicate that selenium deficiency in Kashin-Beck disease regions is more serious than that in Keshan disease regions.

2. Antagonism between Se (IV) and humic acid

Selenium (VI) was found to be reduced and adsorbed slightly by humic acid. The results indicated that the effect of humic acid on physical and chemical behavior of selenium was insignificant (Peng et al., 1986).

The result of the effect of FA on the distribution of selenium in mice (Peng, and Xu, 1984) showed that the fed Se (IV) (5 μ g Se in drinking water / mouse / day for 10 days) was found mainly in kidney, liver, spleen and some other organs, which were in agreement with the results of other reports (Shamberger, 1983). The addition of FA not only decreased the Se levels in liver and heart, but also increased the concentration of Se in spleen. The Se level in kidney, lung, blood and bone in mice were on-

ly slightly changed. Besides, the Se content in bone was much lower than in other organs, which conformed to our previous results of radioautograph experiments. The activity of GSHpx in mice blood was increased by about 30 % when Se (IV) had been added with FA. Because the Se levels in Se (IV) and FA-Se (IV) groups were about the same, we might assume that FA enhanced the activity of GSHpx. The result of the study on toxicity of FA showed that after injection (i.p) of 400 FA mg / kg b. w., the mice died within 1 day, and the mice which received 2.94 mg Se / kg b. w. died within 1 day too. When solution containing selenium (SeO₂) and FA was injected simultaneously at fatal doses, the mice and the rats survived. The results of these experiments are summarized in Table 1.

Table 1. The toxicity antagonism between Se and FA in female mouse and rat

Test No.	Animals	Body Weight (g)	Dose of Se injection (pH = 7) mg Se / kg b.w.	Dose of Yongshou FA injection (pH = 8) mg FA / kg b.w.	Results
1	Mouse	30	0	50	live after 7 days
2	Mouse	30	0	200	live after 7 day
3	Mouse	30	0	400	die within 1 day
4	Mouse	28	0	535.7	die within 1 day
5	Rat	170	2.94	0	die within 1 day
6	Rat	170	2.94	0	die within 1 day
7	Rat	200	3.00	132.6	live after 3 days
8	Mouse	23	5.35	338.3	live after 3 days
9	Mouse	28	0	535.7	die within 1 day
10	Mouse	30	0	400	die within 1 day
11	Mouse	30	0	800.0 ^a (total amount)	die during 12 h after the last in jection
12	Mouse	28	5.071	535.7	live after 7 days
13	Mouse	30	4.733	500.0	live after 5 days
14	Mouse	30	4.000 ^a	800.0 ^a	live after 8 days

a. Several injections at 6-hour intervals.

Mixture of selenite and FA was clearly less toxic than selenite or FA alone. It could suggest that the antagonism between Se and FA played a role in the etiology of Kashin-Beck disease. The luminosity of the T₃ was used as a probe for studying the effects of Se and FA on the biological processes in the organism. Se concentrations < 100 mg / L and FA concentrations < 120 mg / L enhanced the luminosity.

ity. At higher concentrations the luminosity decreased. In a medium containing FA and selenite (at Se concentrations one-tenth of those of FA), the luminosity of the bacteria was considerably higher than the luminosity in media containing FA alone (Fig. 1). Selenite and FA less affected the bacteria in combination than singly, in both mice and rats. The same phenomenon was observed in chondrocytes test.

To compare its physical-chemical characteristics of HA from both disease and non-disease regions (Wang and Peng, 1984), HA was extracted from the water and soil of Yongshou disease region. To this end the total organic carbon in drinking water from both disease and non-disease regions were determined; research was made on spectra characterization using ultra-violet, infrared and fluorescent spectra as well as on ratio value E_4/E_6 , and the content of free radicals, and the gel filtration for the fractionation of HA. Some differences between the humic acids from disease and non-disease regions were observed, and especially a high concentration of free radicals in humic acid from the soil of the disease regions was found. The results were shown in Table 2.

From the above results of our experiments, we suggest a preliminary hypothesis on the cause of Kashin-Beck disease. The disease is caused by the toxicity of the organic compounds in drinking water (and probably in grains) and other pathogenic factors, but in non-disease regions where Se is adequate, the toxicity is antagonized by selenium.

Table 2. The contents of free radical of HA and FA extracted from water of disease and non-disease regions

Samples	Sources	Free radical, spins / g
Soil FA	Jiang Jie Shang ^a	8×10^{17}
Soil HA	Guan Guan Gou ^b	1.76×10^{18}
Soil FA	Guan Guan Gou ^b	6.12×10^{18}
Soil FA	Guan Guan Gou ^b	5.96×10^{18}
Sediment HA	Jiyu river ^c	1.7×10^{15}
Sediment FA	Jiyu river ^c	4.9×10^{14}

a. non-disease region (close to disease region, Yungshou);

b. disease region (Yongshou);

c. health region (far from disease region).

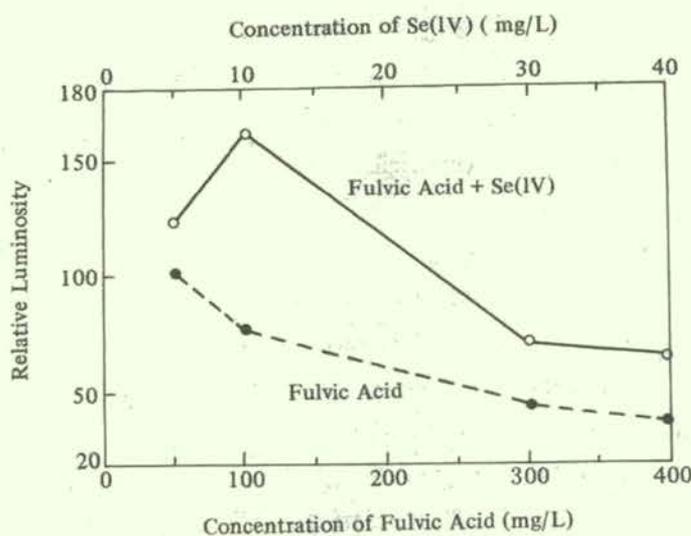


Fig 1. The luminosity of *Photobacterium phosphoreum* T-3 in a medium containing selenite and fulvic acid

REFERENCES

- Bei, N. B., Multinomial statistical analysis of trace elements in the regions of Kashin-Beck disease, *Chinese Jour. Environ. Sci.*, 8: 14-18, 1987 (in Chinese)
- Bei, N. B., Study of the hair data from Keshan and Kashin-Beck discas regions by using pattern recognition method, *Chinese Jour. Endemiology*, 5: 281-284, 1986. (in Chinese)
- Mills, G. C., The purification and properties of glutathione peroxidase of erythrocyte J. *Biol. Chem.* 234:502-506, 1959
- Niu, G. Results of study of two years on the prevention and control of Kashin-Beck disease with selenium seen under X-rays and discussion of etiology, *Chin. J. Endemiol.*, 3: 197-201, 1984, (in Chinese)
- Peng, A., Wang, Y.J., Wang, W. H., Xu, L. Q., and Yang, C. I., Inhibitory effect on the toxicity of Fusarium toxin by selenium, *Acta Scientiae Circumstantiac*, 7:237-239 1987. (in Chinese)
- Peng, A. and Xu, L. Q., The effects of humic acid on the chemical and biological properties of selenium

- in the environment, *The Science of the Total Environment*, 64:89-98, (1987). (in Chinese)
- Peng, A., and Xu, L. Q.**, Effects of humic acid from Kashin-Beck disease regions on the distribution of selenium in mice and the activity of GSHpx in mice blood, *Proceeding of the 1st Symposium of Biochemistry Related to Kashin-Beck and Keshan disease (Chengde, 1984)*, p 267, 1984. (in Chinese)
- Peng, A. and Xu, L. Q.**, Antagonism between selenium and humic acid, *Scientia Sinica*, 15: 66-74, 1987. (in Chinese)
- Shamberger, R. J.**, *Biochemistry of Selenium*, 1983, pp.63, Plenum Press, New York
- Wang, W. H. and Peng, A.**, Investigation of humic acid in water and soil from Kashin-Beck disease regions in Yongshou County, *Bull. Third Conference of Chemical Science Discussion on Humic acid (Lushan, 1984)*, p.333. (in Chinese)
- Wang, Y.**, Outline of the relationship between Kashin-Beck disease and drinking water, *Endemic Disease Newsletter*, 2: 19-22, 1984 (in Chinese)
- Yang, J, He, F., An, M. and Lu, M.**, The progress in studies on effect of Fusarium toxin on Kashin-Beck disease., *Chinese J. Edemiol.* 2:155- 160, 1983 (in Chinese)

STUDIES ON THE BACTERIA TOXIN ETIOLOGY OF KASHIN-BECK DISEASE

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Although the etiology of Kashin-Beck disease is still unknown, the possible relationship between selenium deficiency and this disease has been paid more attention than other hypotheses. However, there are some characteristics of Kashin-Beck disease that cannot be explained by the selenium deficiency hypothesis. Our previous works suggest that the A1 bacteria and its toxin existing in the grains of the endemic areas might be the specific etiological factor and that the low selenium status of local population might be a conditional etiological factor (Niu et al., 1981).

Materials and Methods

1. The isolation of specific etiological factor

Fifty-five grain (wheat, corn, millet and sticky millet) samples collected in Kashin-Beck endemic areas (Heilongjiang, Neimongu) and non-endemic areas (Heilongjiang, Hebei, Henan, Shangdong and Jiangsu) and the bacteria and fungi profiles were studied according to the Methods of Food Hygiene Analysis—Microbiological Section (Ministry of Public Health, 1976). All the isolates were tested in mice (17–18 g body weight) for their toxicity by intraperitoneal injection of the cultures.

2. Pathological studies in rats.

Wistar rats, one week after weaning, were randomly divided into 2 groups with 10 rats per group. Both groups were fed with the same low selenium diet, which was composed of low selenium corn flour (Se content 0.012 ppm) and sodium chloride 2 g/kg, nicotinamide 4 ng/kg, lard 50 g/kg and calcium phosphate 2.3 g/kg, vitamin A 3000 IU/kg and vitamin D 10,600 IU/kg. Selenium free water was used as drinking water. After 2 months of selenium depletion, crude A1 bacteria toxin was added to the drinking water for rats in the experimental group, while rats in the control group continued drinking of selenium free water. All the animals were sacrificed after one month of toxin administration. The selenium content of whole blood was determined by 2,3-diaminonaphthalene fluorometric spectrophotometry (Wilkie, 1970). The heart, liver, lung, kidney and shinbone were fixed

in formalin and their sections observed by microscopy after stained with eosin and hemotoxylin.

Results

1. The isolation of A1 bacteria.

Nineteen types of microorganisms were isolated from 30 endemic and 25 non-endemic study sites. A1 bacteria (an unknown species) was found in 93.33% of the grain samples collected from the endemic sites and in 58.83% (1750 / 3000) of the cultured grain kernels, while in the non-endemic study sites, A1 bacteria was only found in 12.00% of the samples and in 0.032% (8 / 2500) of the cultured grain kernels. The differences were highly statistically significant ($X^2 = 9.98$, $P < 0.001$, for the sample detection rate, and $X^2 = 1190.87$, $p < 0.001$, for the kernel detection rate).

All the 20 mice died within 24 hours after 0.3 ml of crude A1 bacteria toxin were injected intraperitoneally. No death occurred in the control group (20 mice) which was injected with physiological saline.

2. Morphological changes of shinbone in low selenium rats administered with A1 bacteria toxin.

At the time of sacrificing, the average blood selenium concentration of the experimental and control groups were 0.0922 and 0.0789 ppm respectively, showing no significant differences. The main morphological changes in the shinbone of the experimental rats were:

- (1) Necrotic foci were found in the hypertrophy cells of cartilage epiphysis and all cells were in bad order. In the necrotic area of cartilage epiphysis, there was separation between the bone beams and the necrotic areas, and cartilage islands were seen.
- (2) Degenerated cells and necrosis were found in joint cartilages. No pathological changes were found in the control rats.

Discussion

From the above results, a new etiological hypothesis of Kashin-Beck disease is suggested, i.e. low selenium status (the conditional factor) combined with the ingestion of bacteria A1 toxin (the specific pathogenic factor) for at least 3 months. The main evidences are: (1) The Kashin-Beck endemic areas are located in the low selenium zone, where the selenium content of locally produced grains is less than 0.03 ppm and the hair and blood selenium contents of local residents are less than 0.20 and 0.020 ppm respectively; (2) Some Kashin-Beck disease free sites are located in low selenium areas, which suggests that selenium deficiency is not the only etiological factor; (3) The detection rate of bacteria A1 in the grains of endemic areas is significantly higher than that of non-endemic areas; and (4) crude bacteria

A1 toxin can cause pathological changes in cartilage epiphysis and joint cartilage in rats similar to the changes in human Kashin-Beck disease.

REFERENCES

- Ministry of Public Health, Methods of Food Hygiene Analysis—Microbiological Section, 1976, Technical Standard Publishing House, Beijing.
- Niu, G., Zhang, B., Song, Z., Pang, Z., Wang, Y. and Ma, Q., The preventive effect of oral administration of sodium selenite in Kashin-Beck disease, Heilongjiang Medicine No. 6, pp. 15-17, 1981
- Wilkie, J.B., Improvement in the 2,3-diaminonaphthalene reagent for microfluorescent determination of selenium in biological materials. J. Agric. Food Chem. 18:944-945, 1970.

INVESTIGATION ON THE RELATIONSHIP BETWEEN KASHIN-BECK DISEASE AND DRINKING WATER

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One hundred and thirty years have elapsed since the first report of Kashin-Beck disease (KBD). The pathogenic factors of KBD remain unclear (Hypabres, 1956). In our opinion, KBD is an endemic disease closely related to the local natural environment. The pathogens might enter the human body through drinking water or grains, causing damage to the long and short tubular bone of most adolescents in endemic areas, and leading to chronic degenerative necrosis of epiphyseal cartilage, epiphyseal plate cartilage and articular cartilage.

In 1908, Liu Jianfeng, a local administrator, made a statement on KBD in a book named "A Brief of River and Ridge in Chang Bai Mountain". He wrote, "The walnut trees behind the ridge are most harmful to human beings. The branches, leaves, flowers, fruits roots and barks decay in the mountain year after year out of rain and snow immersion. The produced poisonous gas is carried in water along the brooklets to the river and diffuses into wells and springs. People dwelling in the mountain suffered from shortening of hands and feet, enlargement of joints of fingers and toes beginning from the age below 15-16 years of both males and females. It is still harmful to the people. Half of the victims drink mountain water and half drink river water. Few of them have wells and occasionally a shallow well less than 5 feet deep could be found. It is then nothing to be surprised at so many victims." Finally, he pointed out, "Only by drilling deep well, can people be protected" (Liu, 1908). The following is a summary of our studies on the relationship between KBD and drinking water in supporting the above statement.

Methods and Materials

The clinical data were taken from the epidemiological investigations carried out by various medical and geographical teams in the last 15 years in Jilin province. Diagnosis of KBD was based on the changes in the hand roentgenogram of patients. The humic acids in water were concentrated under low temperature, extracted with sodium phosphate and sodium hydroxide and then measured by ultraviolet spectrophotometry (Schnitzer and Khan, 1972; Stevenson, 1982).

Results and Discussion

1. Significant difference in incidence rates of KBD among population using different drinking water sources

It has been observed in many areas of the northeast and northwest parts of China that there is a "healthy island" within endemic areas and a "disease island" within non-endemic areas. The disease area is clearly demarcated with relatively stable endemicity. It shows the characteristics of endemic and focal distribution of KBD. The endemic areas are sometimes very close to the non-endemic areas, and they could even be located in the same village. While there is no apparent difference in agriculture production, life style and varieties (self produced or supplied by others) of food-stuff between the endemic and non-endemic sites, significant differences do exist in drinking water sources.

For example, Dong Shan village where the people drank spring water from rock slit was a "healthy island" without KBD, while the morbidity of KBD in Buó Ji village 1.5 kilometers away from Dong Shan village where the people drank surface water from swamp was 45.10 % in 1970. An analysis was made on the spring water in 1975 with no humic acid detected, while the content of humic acid in the surface well water reached as high as 5.7 mg / L.

The 1st team of Bao An farm exploited the field and built the village in 1960. The people was migrated from non-endemic areas. In 1972, the prevalence of KBD in youths was (72 / 101) and there was no victim in adults. The village is located in the ancient river bed of former Liao river with the water source polluted by the silt layer of river bed. The water had a sanguinary foul odor with humic acid content of 0.2 degree. One fourth kilometer away, the Mi Li Ying village which is a non-endemic village is located on the river bank with shallow wells not polluted by silt layer in river bed, humic acid being 0 degree. There was a very significant difference in morbidity between the two villages ($X^2 = 65.74$, $p < 0.01$).

The village of Fu Xing No. 2 team was divided into a front street and a back street. The drinking water source of the front street was spring water from rock slit which was clear and odorless, while the water source of the back street was from low swamp area with a dirty pool and dung accumulation. There was a very significant difference in X-ray detected rate of KBD between the students in the front street (35.48 %) and those in the back (81.48 %) in 1977 ($X^2 = 12.45$, $p < 0.01$) (Zhai et, al., 1977).

There were three teams in Xing Hua farm. The X-ray detected rates of KBD among students of three teams showed a very significant difference ($X^2 = 128$, $p < 0.01$). No. 26 team was located in the plain and was heavily polluted. No. 3 and 4 teams were located on mountain area. Their drinking water source was spring water from granite slit with no pollution. The food-stuff of each team was supplied by the government. In 1977, the detected rate of KBD in pupil was 71.43 % (9 / 126) in No. 26 team,

11.76 % (8 / 68) in No. 3 team and 13.8 % (9 / 65) in No 4 team. The prevalence was significantly higher in No.26 team than in the 3rd team ($p < 0.001$) and the 4th team ($p < 0.001$).

2. Change drinking water source could alter the morbidity of KBD

In many villages, in case of no changes in foodstuff and life-style, the endemic village became non-endemic, and vice versa, only after an alteration of drinking water sources.

For example, Xiao Ying Zi village in Jing Yu county was severely affected by KBD, and before 1946, the villagers dwelled on the mountain slope and drank mountain water. After 1946, when they migrated to the valley because of exploitation of the forest, they began to drink spring water from pressurized basic rock slit. A general X-ray survey on all villagers in 1974 showed a significant difference in KBD morbidity between those above 26 years old (42.11 %) (drinking surface water during childhood) and those below 25 years of age (0.96 %) (drinking spring water all their life) ($p < 0.01$). But there was no difference between those born in other endemic villages and having migrated to this village after they grew up ($p = 0.341$, $p > 0.05$). Their detected rate of KBD (Ca. 20 %), however, was significantly different from those born in this village below 25 years of age ($p < 0.001$). The people migrated from other villages did not change their water source there and they were affected before the migration. The humic acid in spring water was 0.25 mg / L which indicated a very slight organic pollution.

In Bao An village, before 1943, the inhabitants lived in various valleys and drank surface ditch water. After the building of the village, they drank the underground spring water. It was in 1974 that in the villagers below 30 years old (born after the building of the village), no case of KBD was detected, while in the population above 31 years old, the X-ray detected rate reached 65.2%.

Qian An county is located in Song Nen plain at the middle point between Fuo Long spring and Li Si lake area. Formerly, it was thought that there should be no KBD in water-static and mineral accumulating area of plain grassland. Our institute, however, discovered in 1973 the occurrence of KBD in this plain grassland and found that it was due to the pollution of drinking water source by black and grey silt layer. The humic acid content of the water reached 6.5–7.3 mg / L (Zhai and Zhao et al., 1980).

In Run Zi Jing village, there was no KBD in more than 30 years after it was built. The disease, however, began to appear in 1966 after a change of the drinking water to a deep machinery well of 70 meters in depth. The well passed through two layers of grey and black silt subviscid earth with a thickness of 30–40 meters. Shells and tree leaves and black water with sanguinary foul odor were taken out during drilling. An examination on 283 villagers in 1973 revealed a very significant difference in KBD incidence between those under 20 years and those above 21 years of age ($p < 0.01$). There was no incidence of KBD in the neighboring Jie Zi Jing village in this period with no change of drinking water source. In August of 1973, the villagers started to drink water from other source not polluted by this

kind of earth layers. No new victim was then detected in 1975 and 1978.

Xin Fang Zi village was built in 1960. The inhabitants migrated from Wu Lian county, a non-affected area. Before 1965, there was no KBD when the drinking water was from wells of 7-8 meters in depth. The water source was then changed to other two new wells in 1966 and KBD appeared in 1969. A general survey on 434 youths in 1973 showed an X-ray change rate of 62.20%. According to the drilling data, the new wells reached the grey and black silt layer. The water source was then changed to sand rock layer water 120 meters underground and the invasion of silt layer water was sealed up in 1975. No new victim was detected during the examination in 1978. Wu Feng village, formerly a non-disease area, was flooded in 1968, resulting in the invasion of grassland water from the northeast corner into the drinking water source in the village. The victims were first found in 1970. In 1977, the X-ray detected rate in 210 students was 53.8%. No victim was detected in adults. There was no change in lifestyle and foodstuff of the inhabitants. The disease was apparently due to the effect of flood on drinking water source (Zhai and Chang, 1977).

3. The control of KBD by water alteration

It is a common experience in our province to control the disease by water alteration. Two examples of large-scale water alteration are demonstrated.

The KBD detected rate in Hua Dian town before water alteration was 34.09%. The water from the former water source, Huci Fa river, had been precipitated, filtered, processed by chlorinic disinfection and supplied for drinking in the form of tap water since 1956. A comparative survey was made in 1973 on the youths of 6-16 years old (born in this town after water alteration) who drank tap water and also river or well water (on the same food supply). The X-ray detected rates showed a very significant difference ($X^2 = 17.60$, $p < 0.001$) between populations with different drinking water sources. The detected rate in the tap water group was 0.59% (3/508) and in the well water group 5.63% (16/284). On the contrary, the difference in detected rates was insignificant ($X^2 = 0.392$, $p > 0.50$) when the people living on different food sources were compared (the same well water source). The detected rate in population who consumed government supplied cereals was 5.63% and in population who consumed self-produced cereals 2.17%. (Zhai et al., 1976).

At Jin Yu town, the KBD detected rate in 1964 was 25.20% when their water source was river. It was changed to tap water from deep layer basic rock slit and filtered through manganese sand to eliminate iron. X-ray examination made on population in the age of 6-16 years in 1974 showed a very significant difference ($X^2 = 26.14$, $p < 0.001$) in detected rates in population groups using different water sources. The detected rate in the tap water group was 1.76% (9/512) and in the well (river) water group 10.44% (19/182). The victims in tap water group were distributed between 11-16 years of age with slight X-ray lesion which might be affected before water alteration. The well (river) water group

had victims in all ages with severe X-ray lesions (Zhai, Shao, et al., 1980).

4. A significantly low detected rate of KBD in persons drinking boiled water

In 1973, a survey on water drinking habit of persons below 20 years old in Xing Fan Zi village showed a significant difference ($X^2=8.01$, $p<0.01$) of X-ray detected rates between those drinking boiled water as a habit (14.04%, 8 / 5) and those drinking unboiled water (32.68 %, 41 / 135). It indicates that boiling may eliminate or attenuate the pathogenic effect.

5. All pathogenic drinking water sources were polluted by humic materials

According to a number of surveys, the intimate relationship between KBD and natural environment could be established. There are two basic characteristics in the environment of endemic areas. One is a localized reductive environment rich in humic materials. The other is water stasis.

The total amount of humic acid in drinking water of all KBD endemic areas within and outside Jilin province was higher than 0.5 mg / L, significantly higher than that in the non-endemic areas. The total amount of humic acid was in positive correlation with the incidence of the disease ($r=0.279$, $P<0.01$), which was in positive correlation with the logarithm transformed value of humic acid, $r=+0.348$ and its logarithm regression equation was; $Y=54.03+10.421 \log X$ (The Survey Team of KBD in Yongshou County, 1984).

Humic acid content in drinking water: According to the examinations on nearly one thousand water samples from 600 natural villages of 68 counties all over the country reported by Lin (1985), the humic acid in drinking water source of severe endemic areas averaged above 0.45 mg / L, while that of the non-endemic areas averaged 0.03 mg / L, the difference being highly significant. According to most disease sites in Yongshou county, the X-ray detected rate of KBD and humic acid content in drinking water were correlated in many functions; logarithm ($r=0.569$), exponential ($r=0.508$), linear ($r=0.492$) and exponent ($r=0.359$), the differences being highly significant ($p<0.01$).

The drinking water of the disease area in Qian An county belongs to the 4th epoch q_2 , q_3 layer water, the humic acid content of which is apparently higher than that of the 3rd epoch Nt layer water. In order to identify the relationship between the humic acid in drinking water from q_2 , q_3 layer and the incidence of KBD, a deep well was drilled to the Nt layer, with other layers sealed up. A follow-up observation for 3 years showed that the disease rate in the experimental site decreased significantly as compared with the control site, while at 1500 meters away with no change in water source, new cases were found (no change of food stuff either). The humic acid in experimental site decreased to 1 / 3 of its original value while no apparent alteration was found in the control site. The selenium content of drinking water in the two groups did not vary significantly.

The above studies show that the pathogenic materials of KBD might be some humic acids or their complex compounds in the drinking water. However, it remains to be further investigated.

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REFERENCES

- Hypabres, H.H., A Study and experience for the control of KBD. A Compilation of Papers on KBD, 1956 pp. 52-60, published by the Ministry of Public Health of PR China, Beijing, (in Chinese)
- Lin, N, Advances in the etiology of KBD involving organic material in water and soil from 1968 to 1982, Endemic Diseases Communication 2: 43-45 1985; (in Chinese)
- Liu, J. A brief of river and ridge in Chang Bai Mountain, 1908, p. 141, (in Chinese)
- Schnitzer, M. and Khan, S.U. Humic Substances in the Environment, 1972, Marcel Dekker, Inc., New York
- Stevenson, F.J. Humus Chemistry, 1982, pp. 43, New York.
- The Survey Team of KBD in Yongshou County, The Scientific Investigation on KBD in Yongshou, People's Health Publishing House, 1984, pp. 80-84, Beijing, (in Chinese)
- Zhai, S. and Chang, S. Some Views on the epidemiology and etiology of KBD. Endemic Diseases Newsletter 2:1-8 1977, (in Chinese)
- Zhai, S., Zhao, Z., Shao, G., Yao, J., Cheng, W., Zheng, F., Li, S. and Zhang, H.; The investigation on the relationship between KBD and drinking water or food-stuff in Qian An plain and prairie The collection of Papers on Endemic Disease Published by the Second Endemic Diseases Control Institute in Jilin Province. 2:22-46, 1980, (in Chinese)
- Zhai, S., Shao, G., Yao, J., Cheng, W., Zheng, F. and Li, S. The epidemiologic investigation of KBD in children after drinking tap water for 17 years in Hua Dian county, Endemic Diseases Communications 2:9-16, 1976, (in Chinese)
- Zhai, S., Shao, G., Zhang, G., Cheng, W., Zheng, F., Li, S. and Gao, L. The effect on KBD by drinking tap water from basic rock slit and filtered through manganese sand. The Collection of Papers on Endemic Diseases, published by the Second Endemic Disease Control Institute of Jilin Province 2:59-67, 1980, (in Chinese)

THE PROPHYLACTIC AND THERAPEUTIC EFFECTS OF SELENIUM ON KASHIN-BECK DISEASE

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Kashin-Beck disease is one of the serious endemic diseases in China. The cause is still unknown and specific measures for prevention and treatment are also lacking. In recent years, some authors (Li, et al., 1982; Hou and Zhu, 1982; Ren, et al., 1983) have analysed the drinking water, soil, and grains from endemic areas, and examined the hair and blood of patients with the disease. They have also fed animals with diets low in selenium. It is considered that a diet low in selenium might be the cause of Kashin-Beck disease.

In 1979, Li (Li, 1979) first reported that selenium was effective in the treatment of Kashin-Beck disease. In an attempt to confirm this finding, the authors dispensed selenium tablets or salt to some population groups in an area with severe Kashin-Beck disease in Yongshou county, Shaanxi province from 1980 to 1985. The selenium concentration in the salt and in samples of the patients' hair, blood, and urine were all examined; in addition, blood was analysed biochemically and liver function tests were carried out.

Materials and Methods

In 1980-81, sodium selenite tablets each containing 1mg of Na_2SeO_3 , were orally administered to children at the following dosages:

Age 3-10 years: 1 tablet per week

Age 11-13 years: 2 tablets per week

A total of 325 children with Kashin-Beck disease were observed and they were randomly divided into two groups; one group given selenium tablets, and the other, the control group, given placebo tablets (starch). X-ray examination was done for each child every 3 months (a total of 4 examinations).

From 1981 to May 1985, sodium selenite salt (1:60,000) was provided to all the residents of the area. X-ray changes and new cases (if any) were studied in children aged 3-15 years. Six hundred and 2100 people were observed in 1981-82 and 1983-85 respectively. The prevalence of the disease was observed in the whole population in the area. Once a year, only those children currently within the age limits were examined; thus the number of the subjects was not exactly the same in each year.

The X-ray diagnostic criteria and the effectiveness standards used were the "unified standards" (draft) published in 1983 by the Office of the Leading Group for the Control of Endemic Disease of the Central Committee, CPC.

Before and after the administration of selenium tablets or salt, biochemical analyses including: plasma glutathione peroxidase, serum alanine aminotransferase, and others were done 3-5 times for all cases (about 40-80 cases of patients each time).

The salt and human hair samples were analysed for selenium periodically (selenium salt, monthly; and hair every 3 months).

Results

1. Selenium content of hair

In the group receiving selenium tablets, the selenium concentration in the hair increased from 40 $\mu\text{g}/\text{kg}$ before treatment to 278 $\mu\text{g}/\text{kg}$ after treatment; in the group receiving selenium salt, the levels of selenium in hair increased from 86 $\mu\text{g}/\text{kg}$ to 300 $\mu\text{g}/\text{kg}$. It should be pointed out that agricultural reforms begun in 1982 resulted in increases in the selenium content in the hair of the control group after 1983, though the selenium content did not reach the same level as that in the selenium salt group; however, the hair selenium of the control group did become nearly as high as in people from non-endemic areas (see Table 1).

Table 1. The selenium content ($\mu\text{g}/\text{kg}$) of the hair of children given selenium salt and the control group

Groups	1981	1982	1983	1984	1985
Selenium salt	86 \pm 52 (n = 8)	297 \pm 92 (n = 149)	330 \pm 79 (n = 39)	249 \pm 22 (n = 50)	286 \pm 80 (n = 49)
Control	60 \pm 30 (n = 6)	84 \pm 52 (n = 26)	87 \pm 28 (n = 60)	151 \pm 34 (n = 90)	163 \pm 34 (n = 50)

2. Effectiveness of selenium treatment

The X-ray changes at the metaphyseal part of the tubular bones (especially the smaller ones) were studied, and the results showed that improvements were seen in 81.9% of the selenium tablet group compared with 39.6% of the control group ($p < 0.01$). None of the treated group showed any worsening compared with 18.9% of the control group (Table 2). The improvements were progressive in the group receiving selenium

tablets for 1 year whereas worsening was observed in fewer patients (Table 3). There were no important changes in the control group.

Table 2. Metaphyseal changes in children given selenium tablets and in the control group

	No. of cases observed	No. of cases improved	% improved	No. of cases deteriorated	% deteriorated	No important changes	% no changes
Selenium tablets	166	136	81.9	0	0.0	30	18.1
Control group	159	63	39.6	30	18.9	66	41.5

Table 3. Effectiveness of selenium treatment in KBD patients

		3 months	6 months	9 months	1 year
% Improved	Selenium group	27.9	68.3	79.8	81.9
	Control group	18.7	41.4	40.3	39.6
% Deteriorated	Selenium group	4.4	3.4	0.6	0.0
	Control group	6.6	18.3	18.2	18.9

3. Preventive effect of Se salt

Over the period of 1981-85, 600 children aged 3-15 years were examined and the results at the end of the trial showed that the symptoms and signs of the disease decreased in both the selenium salt group and the control group. Abnormal changes in the metaphyseal region of bones had decreased more in the selenium salt group than in the control group ($p < 0.01$). On the contrary, abnormal X-ray findings of the disease showed no significant changes (Table 4).

Considering the differences in both the number of cases examined each year and the age structure of the two groups, the authors adopted the standardized treatment before analysing the data, in order to eliminate any influence of these factors. The results showed that the severity of the illness was alleviated more evidently in the selenium salt group than in the control group (Table 5).

Table 4. X-ray findings in KBD patients before (1981) and after (1985) selenium treatment

	Selenium salt group		Control group	
	1981	1985	1981	1985
No. of cases X-ray examined	361	372	202	304
No. of abnormal X-ray findings	238	174	153	166
No. of abnormal metaphyseal findings	206	121	125	134
No. of cases with metaphyseal changes	80	30	23	5
No. of abnormal X-ray findings per case examined	0.659	0.468	0.757	0.546
No. of abnormal metaphyseal findings per case examined	0.571	0.325	0.620	0.441

Table 5. Comparison of the severity of illness of the two groups after standardized treatment

	Selenium salt group					Control group				
	1981	1982	1983	1984	1985	1981	1982	1983	1984	1985
% of abnormal X-ray findings	71.8	62.6	54.6	48.6	41.8	79.8	71.5	72.4	62.4	55.3
% of abnormal metaphyseal findings	63.7	52.0	38.3	34.8	29.3	67.9	58.5	63.5	50.0	44.0

Between 1983 and 1985, the number of cases observed in the selenium salt group increased to 2100, and, as a result, the overall severity of illness was lower in both groups. In general, the illness was less severe in 4 years after treatment than it was in 1981 ($p < 0.01$); but there was no difference between the treated and the control groups ($p > 0.05$). After standardization, the data did not show any significant differences.

Observations on the "healthy" children during the period of 1981-1985 did not reveal any new cases of Kashin-Beck disease in the group receiving selenium salt, while four new cases (all of the metaphyseal type, diagnosed by X-ray examination) were found in the control group. The morbidity in the control group, calculated by using the "children-ages" method (i.e., the number of cases observed multiplied by their ages in years), was 2.99%, but the difference between the two groups was not significant ($p > 0.05$) (Table 6).

Table 6. Comparison of morbidity in the selenium treated and the control group

	No.Children	New. cases	Morbidity (per thousand)
Selenium salt group	1253	0	0.00
Control group	1340	4	2.99

4. Laboratory findings in blood and urine

Laboratory investigations showed that the blood selenium level and the glutathione peroxidase activity in plasma and RBC were all increased significantly in those treated with selenium salt ($p < 0.01$). Plasma levels of vitamin E, however, showed no evident changes ($p > 0.05$) (Table 7).

Table 7. Comparison of the levels of blood selenium, certain enzymes, and blood vitamin E after 1 year of selenium treatment

Groups	RBC glutathione peroxidase	Plasma glutathione peroxidase	RBC GST	RBC SOD	Whole blood Selenium	Plasma Vitamin E
Selenium salt	45.22 ± 2.57 (17)	2.02 ± 0.07 (20)	0.25 ± 0.04 (18)	10.49 ± 0.26 (20)	0.056 ± 0.002 (39)	7.7 ± 0.4 (19)
Control	26.09 ± 2.18 (17)	1.29 ± 0.10 (20)	0.45 ± 0.18 (18)	12.89 ± 0.55 (20)	0.036 ± 0.002 (33)	7.0 ± 0.3 (20)
t test	$p < 0.001$	$p < 0.0001$	$p < 0.2$	$p < 0.01$	$p < 0.001$	$p > 0.1$

units used: GSHpx: EM / gHb; GST: EM / gHb; SOD: EM / mgHb; VE: $\mu\text{g} / \text{ml}$; Se: $\mu\text{g} / \text{ml}$.

Tests of liver function—thymol turbidity (TT), zinc sulphate turbidity (ZTT), icterus index and SGPT—carried out in 18 and 24 months after commencement of the treatment, were all within the normal limits, in both the selenium salt group (48 cases) and the control group (28 cases), indicating that there was no liver damage (Table 8).

Table 8. Results of liver function tests after 1 year of selenium salt treatment

Groups	Thymol Turbidity (units)	Zinc Sulfate Turbidity (units)	Icterus Index (units)	SGPT (units)
Selenium salt (n = 48)	2.17 ± 0.50	5.58 ± 2.06	3.48 ± 0.82	25.34 ± 11.33
control (n = 28)	2.41 ± 0.69	5.14 ± 1.99	3.29 ± 1.12	23.08 ± 4.02

In six months after the beginning of selenium tablet treatment, the excretion of aminopolysaccharides and creatinine in urine of the patients had increased significantly ($p < 0.01$) (Table 9).

Table 9. Aminopolysaccharides and creatinine excreted in urine (mg / 24 hours) after 6 months of selenium tablet treatment

Groups	Aminopolysaccharides			Creatinine		
	Before treatment	After 6 months of treatment	P	Before treatment	After 6 months of treatment	P
Selenium tablet	5.23 ± 0.23 (n = 36)	7.67 ± 0.31 (n = 32)	<0.01	363.2 ± 18.7 (n = 34)	412.5 ± 17.4 (n = 32)	<0.01
Control	5.74 ± 0.26 (n = 34)	7.19 ± 0.31 (n = 32)	>0.05	386.8 ± 23.7 (n = 32)	416.9 ± 22.4 (n = 32)	>0.05

Autoanalysis of serum showed no regular changes in eight biochemical indicators (ALP, SGOT, CPK, LDH, rGT, CR, HBDH and SGPT) in either the selenium treated group or the control group.

Discussion

Many experiments have been carried out with selenium for the treatment of Kashin-Beck disease since the first report of Li (Li, 1979) in 1979, but the results have not been consistent owing to the different methods used and the different experimental conditions. The authors of the present paper selected an endemic area for the disease and used methods for parallel comparison and randomized pairing in studying the cases during the selenium treatment period. The results showed that the selenium tablet (1 mg of Na_2SeO_3 taken orally) had a significant therapeutic effect on the metaphyseal changes,

promoting repair and preventing deterioration of the disease: overall improvement was seen in 81.9% (75.9%–87.9%) of those receiving tablets. The benefits increased as the treatment continued, reaching a peak after 1 year; after that, no further improvement was evident. Selenium tablet treatment was not specifically effective in improving the changes at the contra-epiphyseal end of any diseased bone.

So far, there have been no published reports on the prophylactic effect of selenium on Kashin-Beck disease. In these experiments, we did not find a single new case in the selenium group; the morbidity decreased progressively and was clearly less than that in the control group. Based on these results, we consider that selenium has a preventive effect. As mentioned earlier, the decrease of morbidity seen in the control group was probably due to agricultural reforms in the area—changes in composition of the diet (more wheat and less corn being cultivated in recent years), improvements in the standard of living and increased intake of selenium from other sources. The selenium content of human hair increased from 60 $\mu\text{g}/\text{kg}$ to 163 $\mu\text{g}/\text{kg}$ in the whole area, though the selenium content of human hair in untreated people did not reach the same level seen in the selenium group; however, in general population, it did approach the same level found in the non-endemic areas (220 $\mu\text{g}/\text{kg}$). These results lead us to believe that an increase in selenium intake (by whatever method) may possibly lead to elimination of the disease. Since the use of selenium salt is relatively simple, it could be adopted as one of the prophylactic measures in all areas endemic with Kashin-Beck disease.

Laboratory analyses showed that the selenium levels in blood and hair and glutathione peroxidase activity were all below normal prior to the start of selenium salt treatment but increased promptly after the treatment began. Ren et al (1983) reported that animals fed with a low selenium diet showed dysplasia of the epiphyseal cartilage of the tubular bones. Also the findings of experimental studies on human embryos showed that additional intake of selenium could prevent or lessen the damage caused to cartilage cells by hydrogen peroxide. Selenium also promotes the growth and mitosis of cartilage cells and can increase the synthesis of chondroitin sulfate. Mo (1980) suggested that the disease probably begins on the membranous part of the cells. The increase in glutathione peroxidase activity following selenium treatment can restore its protective effect on the cell membrane, thus promoting the repair of diseased cells. All sufferers have some degree of disturbance of sulfur metabolism, and the increased excretion of aminopolysaccharides and creatinine in urine following selenium treatment shows that the metabolism of chondral protein and polysaccharide has improved. These results indicate that the role of selenium is likely to be one of combating the peroxidation damage on the one hand and protecting the cell membrane and improving sulfur metabolism in the cartilage on the other.

Though selenium is one of the essential trace elements for human life, the intake of too much is hazardous. In these experiments, the daily selenium intake per person was 65 μg with the tablets and 76 μg with the salt. These two dosages were within the normal limits of daily physiological requirements

(Kong, 1982). In the 1960s, the same dosage of sodium selenite was employed over a period of 2-3 years for the prevention of the Keshan disease in our province and no case of poisoning was reported. In the present experiments, the hair and blood selenium levels were all below the levels found in city dwellers and approached the levels found in human hair of residents in the non-endemic areas (most of them are peasants). In addition, liver examinations on three occasions (4 analyses each time) revealed no abnormal changes and we therefore believe that the selenium dosage used in these experiments was quite safe.

REFERENCES

- Hou, W. and Zhu, Z.** A study of selenium nutrition states of the population in the Kashin-Beck disease regions. *Chinese Journal of Endemiology*, 2: 84-88 (1982).
- Kong, X. R.** The Nutrition, Physiology and Their Clinical Significance of Essential Trace Elements. (1982, pp296-318.) Anhui Provincial Publishing House of Scientific Technique, Hefei.
- Li, J. et al.** A study of selenium associated with Kashin-Beck disease in different environments in Shaanxi. *Acta Scientiae Circumstantiae*, 2: 93-101 (1982).
- Mo, D.** The Kashin-Beck disease (Special Issue in Celebrating the 30th Anniversary of the Founding of the PRC), 1980.
- Ren, H. et al.** Investigation of experimental pathology for pathogenesis of Kashin-Beck disease-effects of selenium and P-hydroxycoumaric acid (PHCA) on cartilaginous development of rat. *Chinese Journal of Endemiology*, 1: 3-6 (1983).
- Li, Z.** X-ray observations on the effect of sodium selenite and vitamin E in the treatment of 224 cases of Kashin-Beck disease: a discussion about its etiology. *Chinese Journal of Endemiology*, 3: 169-171 (1979).

STUDIES ON THE PREVENTIVE AND THERAPEUTIC EFFECTS OF COMPREHENSIVE METHOD AND SE-FORTIFIED WHEAT ON KASHIN-BECK DISEASE

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Introduction

This study is to compare the effects of the comprehensive method and selenium (Se)-fortified wheat in the prevention of Kashin-Beck disease (KBD) which was preceded by the investigation on Kashin-Beck disease at Yongshou county in 1979-1982 (The Research Group on Ecologic Environment, 1984). Both methods revealed excellent results (Yin et al., 1984).

Methods

Children aged 3-13 in Yongshou county were divided into three groups (Table 1). Group I used the comprehensive method, i.e. to educate the subjects to consume various kinds of food cereals (wheat, rice, millet, corn etc) from different sources and pay more attention to environmental and personal hygiene. The source of drinking water was changed from shallow well to a 114 m deep well with a 39 m³ watertank. The humic acid and oxygen consumption of the water were significantly decreased, as compared to the shallow well water. Besides, an additional soup was given to children twice a week which contained soybean, kelp and multivitamin (VA 2500 IU, VD 1000 IU, VB₁ 0.5 mg, VB₂ 0.5 mg, nicotinamide 3 mg, VE 5 mg and VC 5 mg). Group II was supplemented with Se-fortified wheat. Sodium selenite, about 1 g/mu (0.17 acre), was sprayed to the ears of wheat during blossom. Group III served as control without treatment.

The changes in the right hand X-ray films were used as the main indicator in the observation. The National X-ray Diagnosis Criteria was used in the diagnosis of Kashin-Beck disease (Radiological Research Group of Scientific Research on Kashin-Beck Disease in Yongshou, 1984). The average abnormal detection rate of each population group was calculated for comparison among the 3 population groups. X-ray films were taken every 6 months.

Table 1. General information of all tested groups

Villages	Groups	Treatments	Number of households	Total number of children	KBD (3-13 years old)	
					Cases	%
Nan Shou	I	Comprehensive method	58	334	92	27.59
Qiao Bai	II	Se-fortified wheat	70	349	97	27.79
Qiao Nan	III	Control	197	943	239	25.59
Total			325	1617	428	27.32

Results

In the first group, the total incidence of abnormal X-ray signs in children has been decreased from 80% to 24%, and that of abnormal metaphysis decreased from 74.00% to 8.08% within five years and six months. Both rates started to decline one year after the beginning of the experiment. In group II, the total incidence of abnormal X-ray signs decreased from 62.86% to 41.87%, and that of abnormal metaphysis, from 58.76% to 32.56% within two years. For the control group in which no measures were taken, the change of both rates was non-significant (Table 2).

During five years and six months of observation, there were no new cases occurred in the comprehensive prevention group, but the control group had 12 new patients and the Se-fortified wheat group had one new case.

It is observed that the younger children aged 3-6 years were seldom affected by Kashin-Beck disease since the comprehensive management was carried out. Because the two experiments (comprehensive method and Se-fortified wheat) started at different times, it is difficult to compare them reasonably. Therefore, in Table 3, the value obtained before intervention was taken as 100%. In group I, there was a tendency of decrease of the abnormal detection rate after 1 year which became lower as the time went on. But in group II, the detection rate decreased within the first year, and unchanged later on.

Discussion

1. In Se-fortified wheat, its selenium content increased about 3 times (Table 4). The Se content of corn was consistent and children in group II showed their hair and urine Se elevated after the Se wheat had been taken. Such features were not seen in group I and III.

Table 2. The change of prevalence rate of KBD and the abnormal metaphysis detection rate of children aged 3-13 in group I-III

Year month	Prevalence rate(%)			Abnormal metaphysis (%)		
	I	II	III	I	II	III
80.4	80.00	56.62	66.02	74.09	50.72	45.63
80.10	78.00*	80.00	67.75	62.83*	56.36	44.87
81.4	69.44	62.90	71.83	61.11**	59.68	47.89
81.10	65.68**			56.86**		
82.4	64.0**	60.42	53.33	54.00**	58.33	40.00
82.10	50.49**			34.65**		
83.4	43.68**	62.86*	61.09	31.07**	58.76*	42.67
83.10	42.21**	55.33	58.73	21.05**	42.71**	50.79
84.4	46.15**	51.92	57.98	16.48**	46.15**	48.32
84.10	38.24**	50.00	55.79	13.34**	41.46**	37.89
85.4	31.46**	48.68	47.62**	10.11**	38.16**	39.29
85.9	24.24**	41.87**	43.68**	8.08**	32.56**	36.32
X² trend test						
X ²	60.39	4.04	7.71	155.51	6.56	3.47
P	<0.001	<0.05	<0.01	<0.001	<0.05	<0.5

Note: * Beginning of the experiment

** P < 0.01 (compared with the beginning)

Table 3. Changes of abnormal X-ray detection rates in different groups

Time	I		II		III	
	DR	DRR	DR	DRR	DR	DRR
83.4	31.07	100.00	85.76	100.00	42.67	100.00
83.10	21.05	64.53	42.71	72.69	50.79	119.03
84.4	16.48	53.04	46.15	78.54	48.32	113.21
84.10	13.34	42.94	41.46	73.45	37.89	88.28
85.4	10.11	32.54	38.16	64.94	39.29	92.28
85.9	8.08	26.01	32.56	55.41	36.32	85.12

Note: DR—Detection rate (%)

DRR—Detection rate ratio (%)

Table 4. Changing of Se content of wheat and corn during experiment

Groups	Wheat Se(ppb)		Corn Se(ppb)
	Before	After	
I	25 ± 7.1(8)	17.5 ± 6.4 (12)	29 ± 8 (11)
II	21 ± 4 (8)	75 ± 7 (11)	23 ± 7 (12)
III	27 ± 6 (8)	17 ± 4 (12)	29 ± 8 (13)
IV	41 ± 6 (10)		46 ± 9 (12)

Note: IV—samples from disease-free areas.

There were no relations between detection rate and Se-contents of urine and hair. For example, in 1980–1982, Se content in children's hair was kept in low level, but the abnormal metaphysis detection rate was decreased in group I.

2. By analysing 8 kinds of elements in urine in the three groups, it was found that the value of Zn and Fe elements in group I was higher than that in group II and III (Table 5.). In the Se-fortified wheat group, only the Se content was increased, but the other elements were not.

3. Comparison of amino acids in the urine of disease and disease-free groups: The results showed that valine, methionine and threonine were low in KBD patients, but the non-essential amino acids were normal. In addition, beta-aminoisobutyric acid was higher, but glutamine-asparagine was lower than those in the Se-fortified group (Table 6).

Table 5. Comparison of eight kinds of element contents in the urine of three groups of children ($X \pm SD$ mg / 24 h)

Elements	I(n = 18)	II(n = 16)	III(n = 23)	P ^a
Cu	33.56 ± 16.55	23.35 ± 15.72	30.86 ± 22.56	> 0.05
Zn	332.40 ± 209.02	176.46 ± 109.43	177.37 ± 146.59	< 0.01
Mn	51.43 ± 30.53	35.45 ± 23.62	41.84 ± 20.07	> 0.05
Cd	12.11 ± 6.27	10.08 ± 6.88	16.08 ± 10.73	> 0.05
Fe	171.49 ± 119.04	53.34 ± 32.23	66.16 ± 45.14	< 0.01
Ca	4562.54 ± 2699.99	2984.08 ± 1964.00	3303.43 ± 2490.29	> 0.05
P	367.89 ± 124.67	319.70 ± 130.60	364.25 ± 124.10	> 0.05
S	382.36 ± 101.26	331.18 ± 100.08	345.46 ± 99.61	> 0.05

a. comparing I, and II with III

Table 6. Urinary excretion of amino acids (moles / 24 hr) in KBD patients and children consuming Se-fortified wheat

	Normal ^a	KBD ^b	Se-fortified ^c
Valine	49	33.7 ± 7.02	
Methionine	50	12.35	
Lysine	210	98.5 ± 49.4	
Threonine	150	85.5 ± 21.5	
Glutamine-asparagine	413	185.8 ± 101.3	463.0 ± 277.3
Beta-Aminoiso-butyric acid	17	604.5 ± 394.6	168.7 ± 69.4
Glutamine		278.2 ± 39.5	445.2 ± 1550.0
Hydroxylysine		4.00	37.0 ± 33.1

a. Carver and Paska (1961)

b. Three cases of Kashin-Beck disease

c. Four normal children consuming Se-fortified wheat.

4. As to the main factors acting in the comprehensive management, we suggest that it may be due to the various cereals obtained from different sources and, especially an additional soup which contains more zinc, phosphorus and selenium. These chemical elements are the very elements which are deficient in soil of disease-affected areas (Mo, 1984).

REFERENCES

- Caver, M. J. and Paska, R. Ion-exchange chromatography of urinary amino acids. I. Normal Children, Clin. Chin Acta 6:721, 1961
- Mo, D. The research progress of elements in etiological hypothesis concerning Kashin-Beck disease, Endemiological Bulletin. 2:8, 1984; (in Chinese)
- Research on Kashin-Beck Disease in Yongzhou (1979-1982), 1984; pp.172 People's Health Publishing House, Beijing (in Chinese)
- The Research Group on Ecologic Environment: The relationship between chemical elements in drinking water and Kashin-Beck disease. In: Monograph of Scientific Research on Kashin-Beck Disease in Yongzhou (1979-1982), 1984; pp.73-79. People's Health Publishing House, Beijing (in Chinese)

Yin, P. Guo X. and Li, C. The Trial of comprehensive method to prevent Kashin-Beck Disease in Nan Shao village. In: Monograph of Scientific Research on Kashin-Beck Disease in Yong Shou (1979-1982), 1984; pp.148-151. People's Health Publishing House, Beijing (in Chinese)

BIOCHEMICAL CHARACTERISTICS OF KASHIN-BECK DISEASE

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Early in the 1970s, we proposed the existence of a "sulfur metabolism interfering factor" in the grain and water of the endemic areas. Studies since 1983 on the serum and cartilage sulfation have shown that sulfur metabolism was significantly decreased in both the animal model of Kashin-Beck disease and in patients with the disease. The results confirmed the presence of the sulfur interfering factor in the external environment of endemic areas.

Very few biochemical investigations of Kashin-Beck disease were undertaken in early days. In recent years, progress in that field has been fairly rapid, especially from 1980 onwards. In 1980-82, a comprehensive survey of Kashin-Beck disease was organized in Yongshou county. Nine institutes cooperated on various biochemical studies. Clinical and X-ray examinations were carried out on 3300 inhabitants, and 476 samples of blood, urine, hair, and cartilage were analysed for biochemical variables (Yang, 1984). These researches continued after 1983.

Biochemical Observations on Kashin-Beck Disease

1. Metabolism of cartilage

Kashin-Beck disease is characterized mainly by damage to cartilage. Chondroitin sulfate and collagen are the basic constituents of cartilage and the relationship between them and Kashin-Beck disease has been studied. The results of analysis of the cartilage tissue of five patients are shown in Table 1 (Li et al., 1984a) and compared with the results from control subjects.

The chondroitin sulfate content in the cartilage matrix was lower in the Kashin-Beck patients and the degree of sulfation of chondroitin was also reduced. The collagen content in the cartilage matrix was higher and the urinary excretion of hydroxylysine was also increased in the patients. Hydroxylysine is an important amino acid constituent in the collagen molecule. There is a close connection between the secretion of procollagen and the covalent linkage in collagen fibers.

Urine analysis has shown that there are changes in both the composition of chondroitin sulfate and the amounts present. The degree of sulfation of the chondroitin sulfate was reduced in patients with X-ray film

changes, being lowest in patients with X-ray findings typical of Kashin-Beck disease. The results in patients were significantly different from those in the normal population in the endemic area. The concentrations of both total phospholipids and DNA in cartilage were lower than in the normal controls. SM / PC ratio is significantly increased.

All these changes constitute an important metabolic basis for the lesions in cartilage tissue in patients with Kashin-Beck disease. They are directly correlated with the damage to the cartilage tissue, and are important with a view to future research on the etiology and pathogenesis of Kashin-Beck disease.

Table 1. Biochemical studies of cartilage tissue in Kashin-Beck disease patients

Groups	No. of subjects	DNA (%)	Phospholipids (mg / g)	SM / PC	CHS SO ₄ (%)	Collagen (mg / g)	
Controls ^a	Articular cartilage	7	0.173	0.74	2.67	4.87	407.6
	Epiphyseal cartilage	7	0.209	0.90	1.00	4.51	375.1
Patients	Articular cartilage	5	0.096	0.56	5.79	3.67	534.9
	Epiphyseal cartilage	5	0.104	0.86	3.60	3.39	484.5

a. Pediatric patients from non-endemic area without osteoarticular disease.

2. Metabolic abnormalities in other tissues

The constituents of the blood come from tissue cells of the body and any change in the level of the constituents of blood reflect clearly the functional status of tissue cells. For example, creatine kinase and creatinine come mainly from muscle tissue; serum alkaline phosphatase and the osteogenic isoenzymes of children come mainly from cartilage tissue; serum LDH, GOT, HBDH, etc., come mainly from soft tissue and red cells (Hou et al., 1984a). Some of these data are shown in Table 2 (Xi, 1984).

It is evident that in Kashin-Beck disease there is not only a metabolic disorder in the cartilage, but also significant changes in other tissues of the body.

Table 2. Composition of blood from patients with Kashin-Beck disease

		Non- endemic healthy	Endemic healthy	Normal X-ray	Patients with	
					Metaphysial	Typical X-ray
ALP	u/l ^a	157.9	157.3	188.8	187.7	226.0
GOT	u/l	18.1	39.3	50.0	52.0	46.7
GPT	u/l	17.5	19.8	23.2	22.9	20.8
LDH	u/l	96.1	107.4	119.2	119.9	128.6
CK	u/l	47.1	65.9	69.0	82.5	69.4
HBDBH	u/l	212.4	267.8	265.8	267.6	250.9
Ca	mg/dl	9.27	9.75	10.65	10.16	10.11
Pi	mg/dl	5.43	5.64	5.34	5.15	5.01
G	mg/dl	87.2	99.1	100.4	92.8	90.5
Urea-N	mg/dl	14.5	15.7	12.7	13.7	13.3
Creatinine	mg/dl	0.658	0.594	0.663	0.597	0.565

a. u—enzyme units

3. Metabolic disorders in patients with Kashin-Beck disease associated with low selenium

It is clear from Table 3 (Han et al., 1984) that the concentration of selenium in blood, urine and hair were low and that the activity of GSH-px in blood was reduced, while blood TBA and plasma FFA levels were raised in people in the endemic area.

GSH-px is an important selenium-containing enzyme and an enzyme exerting a protective action in the body. Selenium is capable of removing hydrogen peroxide and TBA produced in the tissues through the activity of GSH-px. It is clear, therefore, that all the findings such as low concentrations of selenium in the blood, low urinary selenium levels, decreased activity of GSH-px, and the accumulation of TBA in the population resided in the Kashin-Beck disease endemic areas, are closely related to low selenium. The metabolic disorder seems to be related to a series of changes centered on selenium levels in the body.

4. Damage to red cell membrane

It has been shown by analysis that the red cell membrane in patients with Kashin-Beck disease has a low total lipid content and in particular a low level of phosphatidylcholine (Table 4) (Li et al., 1984), so that the percentage of sphingomyelin in the membrane is somewhat elevated. Among the lipid constituents of the membrane, the ratios of cholesterol/phospholipid and SM/PC are increased. The selenium content of red cells is decreased (Yang et al., 1984).

Table 3. Metabolic changes associated with low urinary levels of selenium

Groups	GSH-px (units)	TBA (nmole / ml)	FFA (mM / l)	Urinary Se (ng / day)
Non-endemic area, healthy	129.3 ± 13.8 (35) ^a	2.32 ± 0.8 (20)	0.56 ± 0.22 (20)	6.53
Endemic area, healthy	77.9 ± 18.2 (35)	4.54 ± 1.4 (20)	0.80 ± 0.21 (20)	3.01
Mild cases	72.8 ± 17.0 (30)	4.80 ± 1.03 (19)	0.89 ± 0.024 (22)	3.10
Moderately severe cases	94.5 ± 15.0 (30)	4.22 ± 0.97 (20)	0.88 ± 0.22 (16)	2.99
Severe cases	105.0 ± 11.2 (35)	4.18 ± 0.77 (20)	1.05 ± 0.26 (20)	2.97

a. The figures in parentheses indicate the number of subjects.

The fragility of the red cells is increased. There is a "non-invasive release" of certain enzymes (Yang and Yan, 1984) (LDH, HBDH, GOT) from the red cell (Hou, 1984) and it can also be shown from analysis of SEM and TEM, that there is damage to both the functioning and the composition of the red cell membrane.

5. Changes in the metabolic regulatory system

Changes in the levels of some metabolic regulators such as urinary cAPM, cGMP, 17KS, 17-OHCS, and urinary aromatic acids, and blood T₃ and T₄, etc., are found to various extent in Kashin-Beck disease patients.

The Nature of Metabolic Disorders in Patients with Kashin-Beck Disease and their Significance

I. Systemic metabolic disorders in Kashin-Beck disease patients

Table 4. Lipid components (mg / 10⁷ cells) of the red cell membrane in patients with Kashin-Beck disease

Groups	N	Age (years)	X-ray finding	Total lipids	Phospho-lipid	Cholesterol	Chol / PL (molecular)
Patients ^a (active state)	10	9-10	+	5.43 ± 0.45	1.44 ± 0.41	1.35 ± 0.06	2.00
Patient ^b (mild)	6	10-12	-	6.23 ± 0.40	1.72 ± 0.20	1.49 ± 0.18	1.67
Healthy children (endemic area)	6	8-12	-	6.54 ± 0.34	2.48 ± 0.13	1.45 ± 0.04	1.11
Healthy adults ^c (non-endemic area)			-		2.72 ± 0.16	1.49 ± 0.03	1.04

a. With abnormal X-ray changes

b. First degree of clinical types

c. Normal values cited from reference (Li et al., 1984b)

There are obvious changes in the composition of blood in patients with Kashin-Beck disease. These include changes in enzyme activity and in the composition of body tissues, e.g. changes in serum enzymes (ALP, GOT, LDH), red cell enzymes (LDH, GSH-px, GOT), and other constituents (serum FFA, TBA, creatinine, red cell membrane lipids). The differences between the group of patients and the control group were statistically significant.

The recognition of these differences is further strengthened by recent work on Kashin-Beck disease in five provinces and districts in China (Hou et al., 1985a; Sun et al., 1985a; Xi et al., 1985a; Xi et al., 1985b). From the biochemical point of view, it can thus be said that Kashin-Beck disease involves not only damage to the cartilage tissue, but also abnormalities of other tissues.

These other metabolic disorders are mostly rather slight, as seen from the degree of variation. As regard to the enzyme spectrum, such as LDH and GOT, the variation in activity on the average does not exceed two times the value of the control, or in some cases the values are at the upper limit of the normal range. This situation clearly differs from that seen in some acute diseases seen in the clinic (Yang and Yan, 1984). In acute diseases there is an abrupt and acute rise in the enzyme activities, for example, in acute myocardial infarction or in case of hepatic damage. In such instances, there is not only an abrupt rise in the enzyme activities, but also the rise in activity which may reach a value well over 10 times of the normal value. In contrast, the changes in Kashin-Beck disease are usually slight but of

long duration; nevertheless there are statistically significant differences as compared with the normal controls.

It can be seen from a dynamic analysis of the blood that there is seasonal variation in the composition of the blood. The changes in serum enzymes and in the lipid composition of the red cell membrane become less prominent in autumn, even approaching normal. This is in agreement with the observations of the epidemiological survey that there are seasonal variations in the disease occurrence, with relapses in spring and remissions in autumn (Yang, 1984a).

It can be concluded from the results given above that:

(a) The abnormalities in some of the chemical constituents of the blood in Kashin-Beck disease patients may improve to some extent, and that under certain conditions the levels may approach normal. These observations suggest that at the early stages of the disease the damage may be functional.

(b) The recovery towards normal of the blood composition in Kashin-Beck disease patients corresponds with their clinical status. Thus, the biochemical findings may be used as indices of the development and progression of the disease. These observations could thus form a theoretical basis for the application of biochemical methods for monitoring and for the early diagnosis of Kashin-Beck disease.

2. The relationship between low selenium and Kashin-Beck disease

The areas in which Kashin-Beck disease is prevalent are low in selenium (Environmental Ecology Section, 1984) and favourable results are obtained by the administration of selenium salt (sodium selenite) for the prevention of the disease. These facts have aroused considerable interest in the relationship between Kashin-Beck disease and low selenium levels. Biochemical examinations have shown that the selenium levels in blood and urine, and the level of GSH-px activity in blood are low, and that the levels of lipid peroxide and plasma FFA are high in Kashin-Beck disease patients. All of these findings are closely related to the low selenium, indicating that the internal environment of Kashin-Beck disease patients is low in selenium.

Table 3 (Hang et al., 1984) shows that the biochemical indices of urine selenium and blood GSH-px and TBA in the population of the endemic area are significantly different from those in populations in non-endemic regions. Within the endemic area, however, there is uniformity of the indices among different groups (those with mild, moderate or advanced manifestations of the disease, or those without the disease) of the population in the same locality. Thus, all the inhabitants of the endemic region show metabolic disorders related to low selenium, regardless of the clinical manifestations of the disease. All these facts indicate that the metabolic disorder is much more closely related to the low levels of selenium than the development of Kashin-Beck disease itself.

It is evident from epidemiological surveys that there is seasonal variation in the clinical manifestation of Kashin-Beck disease, which turns to be worse in spring and improved in autumn. The activity

of some of the blood enzymes fluctuates in the same way as the clinical status, but there are almost no seasonal variations in the metabolic disorders associated with the low selenium content.

The above observations indicate that the low selenium levels are related to environmental factors, in both the endemic areas and the non-endemic regions, rather than to the clinical manifestation of the disease. In regions with low selenium levels, there may be patients with Keshan disease or with cancer. Both these kinds of patients may show the metabolic abnormalities associated with low selenium, but they do not necessarily have cartilage tissue lesions. It can thus be deduced that there may be factors other than low selenium that are specific for Kashin-Beck disease.

3. Abnormalities of cartilage metabolism in patients with Kashin-Beck disease

Cartilage tissue is the target tissue that shows most damage in Kashin-Beck disease patients and that needs further study.

There have been many studies over the years of the metabolic changes related to cartilage damage by means of direct examinations of autopsy materials, and by analysis of the main metabolites of cartilage tissue in tissue fluids, and by experimenting animal models. These studies have provided the basic information on the metabolic features of cartilage damage in Kashin-Beck disease.

(a) Abnormalities in the important basic component of cartilage matrix—chondroitin sulfate.

Chondroitin sulfate is an important constituent of the connective tissue, including cartilage tissue. Studies of changes in the chondroitin sulfate in body fluids of patients can be helpful in understanding the metabolic status of cartilage matrix.

The excretion of chondroitin sulfate in the urine of patients with Kashin-Beck disease is usually high (Sun et al., 1985b; Yang and He, 1984). This high level of excretion is especially obvious in certain endemic areas, such as the endemic areas in Shangzhi county of Heilongjiang province. Being expressed in the terms of glucuronic acid, the amount of chondroitin sulfate excreted in the urine of control population is 3.88 ± 1.41 mg / 24 hours and that of the population of the disease area is 5.87 ± 1.44 mg / 24 hours; when expressed in terms of hexamine, the amounts are 1.765 ± 0.684 mg / 24 hours and 2.635 ± 1.045 mg / 24 hours, respectively. Both methods of calculation indicate significant differences in the levels of excretion (Zhao et al., to be published).

Other studies have also shown some changes in the composition and molecular weight of the chondroitin sulfate molecule. Cellulose acetate membrane electrophoresis has been used to measure the degree of sulfation of the chondroitin sulfate and Sephadex gel chromatography and PAG electrophoresis has been used to study the physico-chemical properties of chondroitin sulfate molecule in urine of Kashin-Beck disease patients. These studies have shown that in some of the endemic areas, the chondroitin sulfate molecule is smaller than in the control subjects (Table 5) (Zhao et al., to be published).

Table 5. The size of the chondroitin sulfate molecule in Kashin-Beck disease patients

Groups	No. of subjects	Mobility of CHS (F value)	t test
(1) Non-endemic area, healthy	6	0.962 ± 0.057	(1):(2) p > 0.05
(2) Endemic area, healthy	10	1.150 ± 0.249	(2):(3) p > 0.05
(3) Patients	9	1.340 ± 0.379	(1):(3) p < 0.05

(b) The metabolic abnormalities of cartilage are focused on chondroitin sulfate

Kashin-Beck disease is definitely an endemic disease and is closely related to factors associated with the soil and the water. These factors are thought to operate through the food consumed by the population, and these facts lead to the hypothesis that Kashin-Beck disease is a bio-geochemical disease.

Animal experimentation has shown that abnormalities in the metabolism of chondroitin sulfate occur in animals fed on a diet of grain and water collected from endemic areas (Yang et al., 1972; Xian Medical College, 1972).

When animals were fed on cereals collected from endemic regions such as Jilin, Heilongjiang, Shaanxi provinces and the Inner Mongolia Autonomous Region, the following changes were found:

(i) Experimental animals showed a low rate of incorporation of ^{35}S into chondroitin sulfate, as detected in the urine and cartilage (Chang et al., 1984; Yang et al., 1972; Xian Medical College, 1972).

(ii) In experimental animals, the excretion of chondroitin sulfate in urine increased and the content of chondroitin sulfate in cartilage decreased. These changes in chondroitin sulfate are similar to the findings in Kashin-Beck disease (Zhao et al to be published).

(iii) Autoradiographic observations showed that the absorption of ^{35}S by cartilage was somewhat decreased in experimental animals (Li et al., 1984c).

(iv) The uptake of ^3H -UdR by cartilage from experimental animals in tissue culture was less than that by cartilage from control animals. The level of chondroitin sulfate in the interstitial tissue was also decreased (Nie and Chang, 1984).

(v) The proportion of PGs in cartilage stroma was decreased while the proportion of proteoglycanmonomer PG was increased, and the molecular weight of PG was decreased (Yan, 1985a).

(vi) Electronic microscopic observations showed that the rough endoplasmic reticulum of the carti-

lage cells of experimental animals were smaller, shorter, and irregularly lined. Sometimes dispersion was evident. Development of the region including the Golgi apparatus was poor (Zhang et al., 1985). There were fewer proteoglycan granules in the outer circumferential stroma of the cartilage cell.

(vii) The activity of serum ALP and GOT was found to be increased in experimental animals (Hou et al., 1984b; Yan, 1985b).

(viii) The activity of the serum sulfation factor was lower in experimental animals (Hou et al. 1985b).

(ix) Microscopically, there was no significant structural damage in the bone and articular cartilage.

These experimental results indicate that a series of metabolic disorders are seen in the cartilage tissue when rats are fed on a diet similar to that of the inhabitants of the endemic area of Kashin-Beck disease. The main changes are in the metabolism of chondroitin sulfate and proteoglycan of the cartilage stroma, and many of the metabolic abnormalities are similar to those found in Kashin-Beck disease patients. Microscopic examination of the experimental animals demonstrated no obvious abnormality of the cartilage tissue, but electronic microscopic examination shows a decrease in number of the proteoglycan granules and early morphological changes of the endoplasmic reticulum of the cartilage.

On the basis of the biochemical and pathological observations in human and animal bodies, it can be deduced that the metabolic disorders in the cartilage in animals fed on a diet of grain from endemic areas is most probably the early manifestations of the metabolic disorders of Kashin-Beck disease patients.

(c) Sulfur metabolism interfering factor (Yang et al., 1972)

In a study with an experimental animal model of Kashin-Beck disease in the early 1970s, a diet from regions endemic for Kashin-Beck disease was found to interfere with the utilization of sulfur in the rat. The existence of an animal sulfur metabolism interfering factor in grain and water in the endemic regions of Kashin-Beck disease was proposed. The "sulfur metabolism interfering factor" was thought to influence mainly the anabolic metabolism of chondroitin, that is, the sulfation of chondroitin. Studies of a possible "sulfation factor" in the serum and cartilage of animals have been carried out since 1983, using cartilage tissue cultures and a cell-free cartilage enzyme system. The results are shown in Table 6 (Hou et al., 1985b). It was found that the activity of the "sulfation factor" in the serum of animals fed on grain from Kashin-Beck disease endemic regions is 161.5 ± 66.8 (^{35}S cpm / 100 mg cartilage), a value significantly lower than the 212.2 ± 58.9 (^{35}S cpm / 100 mg cartilage) of the animals fed on grain from non-endemic regions. The difference between these two values was statistically significant.

Table 6. Activity of the serum "sulfation factor" in an experimental animal model

Animals	Sources of cartilage	N	Incorporation of ^{35}S (cpm / 100 mg cartilage) ($\times 10^2$)	t test
Rats fed on grain from non-endemic areas	Chicken embryo	22	212.2 ± 58.9	$P < 0.02$
Rats fed on grain from endemic areas	Chicken embryo	22	161.5 ± 66.8	

Recently, the activity of the serum "sulfation factor" was determined in patients with Kashin-Beck disease, and the preliminary results show that the values of most of the patients were lower than those of the controls.

These results do demonstrate the existence in the grain of endemic regions of an "animal sulfur metabolism interfering factor", which causes a metabolic disorder of the chondroitin sulfate in cartilage. This simulates closely the metabolic disorder found in cartilage of Kashin-Beck disease patients. It is, thus, worthwhile to study the relationship between this "sulfur metabolism interfering factor" and the commonly called "Kashin-Beck eliciting factor" that is present in the environment in the endemic regions.

Annex 1 **Abbreviation used in this paper**

GOT:	Glutamate-oxaloacetate aminotransferase
LDH:	Lactic dehydrogenase
HBDH:	Hydroxy-butylate dehydrogenase
GT:	Gamma-glutamyl transferase
CK:	Creatine kinase
GSH-px:	Glutathione peroxidase
GPT:	Glutamate-pyruvate aminotransferase
ALP:	Alkaline phosphatase
TBA:	Thiobarbituric acid
FFA:	Free fatty acid
G:	Glucose
cGMP:	Cyclic Guanosine monophosphate
cAMP:	Cyclic adenosine monophosphate
17OHCS:	17-Hydroxysterol
17KS:	17-Ketosterol
SM:	Sphingomyelin
PC:	Phosphatidylcholine
OHPr:	Hydroxyproline
OHLy:	Hydroxylysine
CHS:	Chondroitin sulfate
PGs:	Proteoglycanpolymer
PG:	Proteoglycanmonomer

REFERENCES

Chang, S.F. et al. Pathogenesis of Kashin-Beck disease of feeding water and cereals from endemic areas. In: Assays of Kashin-Beck Disease. Supplement to Journal of Bethune Medical University, pp 25-31, 1984.

Environmental Ecology Section. Comprehensive survey report of environmental ecology: Assays of Scientific Investigations of Yunshou Kashin-Beck Disease. 1984, pp 55-103. People's Health Publishing House, Beijing.

- Han, C. et al. Quantitation of blood glutathione peroxidase, lipid peroxide and free fatty acid contents in Kashin-Beck disease children. *ibid*, 1984, pp 333-337.
- Hou, L.Zh. et al. Changes in erythrocytic enzyme activity in Kashin-Beck disease. *ibid*, 1984a, pp 316-321.
- Hou, L.Zh. et al. Features of serum enzyme spectrum of Kashin-Beck disease children in Jilin Province. In: Assays of the First National Academic Meeting devoted to Biochemical Research on Kashin-Beck Disease and Keshan Disease. Special issue of Chinese Journal of Endemiology, 4: 160-163, 1985a.
- Hou, L.Zh. et al. Studies on the blood sulfation factor in the rat feeding cereals from Kashin-Beck disease areas: *ibid*, pp 242-247, 1985b.
- Hou, L.Zh. et al. Serum enzyme spectrum of the rat feeding cereals from Kashin-Beck regions. In: Assays of Kashin-Beck Disease. Supplement to Journal of Bethune Medical University, pp 19-21, 1984b.
- Li, F.S. et al. Molecular biological features of the cartilage lesions of Kashin-Beck disease children with a discussion of the etiology of the disease. In: Assays of Scientific Investigations of Yunshou Kashin-Beck Disease, pp 372-375, 1984a.
- Li, F.S. et al. Abnormality and significance of chromatographic spectrum pattern of erythrocyte membrane phospholipid in Kashin-Beck disease children: *ibid*, pp 343-349, 1984b.
- Li, Zh.H. et al. Autoradiographic observation on sulfur metabolism of the cartilage in animals feeding on cereals from Kashin-Beck disease endemic areas. In: Assays of Kashin-Beck Disease. Supplement to Journal of Bethune Medical University, pp 32-36, 1984.
- Nie, Y.Q., and Chang Y.L., Application of cartilage culture in Kashin-Beck disease. In: Assays of Kashin-Beck Disease. Supplement to Journal of Bethune Medical University, pp 53-55. 1984.
- Sun, K. et al. Comparison of urinary glucosaminoglycan of children from districts with and without Kashin-Beck disease. In: Assays of the First National Academic Meeting devoted to Biochemical Research on Kashin-Beck Disease and Keshan Disease, Special issue of Chinese Journal of Endemiology, 4: 217-219, 1985a.
- Sun, K. et al. Serum enzyme activity of Kashin-Beck Disease children in Heilongjiang province, *ibid*, pp 156-159, 1985b.
- Yan, W.Q. Studies of cartilage proteoglycans in animals feeding on cereals from Kashin-Beck disease endemic areas. M.Sc. thesis, Norman Bethune University of Medical Sciences. 1985a.
- Yan, W.Q. Studies on enzyme spectrum of the cartilage tissue in animal feeding on cereals from Kashin-Beck disease endemic regions. M.Sc. thesis, Norman Bethune University of Medical Sciences, 1985b.

- Yang, F.Y. et al. Changes of erythrocyte membrane of Kashin-Beck disease children in Yongshou county. In: Assays of Scientific Investigations of Yunshou Kashin-Beck disease, pp 366-371, 1984, People's Health Publishing House, Beijing.
- Yang, J.B. (Epidemiology Section). A sketch of epidemiological research; *ibid*, 15-21, 1984.
- Yang, J.B. and He, F.L. Studies for the early diagnosis of Kashin-Beck disease using urinary biochemical indices. *Journal of Harbin Medical University*, 18 (4)(Suppl.): 1-5, 1984.
- Yang, T. S.(Biochemistry Section). Advances in biochemistry of Kashin-Beck disease: In: Assays of Scientific Investigations of Yunshou Kashin-Beck Disease. pp 275-286, 1984, People's Health Publishing House, Beijing.
- Yang, T.S. and Yan, W.Q. Biochemical indices of myocardium damage. In: Assays of Myocardium Biochemical Research. Supplement to *Journal of Bethune Medical University*, pp 9-21, 1984.
- Yang, T.S. et al. Kashin-Beck disease and sulfur metabolism. In: Academic Meeting on Epidemiology, Jinlin Province. 1972.
- Zhang, Z.Yi. et al. Studies of the ultramicroscopic structures of epiphyseal cartilage in the rat feeding on cereals from Kashin-Beck disease areas. In: Assays of the First National Academic Meeting devoted to Biochemical Research on Kashin-Beck Disease and Keshan Disease. Special issue of *Chinese Journal of Endemiology*, 4: 236-241, 1985.
- Zhao, Q.L. et al. Studies of the constituents of urinary glucosaminoglycan of Kashin-Beck disease patients: to be published.
- Zhao, Q. L. et al. Effect upon urinary and cartilage glycosaminoglycan in experimental animals feeding on cereals from Kashin-Beck disease areas: to be published.
- Xian Medical College (Basic research group). Changes of sulfur metabolism of bone growth and the cartilage in animal feeding on water and cereals from Kashin-Beck disease areas. *Shanxi Modern Medicine*, 4: 40-44, 1972.
- Xi, G.Z. et al. Plasma enzyme activity of Kashin-Beck disease. In: Assays of Scientific Investigations of Yongshou Kashin-Beck Disease. pp 287-300, 1984, People's Health Publishing House, Beijing.
- Xi, G.Z. et al. Selenium status and serum enzyme spectrum of Kashin-Beck children in Sichan Province. In: Assays of the First National Academic Meeting devoted to Biochemical Research on Kashin-Beck Disease and Keshan Disease. Special issue of *Chinese Journal of Endemiology*, 4: 147-152, 1985a.
- Xi, G.Z. et al. Enzyme spectrum of Kashin-Beck disease children in Inner Mongolia: *ibid*, 152-155, 1985b.

ABNORMALITIES OF ERYTHROCYTE MEMBRANE FROM PATIENTS WITH KASHIN-BECK DISEASE AND THE ROLE OF SELENIUM IN THE STABILIZATION OF HUMAN ERYTHROCYTE MEMBRANE SKELETON^①

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Introduction

It was reported that the lipid composition of erythrocyte membranes of children suffering from Kashin-Beck disease (KBD) was abnormal and the morphology of these erythrocytes studied by scanning electron microscopy was also changed (Li, Wei et al., 1984). Therefore, it was tentatively suggested that KBD might be a kind of membrane disease. (Li, Wei et al., 1984).

It is generally recognized that the endemic areas of KBD are located in a Selenium (Se) deficiency belt and Na_2SeO_3 supplementation is effective for its prevention (Li, Huang et al., 1984).

In the present paper, Na, K-ATPase activity, membrane fluidity and the Se content of erythrocytes as well as erythrocyte membranes of control children and patients with KBD were determined and compared. The investigation was carried out in the endemic region of Yongshou county of Shaanxi province. The results showed that a correlation might exist between the decrease of Se content and some defects in erythrocyte membranes from patients with KBD. The role of Se in the stabilization of human erythrocyte membrane was studied in vitro on this basis.

Experimental Procedures, Materials and Methods

Experimental procedures

The investigation was carried out in the endemic region of Yongshou county (Han Gou) of Shaanxi province in April, 1983.

Sixteen patients with KBD, between the ages of 8 and 12, were chosen for study. According to the X-ray diagnostic criteria, there were 11 patients with pathological changes in their metaphysis (metaphysis+) and 5 patients with pathological change in bone end (bone end+). Five healthy children from the same endemic region and 20 children from the nonendemic region (Lan Tian), aged 8-12,

^①This work is the result of a collaboration with Dr. Huang, F., Lin, Z.H., and Wo, W.H. Details of the experiments will be published elsewhere.

were chosen as controls.

Materials and methods

Preparation of erythrocyte membranes, determination of Se content, Na, K-ATPase activity and membrane fluidity were carried out as reported by Yang et al. (Yang, Huang, et al., 1984).

Sensitivity of Na, K-ATPase to ouabain was determined and calculated with the method described by Pearson (1978).

Test on the aging of erythrocyte membranes was carried out as follows: after washed for three times, erythrocyte ghosts were suspended in 10 volumes of Tris-HCl solution (10 mM, pH 7.4) and were aged in the presence or absence of Se at 4°C for 4-21 days.

Membrane skeletal components were analyzed according to Pharmacia's method (Polyacrylamide Gel Electrophoresis-Laboratory Techniques, Pharmacia Fine Chemicals, 1983) by using SDS-polyacrylamide concentration gradient gel electrophoresis. The gels were scanned by Shimadzu CS-910 TLC Scanner.

Results and Discussion

Measurement of selenium content of erythrocytes and erythrocyte membranes of the KBD patients

The results shown in Table 1 indicate that there were six experimental groups examined, including Beijing adults, Beijing children, control subjects from endemic and nonendemic regions, and KBD patients with lesions in metaphyses (metaphysis+) or bone end (bone end+). The Se content of patients erythrocyte was found to be 66% less than the Se values from control subjects from the nonendemic region. Because of a large quantity of samples is required for determining the Se content, only the Se content of erythrocyte membranes of some of the KBD patients was compared with the control children of the nonendemic region. Table 1 shows that the Se content of erythrocyte membranes of the former is about 50% of the latter. It is interesting to see that the Se content of erythrocytes and erythrocyte membranes of the children from the nonendemic region (Lan Tian) is invariably lower than that of the adults or children of the Beijing region. Se content of erythrocytes of the former is only 25% of the latter and that of erythrocyte membranes about 50%.

Changes of Na, K-ATPase activity of erythrocyte Membranes in KBD Patients

The activities of Na, K, Mg²⁺-ATPase in the presence and absence of ouabain of erythrocyte membranes were determined. From Table 2, it can be seen that the Na, K-ATPase activity of erythrocyte membranes of the KBD patients (metaphysis+) was decreased when compared to the control values.

Table 1. Se content of erythrocytes and erythrocyte membranes from KBD patients in Yongshou

Groups	Erythrocytes Se ($\mu\text{g} / \text{mg}$ hemoglobin)	Erythrocyte membrane Se content ($\mu\text{g} / \text{mg}$ membrane protein)
Control children in endemic region	0.0293 ± 0.0091 (5) ^a	
Control children in nonendemic region	0.0730 ± 0.0064 (20)	0.933 ± 0.161 (20)
KBD patients (metaphysis+)	0.0227 ± 0.0053 ^b (11)	0.562
KBD patients (bone end+)	0.0279 ± 0.0091 ^a (5)	
Beijing adults	0.310 ± 0.06 (36)	1.85 ± 0.967 (36)
Beijing children	0.319 (1)	

- The numbers in parentheses denote the number of samples measured.
- The difference between the Se content of erythrocytes of KBD patients (metaphysis+) and that of controls of the nonendemic region is statistically significant at $p < 0.001$
- The difference between the Se content of erythrocytes of KBD patients (bone end+) and that of controls of the nonendemic region is statistically significant at $p < 0.001$.

Fluidity change of erythrocyte membrane lipid in KBD patients

The lipid fluidity of the erythrocyte ghosts was measured by fluorescence polarization using DPH as a probe. From Table 3, it can be seen that the fluorescence polarization (P) values of erythrocyte membranes of KBD patients (metaphysis+) were higher than those of control children. The difference in fluorescence polarization is quite small, but statistically significant. This indicates that the fluidity of erythrocyte membrane of KBD children was less than that of the controls.

From the above-mentioned results it seems that a correlation may exist between the decrease of Se content and some defects in erythrocyte membranes of KBD patients. So, the role of Se in the stabilization of human erythrocyte membrane skeleton was studied *in vitro* as follows.

Effects of Se on the ouabain sensitivity of Na, K-ATPase of aging ghosts

Ghosts isolated from human erythrocytes were aged in Tris-HCl (10 mM, pH 7.4) medium, containing 0.16 ppm Se (Na_2SeO_3) or NaCl (same ionic strength as Na_2SeO_3). The latter sample was used as control and the aging was carried out at 0°C for 4-21 days. After aging, the samples were

centrifuged and in the remaining pellets, the Na, K-ATPase sensitivity to ouabain, DPH fluorescent polarization and membrane skeletal components were assayed and determined respectively (Wo, and Yang, 1986).

Table 2. Na, K-ATPase activity of erythrocyte membranes of infantile patients with KBD

Groups	Specific activity of Na, K-ATPase	t test
Control children in endemic region (5) ^a	0.550 ± 0.205	P < 0.01
KBD patients, (metaphysis+) (8)	0.316 ± 0.213	

a. The numbers in parentheses denote the number of samples.

It can be clearly seen from Fig. 1 that the sensitivity of Na, K-ATPase to ouabain was gradually decreased with the increasing of aging time. After aging in the Se-"free" medium for two weeks, the Na, K-ATPase was even activated by ouabain, while in the Se-containing (0.16 ppm Se, Na₂SeO₃) medium, the decrease in sensitivity of Na, K-ATPase to ouabain was obviously delayed.

Effect of Se on the lipid fluidity of aging erythrocyte membrane

Table 4 shows that DPH fluorescent polarization of aging ghosts in Se-"free" medium was gradually enhanced with the increase in aging time, while the supplementation of a trace amount of Na₂SeO₃(0.16 ppm Se) in the aging medium could also delay such changes.

Effect of Se on the change of membrane skeletal components of aging ghosts

The SDS-polyacrylamide gel patterns of the skeletal membrane components of the ghosts aged in the Se-containing and Se-"free" media were scanned by TLC scanner and the results are shown in Fig. 2. It was noted that the main difference between these two samples was in the relative percentage of Bands 1 and 2 (i.e. spectrin). After aging of ghosts for 4 days, the percentage of spectrin remaining in the ghosts aged in Se-supplemented medium was 21.4 while that in Se-"free" medium was 14.5. This indicates that Se may play some role in the association of spectrin with the erythrocyte membrane.

The following experimental results provided further evidence in favor of the postulation that Se could prevent spectrin dissociation from aging ghosts.

Table 3. Fluidity change of erythrocyte membrane lipid in KBD children

Groups	Fluorescence polarization (P)	Student's t test	
		Comparison with control children in endemic region	Comparison with control children in nonendemic region
Control children in endemic region	0.294 ± 0.009 (5) ^a		
Control children in nonendemic region	0.294 ± 0.003 (20)		
KBD patients (metaphysis+)	0.298 ± 0.004 (11)	NS	P < 0.01

a. The numbers of parentheses denote the number of samples measured.

Table 4. Comparison of fluidity changes of human erythrocyteghosts preserved in -Se and +Se media

Aging Time (weeks)	Increase in DPH fluorescent polarization		t-Test	Number of experiments
	-Se	+Se (0.16ppm Se)		
0	0	0		
1	0.004	0.002	P < 0.05	5
2	0.014	0.005	P < 0.001	5
3	0.020	0.008	P < 0.001	5

Fresh ghosts were suspended in 1 mM EDTA (pH 9.0) or 1 mM EDTA with supplementation of Se (0.1-1.0 ppm Se) overnight at 4°C. After centrifugation, the obtained pellets were analyzed by SDS-polyacrylamide concentration gradient gel electrophoresis. Results shows that there is a correlation between the relative levels of the remaining spectrin on the membranes after 'aging' and the amount of the supplemented Se. This may further indicate that Se is able to maintain the integrity of the membrane skeleton and prevent the dissociation of spectrin from the erythrocyte membranes. No similar results could be observed if NaCl with the same ionic strength was added instead of Na₂SeO₃.

From the above-mentioned results, we might tentatively suggest that supplementation of Se in the aging medium could prevent the dissociation of spectrin from the erythrocyte membrane, so the changes in the lipid fluidity and also the Na, K-ATPase activity would consequently be delayed.

Generally, the physiological functions of Se are interpreted in terms of the activity of the Se-containing glutathione peroxidase (GSHpx) which can protect biomembranes as a result of its catalytic reaction of lipid peroxide with glutathione (Wo, and Yang, 1986). However, GSHpx distributes mainly in the cytoplasm of erythrocytes (Beilstein and Whanger, 1983). Similar results were also obtained in our laboratory. Therefore, it seems that the maintenance of membrane integrity of erythrocytes by supplementing Se might not be related to the activity of GSHpx.

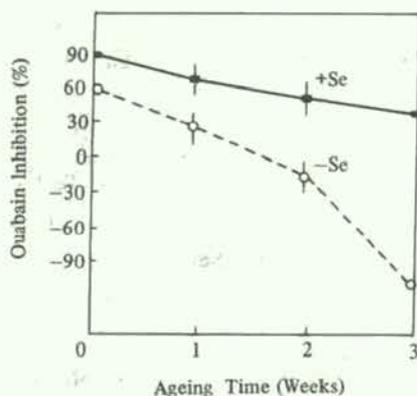


Fig.1. Change of ouabain sensitivity of Na, K-ATPase of erythrocyte ghosts aged in Na_2SeO_3 "free" (---) or Na_2SeO_3 -supplemented (—) media.

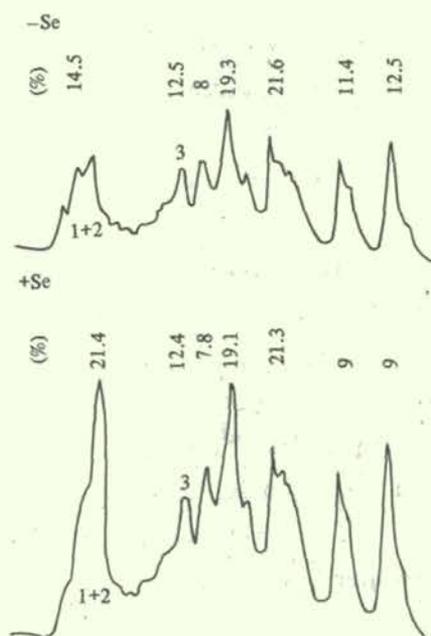


Fig.2. Densitometric scan of SDS-PAGE of ghosts aged (4°C, 4 days) in Na₂SeO₃-“free” or Na₂SeO₃-supplemented (0.16 ppm Se) media.

Presumably, there are two main physiological functions of Se for the biomembranes: protective effect interpreted in terms of GSHpx activity and stabilizing effect on the membrane structure. It is tentatively suggested that Se-deficiency might lead to a shortage of both ‘protective’ and ‘stabilizing’ agents for the biomembranes, and consequently, they are easily to be impaired by environmental pathogenic factors. And, the effectiveness of Se for prevention of Kashin-Beck disease might be the consequence of its ability to protect and stabilize the membrane structure.

REFERENCES

- Beilstein, M. A. and Whanger, P. D., Distribution of selenium and glutathione peroxidase in blood fractions from humans, rhesus and squirrel monkeys, rats and sheeps. *J. Nutr.* 113: 2138-2146, 1983.
- Li, F.S., Wei, F.Q., Fu, Z. L., Bai, S. C., Bai, Q. F., Guan, J.Y., Duan, Y.J., Zou, L. M., Zhao, Y. H., Li, L. and Jin, Q. (1984) Approaches to mechanisms of prevention of Keshan and Kashin-Beck diseases by selenium. In: *Selenium in Biology and Medicine*, Van Nostrand Reinhold Company, pp. 957-967.
- Li, C. Z., Huang, J. R. and Li, C. X. 1984 Sodium selenite as a preventive measure for Kashin-Beck

disease as evaluated in X-ray studies. *ibid*, pp. 934-937.

Pearson, T. W. 1978 Sodium-potassium ion-dependent ATPase of Duchenne muscular dystrophy erythrocyte ghosts. *Life Sci.* 22, 127-132.

Polyacrylamide Gel Electrophoresis-Laboratory Techniques, Pharmacia Fine Chemicals 1983, pp. 25-27.

Rotruck, J. T., Pope, A. L., Ganther, H. E., and Hoekstra, W. G., Prevention of oxidative damage to rat erythrocytes by dietary selenium. *J. Nutr.* 102, 689-696, 1972.

Wo, W. H. and Yang, F. Y. Study on the interaction of Se and erythrocyte membrane——protective effect of Se on the erythrocyte membranes, *Sci.* 29, 1179-1185, 1986.

Yang, F. Y., Huang, F., Lin, Z.H., Zhang, K., Shi, B. S., Fu, Y. G., Li, S. G., Sun, S., Chen, J. W., Zhang, L. P., Guo, B. G., Wo, W. H., Zhang, R. Q., Xing, Q. R., Chen, W. W. and Zhou, J. 1984 Abnormalities of erythrocyte membranes from patients with Kaschin-Beck disease, In :Selenium in Biology and Medicine, Van Nostrand Reinhold Company pp. 947-956.

MEMBRANE DAMAGE IN KASHIN-BECK DISEASE AND ITS ETIOLOGICAL SIGNIFICANCE

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Kashin-Beck disease, which occurs in remote areas among the mountainous areas in China and in the eastern part of Soviet Union, is basically a deformational osteoarthritis, and is found predominantly in children. The main pathological change is the necrosis of chondrocytes in epiphysis. The chondrocyte membrane damage may be related to metabolic disorders caused by the local ecological environment (Li et al., 1984). We have investigated children suffering from KBD in Inner Mongolia and Shaanxi province for several years. The lipid components and selenium content of the membranes were analysed. The shape and fragility of the erythrocytes of children with Kashin-Beck disease have been examined using scanning electron microscope and the cartilage components of patients were determined.

Materials and Methods

Blood specimens were collected from KBD children and healthy age-matched control children (6-12 years old) in Zhaowuda Meng of Inner Mongolia and Yongshou county of Shaanxi province. Autopsy samples were collected from children (aged 1-6 years old, 1 male, 4 females) in Yongshou and Huluenbeier Meng, as well as samples from seven control children collected in Shenyang city (1-4 years old, 6 males, 1 female).

The total lipids was extracted from erythrocytes and tissues by the method of Folch et al. (1957) and determined by the method of Pande et al. (1973). Fractionation of phospholipid (PL) was performed by thin layer chromatography and detected with a dual wavelength chromatoscanner (Shimadzu CS-910). The total phospholipid was determined by the method of Stewart (1980) and the cholesterol content by Zak's method (Zak, 1975). Selenium content (Wilkie and Young, 1970), and fragility of erythrocytes (Parpart, et al., 1974) were determined, and hexuronic acid (Bitter and Muir, 1962), hexosamine (Gatt and Bergman, 1968), sulfuric acid (Dongson and Price, 1962), collagen (Brandt and Muir, 1971) and DNA (Santen and Agranoff, 1963) were measured in cartilage. The shape of erythrocytes was observed with a JEM scanning electron microscope.

Results

1. Content of selenium in plasma and erythrocytes

The children living in endemic areas all suffered from selenium deficiency. The content of selenium in blood of both children (including KBD children and controls) in endemic area was found to be low (Table 1).

Table 1. Content of selenium in plasma and erythrocytes in children with Kashin-Beck disease and control children ($\bar{x} \pm SD$)

Subjects	No of children	Age (years)	Selenium($\mu\text{g} / \text{ml}$)	
			Plasma	Erythrocytes
KBD patients	19	6-12	0.017 ± 0.002	0.076 ± 0.012
Controls in endemic areas	13	7-13	$0.031 \pm 0.002^{**}$	$0.042 \pm 0.008^*$
Controls in non-endemic areas	17	9-12	$0.119 \pm 0.033^{***}$	$0.148 \pm 0.005^{***}$

Compared with KBD: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

2. Lipid component, fragility, and shape of erythrocytes

Table 2 and Table 3 show the results of membrane lipid component analysis of erythrocytes in children with Kashin-Beck disease from the endemic areas in Inner Mongolia and Shaanxi province. The phospholipid content of the patients was found to be lower and the molar ratio between cholesterol(Ch) and phospholipid (PL) higher.

Fractionation of phospholipid was performed by thin-layer chromatography. The results (Table 4) indicated that in patients the phospholipid content of erythrocytes was decreased; the phosphatidylcholine (PC) content was decreased significantly and the molar ratio between sphingomyelin and PC (SM / PC) was increased. This relationship can clearly seen from the analysis of the chromatogram (Fig. 1). Fig. 2 shows the data from the determination of the molar ratio of Ch / PL and SM / PC in the lipids of erythrocytes of patients with Kashin-Beck disease and controls in Yongshou county.

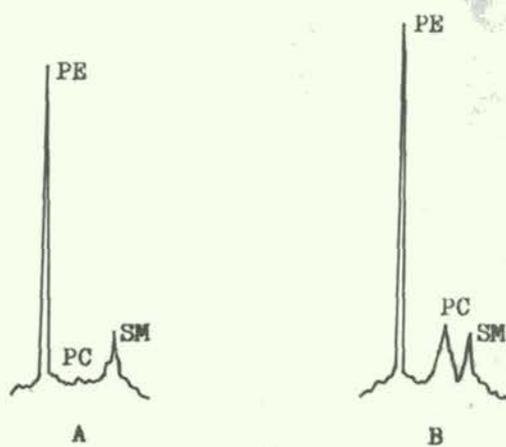


Figure 1. Phospholipid chromatogram of erythrocytes of Kashin-Beck disease (A) and control (B) children
SM: sphingomylin, PC: phosphatidycholine
PE: phosphatidylethanolamine

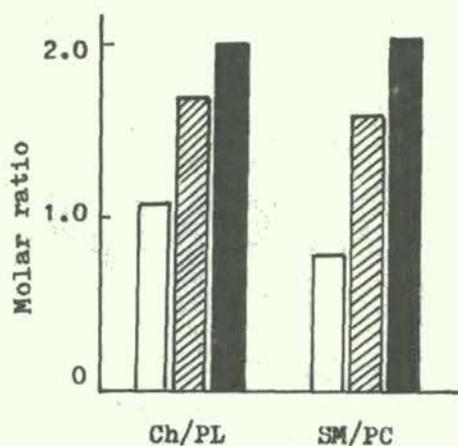


Figure 2. Molar ratio of lipid component cholesterol / phospholipid (Ch / PL) and sphingomelin / phosphatidylcholine (SM / PC) in erythrocytes of Kashin-Beck disease patients ■ and controls □ .

Table 2. Lipid components of erythrocytes in children with and without Kashin-Beck disease ($\mu\text{g} / 10^7$ cells, mean \pm S.D.)(Inner Mongolia, 1980)

Subjects	No. of children	Age (years)	Total lipid	Total phospholipids
KBD patients	14	6-12	3.87 ± 0.17	1.86 ± 0.16
Controls in endemic areas	13	7-13	4.28 ± 0.27	$2.48 \pm 0.17^*$
Controls in non-endemic areas	16	9-12	$4.55 \pm 0.22^*$	$2.73 \pm 0.12^{**}$

Compared with KBD patients: * = $p < 0.05$; ** = $p < 0.01$.

Table 3. Lipid components of erythrocytes in children with and without Kashin-Beck disease ($\mu\text{g}\cdot 10^{-7}$ cell, mean \pm S.D.)(Shaanxi, 1982)

Subjects	No. of children	Age (years)	Phospholipids (PL)	Cholesterol (Ch)	Ch / PL molar ratio
KBD patients	10	9-12	1.44 \pm 0.14	1.35 \pm 0.06	2.0
Controls in endemic areas	6	8-12	2.48 \pm 0.13 *	2.48 \pm 0.17 *	1.1
Controls in non-endemic areas	16	9-12	2.73 \pm 0.12 **	2.73 \pm 0.12 **	1.0

Compared with KBD patients: * = $p < 0.05$; ** = $p < 0.01$

Table 4. Contents of main phospholipids in the erythrocytes(plasma membrane) of children with Kashin-Beck disease (Mean \pm S.D.)

Subjects	No. of children	Main phospholipid ($\mu\text{g} / 10^7$ cells)			
		Sphingomyelin (SM)	Phosphatidyl-choline (PC)	Phosphatidyl-ethanolamine	Ch / PL molar ratio
KBD patients	15	0.44 \pm 0.07	0.28 \pm 0.04	0.70 \pm 0.05	1.41
Controls in endemic area	15	0.40 \pm 0.04	0.47 \pm 0.07 *	0.50 \pm 0.09	0.76
Controls in non-endemic area	15	0.67 \pm 0.07	0.53 \pm 0.07 *	0.80 \pm 0.07	1.18
Adult controls	7	0.79 \pm 0.14	0.57 \pm 0.09	1.17 \pm 0.17	1.23

Compared with KBD patients: * $p < 0.05$

These aging changes in the erythrocytes membrane of patients were also evident in observations of function and ultrastructure, such as the increased osmotic fragility (Fig. 3) and the great numbers of acanthocytes or spur cells, seen under the scanning electron microscope.

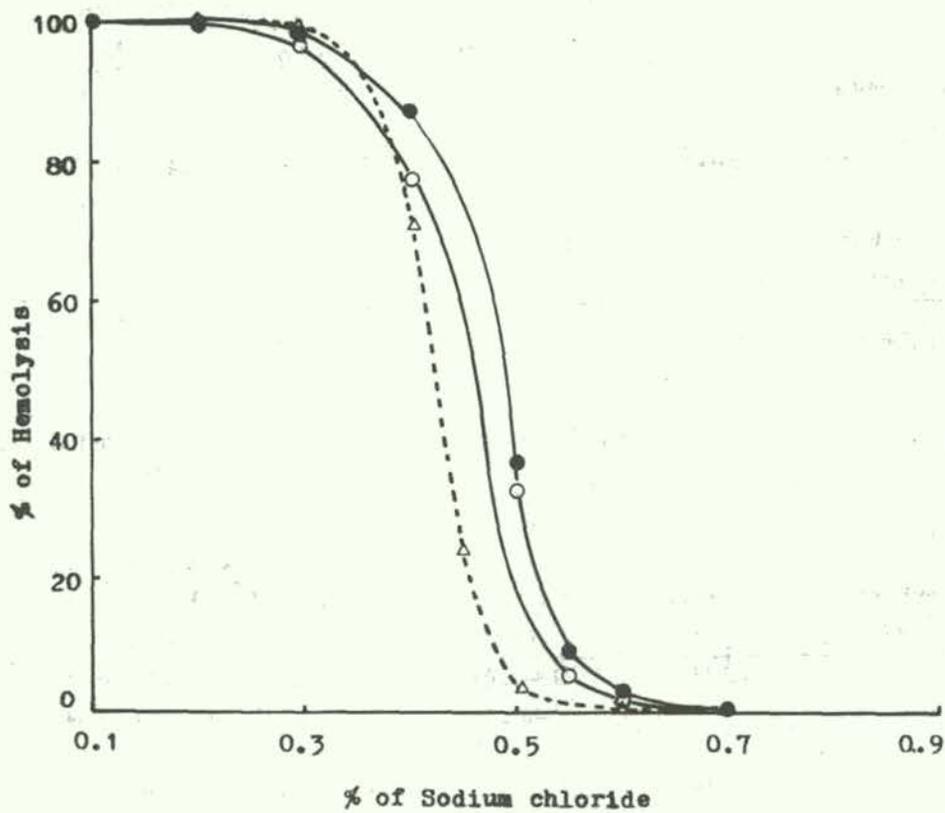


Figure 3. Osmotic fragility of erythrocytes of the Kashin-Beck disease patients (●—●) and controls in endemic area, (○—○) and in non-endemic area (△...△).

3. Changes in the components of cartilage in children with Kashin-Beck disease

a) Lipid components of cartilage

In the last few years, it has been discovered that there are biomembrane disorders in some diseases, including changes in erythrocyte membrane. It is important to know whether the membrane lipid of cartilage tissue in the KBD patient with abnormal red cell membrane has the same disorder? Our analysis showed a series of changes in the cartilage tissue of the patients (Table 5). The difference between the total lipid in cartilage in patients and that in the controls was not significant, but the phospholipid levels in the patients were lower than in the controls; in particular, the phospholipid content of the surface layer of the joint cartilage was significantly lower. Similar changes in red cells membrane of the patients were found in the PL components of cartilage (Table 6). The phosphatidylcholine (PC) was decreased and the sphingomyelin (SM) increased. The molar ratio of SM / PC in either joint cartilage or epiphyseal cartilage was all increased as compared with controls (Fig. 4).

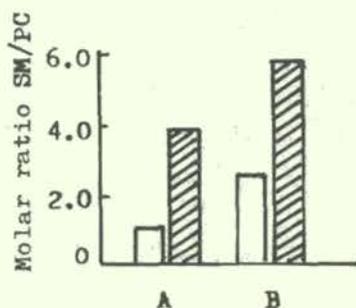


Figure 4. Molar ratio of sphingomyelin / phosphatidylcholine (SM / PC) in phospholipid fraction of Kashin-Beck disease (▨) and control (□) children.

A: Epiphyseal cartilage, B: Joint cartilage

Table 5. Content of total lipid and phospholipid in cartilage of children with and without Kashin-Beck disease (mg / g dry weight, mean \pm S.E.)

Subjects	No. of children	Joint cartilage			Mean	Epiphyseal cartilage
		Surface layer	Middle layer	Deep layer		
Total lipid						
Control	7	3.70 \pm 0.96	4.48 \pm 0.52	4.36 \pm 0.59	4.24 \pm 0.33	4.12 \pm 0.62
KBD	4	3.71 \pm 0.74	3.78 \pm 0.41	3.21 \pm 0.57	3.57 \pm 0.35	4.41 \pm 0.36
Phospholipid						
Control	7	1.10 \pm 0.20	0.69 \pm 0.16	0.53 \pm 0.11	0.74 \pm 0.15	0.90 \pm 0.1
KBD	4	0.71 \pm 0.07*	0.42 \pm 0.03	0.56 \pm 0.07	0.56 \pm 0.05	0.86 \pm 0.08

Compared with controls: * $p < 0.05$

Table 6. Content of main phospholipids in cartilage of children with and without Kashin-Beck disease (mg / g dry weight, mean \pm S.E.)

Subjects	Joint cartilage			Mean	Epiphyseal cartilage
	Surface layer	Middle layer	Deep layer		
Controls (N = 7)					
Lysolecithin	0.04 \pm 0.01	0.06 \pm 0.04	0.02 \pm 0.00	0.04 \pm 0.02	0.17 \pm 0.10
Sphingomyelin	0.32 \pm 0.02	0.23 \pm 0.07	0.05 \pm 0.03	0.20 \pm 0.07	0.12 \pm 0.03*
Phosphatidyl-choline	0.14 \pm 0.03	0.05 \pm 0.03	0.02 \pm 0.01	0.07 \pm 0.03	0.11 \pm 0.05
Phosphatidyl-ethanolamine	0.11 \pm 0.10	0.11 \pm 0.12	0.02 \pm 0.01	0.12 \pm 0.08	0.02 \pm 0.01
Total	0.61 \pm 0.06	0.48 \pm 0.19	0.11 \pm 0.04	0.43 \pm 0.11	0.42 \pm 0.10
KBD(N = 3)					
Lysolecithin	0.04 \pm 0.02	0.02 \pm 0.02	0.04 \pm 0.02	0.03 \pm 0.03	0.03 \pm 0.01
Sphingomyelin	0.32 \pm 0.06	0.24 \pm 0.11	0.08 \pm 0.04	0.21 \pm 0.07	0.24 \pm 0.03*
Phosphatidyl-choline	0.08 \pm 0.04	0.01 \pm 0.01	0.01 \pm 0.01	0.03 \pm 0.02	0.06 \pm 0.03
Phosphatidyl-ethanolamine	0.17 \pm 0.17	0.06 \pm 0.06	0.03 \pm 0.03	0.03 \pm 0.09	0.09 \pm 0.09
Total	0.61 \pm 0.17	0.33 \pm 0.01	0.16 \pm 0.04	0.36 \pm 0.10	0.47 \pm 0.09

* $p < 0.05$

Table 7. DNA content in cartilage of children with Kashin-Beck disease (mean \pm SE, % dry weight)

Subjects	No. of children	Joint cartilage			Mean	Epiphysial cartilage
		Surface layer	Middle layer	Deep layer		
Control	7	0.23 \pm 0.04	0.15 \pm 0.03	0.17 \pm 0.03	0.21 \pm 0.03	0.21 \pm 0.03
KBD	5	0.11 \pm 0.01*	0.07 \pm 0.01	0.09 \pm 0.03	0.10 \pm 0.02	0.16 \pm 0.04

* $p < 0.05$

b) DNA content of cartilage

The DNA content in every layer of joint cartilage and epiphysial cartilage of KBD patients was less than that of the controls (Table 7).

c) Matrix content of cartilage

The sulfuric acid level of mucopolysaccharides in cartilage of children with Kashin-Beck disease was decreased, but not statistically significant (Table 8), whereas the collagen content increased (Table 9). The ratio of chondroitin sulfate and collagen was below 0.5, which was lower than the controls. These findings showed a typical aging changes of the connective tissue.

Table 8. The mucopolysaccharide content in cartilage of children with Kashin-Beck disease and of control children (% dry weight, mean \pm S.E.)

Subjects	Joint cartilage			Mean	Epiphyseal cartilage
	Surface layer	Middle layer	Deep layer		
Control (N = 7)					
Hexuronic acid	7.96 \pm 0.68	8.65 \pm 0.78	9.25 \pm 0.86	8.62 \pm 0.37	10.46 \pm 0.95
Hexosamine	7.78 \pm 0.92	8.64 \pm 0.37	9.29 \pm 0.35	8.57 \pm 0.44	10.29 \pm 0.52
Sulfuric acid	4.82 \pm 0.92	4.24 \pm 0.59	5.56 \pm 0.69	4.97 \pm 0.39	4.51 \pm 0.91
KBD(N = 4)					
Hexuronic acid	8.20 \pm 0.61	8.64 \pm 0.93	9.36 \pm 0.95	8.73 \pm 0.34	8.24 \pm 1.22
Hexosamine	7.83 \pm 1.16	9.33 \pm 1.93	9.71 \pm 1.51	8.96 \pm 0.57	8.20 \pm 1.47
Sulfuric acid	3.76 \pm 0.41	3.18 \pm 0.77	4.08 \pm 0.56	3.67 \pm 0.26	3.39 \pm 1.01

* $p < 0.05$

Table 9. The collagen content in cartilage and ratio of chondroitin sulfate and collagen in cartilage of KBD patients and controls ($\bar{x} \pm S.E.$)

Subjects	No. of children	Joint cartilage			Mean	Epiphyseal cartilage
		Surface layer	Middle layer	Deep layer		
Collagen in cartilage mg / g dry weight						
Control	7	432.4 \pm 8.43	396.6 \pm 17.9	393.9 \pm 25.5	407.6 \pm 12.4	375.1 \pm 14.4
KBD	4	549.0 \pm 13.0**	544.5 \pm 28.4*	511.0 \pm 36.2	534.9 \pm 10.7	484.5 \pm 23.3**
Ratio of chondroitin sulfate to collagen						
Control	7	0.49	0.58	0.62	0.56	0.74
KBD	4	0.40	0.43	0.49	0.44	0.46

Compared with KBD patients * $p < 0.05$; ** $p < 0.01$.

Discussion

The hypothesis that Kashin-Beck disease may be a disease of biomembrane disorder caused by the comprehensive ecological effects of the environment was suggested by us recently on the basis of the pathological changes seen in patients and related epidemiological characteristics (Li et al., 1984). The results reported in this paper support this hypothesis.

The selenium content and the activity of glutathione peroxidase in the blood of children in areas with Kashin-Beck disease (including normal children) were lower than those in children in the non-endemic areas. However, children with selenium deficiency in the endemic area do not necessarily suffer from Kashin-Beck disease. In the present studies, striking differences were found in the membrane lipid components, and in the fragility and shape of erythrocytes between the children with the disease and the control children in the same endemic area. In the patients, the shape and function of the erythrocyte membrane revealed abnormal changes.

The phospholipid (PL) content of erythrocytes in the patients was lower than that in the controls in non-endemic areas and endemic areas; in particular, the phosphatidylcholine (PC) decreased significantly, while, on the contrary, the sphingomyelin (SM) increased. The molar ratios of cholesterol (Ch) / PL and SM / PC increased as did the number of acanthocytes. According to a recent report, the increases in the level of sphingomyelin in the acanthocytes and the increases in the molar ratios of Ch / PL and SM / PC are proportional to increases in age (0-90 years) (Barenholz and Thompon, 1980).

Changes similar to those seen in erythrocytes were also seen in cartilage in Kashin-Beck disease patients. For example, the DNA content decreased as the number of cell nuclei decreased. In addition, the phospholipid content of the cell membrane also decreased. This is consistent with the increase of uric acid and fatty acids in the blood of patients (Han, 1983). The sulfuric acid level of the mucopolysaccharides decreased in the patients and the collagen content increased, both of these are regarded as metabolic products of chondrocytes. The ratio of chondroitin sulfate to collagen was lower in children with the disease. These changes are identical with the typical features of aging of connective tissues (Orij, 1968; Stimunek and Muir, 1972).

The results indicate that the molecular biological changes in Kashin-Beck disease are similar to early aging phenomena. The early osteogenesis and early aging phenomena seen in Kashin-Beck patients were pointed out 45 years ago by Japanese pathologists (Suzuki, 1940).

Changes in the biomembrane lipids are known to affect membrane structure and function, leading to changes in the permeability, fragility, fluidity, binding enzymes and lysosome enzymes that lead to cell deformation and death (Glaser and Vegelos, 1974). While the changes in the lipid component and in the function and shape of membranes appeared in patients, and the activity of the some enzymes in cell decreased, the activity of the same enzymes in the plasma increased, and the cGMP content in urine decreased (Zhang and Yang, 1984). All these changes indicate that there may be a disorder of structure and function of the cell membranes in patients with Kashin-Beck disease. From the etiological point of view, this membrane defect may be caused by environmental factors that result in a deficiency of selenium and some membrane materials.

The findings reported here may offer new possibilities of early diagnosis and suggest ways of preventing Kashin-Beck disease and some other aging diseases.

REFERENCES

- Barenholz, Y. and Thompson, T.E. Sphingomyelin in bilayers and biological membranes. *Biochim. Biophys. Acta*, 604:129-158, 1980.
- Bitter, T. and Muir, H. A modified uronic acid carbazol reaction. *Analyt. Biochem.* 4:330-334, 1962.
- Brandt K. D. and Muir, H. Heterogeneity of protein-polysaccharides of porcine articular cartilage. *Biochem. J.*, 121:261-270, 1971.
- Dogson, K. S. and Price, R. G. A note on the determination of the ester sulfated polysaccharides. *Biochem. J.*, 84:106-110, 1962.
- Folch, J. et al. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-509, 1957.

- Gatt, R. and Bergman, E. R.** A rapid procedure for the estimation of amino sugars on a micro scale. *Analyt. Biochem.* 15:167-171, 1968.
- Glaser, M. and Vegelos, P. R.** Manipulation of the phospholipid composition of tissue culture cells. *Proc. Natl. Acad. Sci. USA*, 71: 4072-4076, 1976.
- Han, C. et al.** Determination of blood glutathione peroxidase, vitamin E, lipid peroxide and free fatty acids in children in the Kashin-Beck disease affected areas. *Chinese J. Endemiol.*, 2:65-69, 1983. (in Chinese)
- Li, F. S. et al.** The pathogeny of Kashin-Beck disease—A comprehensive ecologic effect of endemic area. *J. Ecology*, 6:10-14, 1984. (in Chinese)
- Orli, T.** Non-sulfated acid mucopolysaccharides excretion in normal male children and adults. *Biochim. Biophys. Acta*, 170:204-206, 1968.
- Pande, S. V. et al.** Microdetermination of lipid and serum total fatty acids. *Analyt. Biochem.*, 6:415-423, 1973.
- Parpart, A. K. et al.** The osmotic resistance (fragility) of human red cells. *J. Clin. Invest.* 26:636-640, 1974.
- Santen, R.T. and Agranoff, B. W.** Determination of nucleic acids. *Biochim. Biophys. Acta*, 72:254-262, 1963.
- Simunek, Z. and Muir, H.** Changes in the protein-polysaccharides of pig articular cartilage during prenatal life, development and old age. *Biochem. J.*, 126:515-523, 1972.
- Stewart J. C. M.** Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Analyt. Biochem.*, 104:10-14, 1980.
- Suzuki, M.** Pathologic and histologic studies on the Kashin-Beck disease. *Nissin Igaku (Japan)*, 29: 1485-1583, 1940.
- Wilkie, J. B. and Young, M.** Improvement in the 2,3-diaminonaphthalene reagent for microfluorescent determination of selenium in biological materials. *Agric Food Chem.*, 18:944-948, 1970.
- Zhang, J. S., and Yong, M.** Cyclic-GMP contents in urine of Kashin-Beck disease patient. In: *Proceedings of The Investigation for Kashin-Beck Disease in Yongshou County*. 1984, pp.386. People's Health Publishing House, Beijing.
- Zak, B.** Simple rapid microtechnic for serum total cholesterol. *Am. J. Clin. Path.*, 27: 583-588, 1975.

AN OUTLINE OF HISTORY OF RESEARCH ON KASHIN-BECK DISEASE IN JAPAN

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Kashin-Beck disease (Daguñebing) has been known since Yusensky found patients of the dwarf form in the region of the Urov River in the Transbaikal district of Eastern Siberia in 1849.

The history of the investigation of the disease by the Japanese doctors goes back to 1919, when Dr Ikano, an army surgeon, found patients suffering from an endemic chronic progressive and deforming polyarthrititis in the northern part of the Korean Peninsula. He reported that the clinical symptoms corresponded to those of Kashin-Beck disease. Dr Nakamura visited the southern part of the above mentioned area in 1927, Dr Kato in 1928 and Dr Kim in 1929, they observed cases of the same disease. These reports indicated that Kashin-Beck disease was distributed in the northern part of the Korean Peninsula, i.e. in regions other than Siberia.

Since 1935, many papers were published by medical staffs of the Manchurian Medical College. The first paper was presented by Prof. Takamori at the Annual Meeting of the Japanese Society of Internal Medicine in 1935 which revealed that Kashin-Beck disease patients were also present in northeast part of the People's Republic of China. In the same year, Aiso and Hayashi reported at the Assembly of the Manchurian Medical Society that they had examined fifteen adult cases of the disease and that it may be caused by excessive iron content in the drinking water, as suggested by Prof. Hieda.

Takamori expressed his view on the etiology of Kashin-Beck disease at the Annual Meeting of the Japanese Society of Internal Medicine in 1936. He stated that the early ossification starts from the epiphyseal cartilage and then involves the joint. He suggested that these changes, essentially a dystrophy of the bone and cartilage, were caused by functional disorders of the endocrine(s). Takamori observed abnormal functions of the autonomous nerve and of the liver, an accentuation of the basic metabolism, low vitamin contents in the serum and acidosis of the blood in the patients. Six autopsy cases of young patients were reported in 1940 by Suzuki who reported that developmental disturbance and deformity resulted from regressive changes mixed with reparative processes of the bone and there were changes in the endocrines, especially in the anterior lobe of the hypophysis, thyroid gland and parathyroid.

Prof. T. Ogata at the Pathology Department, Tokyo University Medical School, made an hypothesis based on the fact that the salivary gland had incretory as well as excretory function. As the

"Streinfensueck" of the gland is an incretory apparatus, degeneration of this part results in hypo- or aptyalism which causes defective growth of the bone, if the change began in infancy. Ogata ventured the hypothesis that the Kashin-Beck disease may be caused by the injury of the incretory function of the salivary gland. He and his group including Takizawa, who was later Professor at China University Medical School, often visited northeast China to investigate the disease from 1941 through 1945. Ogata and others asserted that the main lesions of the bone and of the cartilage in Kashin-Beck disease patients were closely related with the change in the "Streinfensueck" of the salivary gland. The whole features was, according to them, similar to the so-called arthritis deformans. They also asserted, on the basis of their epidemiological studies, that the cause of the hypo- or aptyalism may be caused by organic substance(s) in the drinking water.

The cause of Kashin-Beck disease was attributed by many investigators to drinking water, thyroid toxicosis, combined avitaminosis, mycotic toxin, or inorganic substances.

As far as drinking water is concerned, organic substances from putrid animal bodies or from decayed plants and excessive iron content were suspected. Prof. Noguchi, a geological chemist, compared the water from two hundreds points including the endemic and non-endemic areas in northeast China. He observed that the content of organic substances as measured by biological oxygen demand (BOD) exactly paralleled the morbidity of the disease. He purified two chemical substances, feruric acid and p-hydrocinnamic acid, from decayed plants and proposed them as causative agents of the Kashin-Beck disease in 1967.

Doubtful Patients with Kashin-Beck Disease in Japan

In 1970, Prof. Takizawa and his collaborators announced that they had found a number of school children suffering from Kashin-Beck disease in Tokyo and in a neighbouring city, and that the tap water in these areas was polluted by organic substances. The problem was so serious that committees at the governmental and metropolitan levels started to investigate whether patients with Kashin-Beck disease did really exist in these areas and whether the tap water was safe for drinking or not. Two committees consisting of orthopedists reached the conclusion that there were no Kashin-Beck disease patients in Japan, after they had carefully checked children identified by Takizawa. Another group consisting of pathologists and pharmacologists carried out tests for subacute toxicity of feruric acid by p.o. and s.c. routes in Wistar rats. The growing rats given the highest dose (0.1 M) for two months showed a marked depression of the weight increase and water consumption, but minimum changes in epiphyseal cartilage of the tibia. It was concluded that tap water in Tokyo and other cities was not harmful to the people. A remark was added that the relationship between the changes observed in the

experiments and Kashin-Beck disease was not discussed.

REFERENCES

- Egashira, Y. and Kawamura, B. (1981) Studies on etiology, prophylaxis and treatment of Kashin-Beck disease (WHO Assignment Report; 9-25 June, 1981) for the project of Kashin-Beck disease control.
- Kashin-Beck Disease Research Committee (Chairman Shigematsu) (1972) Research on the substance of Kashin-Beck Disease. In: Reports of Research through the Special Expenditure of the Ministry of Welfare in 1971 (in Japanese).
- Takamori, T. (1968) Kashin-Beck Disease. Memorial Fund for Professor Takamori's Retirement, The 2nd Internal Medicine. University of Gifu.
- Takizawa, N. (1970) A Research on the Kashin-Beck Disease in Japan. Ogata Book Co. Tokyo (in Japanese).

KASHIN-BECK DISEASE IN THE USSR

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Introduction

Kashin-Beck (Urov) disease is a systemic disease that primarily affects the bone and joints in the form of deforming osteochondroarthrosis. It is endemic throughout eastern Zabaikalye, the southern parts of the Amur region of the USSR, and the northern and central regions of China.

Kashin-Beck disease is characterized by symmetrical degenerative changes with a marked proliferation of the epiphyses of the short and long tubular bones and their joint surfaces. The pathological process also involves other organs and the cardiovascular, nervous, alimentary and endocrine systems.

The Occurrence of Kashin-Beck Disease

The first mention of deforming polyarthritis affecting inhabitants of eastern Zabaikalye was found by the Irkutsk physician S.G. Filatov in the decembrist Rayevsky's correspondence (1830).

The first physician to examine several Urov patients was M.A. Dokhturov, a physician from Nerchinsk (1836).

The main clinical manifestations of the disease were described by I.M. Yurensky in 1849. He noted the unusual structure of the bone and joints in some residents of the Urov river settlements, who also had goitre and some kind of exostoses in other parts of the body. Yurensky stated that such people had shortened fingers, a characteristic gait, and could neither walk nor work properly.

After the Zabaikalye Cossack military detachment was established in 1856, the military headquarters paid attention to the fact that a certain segment of the local population was unable to serve their term in the army, because of deformity. A special order was issued to investigate the disease. The duties were assigned to N.I. Kashin, a doctor of the 4th infantry battalion. N.I. Kashin thoroughly examined the patients, the hygienic conditions under which they were living, their occupational environment, locality, and other environmental factors. In 1859, N.I. Kashin came to the conclusion that "goitre, rheumatic pain and cretinism" was an endemic disease, spreading in the area of the Urov and

Uryumkan rivers. The disease was called "Urov disease" after the river where it occurred most often.

Some time later, E.V. Beck (1899–1903) found similar patients in other settlements located in the tributaries of the Shilka and Argun rivers including the Gazimur, the Unda and the upper and middle Borzya. Beck stated that in the endemic district the morbidity ranged from 6.5 to 46%, with an average of 31.6%.

Only after Soviet power was established in Zabaikalye, the study of the Urov disease became systematic. The researchers were local physicians L.O. Dobrovolky (1925), N.L. Sakovich (1927) and A.K. Belyavsky (1929, 1932), and Professor V.G. Shchipachev (1925).

In 1929, a research station was established to facilitate the study of Urov disease. All the population of the endemic district (numbering 33,238) was examined and data were collected. The peak of morbidity was found in mountainous districts with boggy valleys. In drier steppe zones, the degree of endemicity was lower. The endemicity was found to have a mosaic character, i.e. affected settlements were located next to healthy ones (N.I. Damperov, 1939; F.P. Sergievky, 1952).

Further medical examinations covering 32,844 people, carried out under the guidance of F.P. Sergievsky in 1939, showed that, over the intervening years, the total number of affected patients had fallen by 52%. Morbidity in the 1–10 year age group had fallen by 59% and the incidence rate and number of severe cases had considerably decreased.

Successive check-ups revealed a wave character in the morbidity: During the period of 1958–62, there was a 9.6% reduction in morbidity (L.F. Kravchenko), while over another decade, there was a 20.3% elevation (Yu. A. Petrov).

On the basis of a number of investigations carried out in other regions of the USSR and abroad, it was possible to extend the borderline of the endemic area. Several cases of Kashin–Beck disease were found by S.I. Verzhbitsky (1936) in the Amur region. A thorough study of this endemic district was made in 1948 by N.I. Zhuravleyov, the centre being the Zeya district. Single cases were found in the Kuibyshev, October, and Tambov districts of the Amur region. Morbidity in the region was stated to be as high as 19.8%. Another study carried out in 1958 showed a decrease in morbidity to 11.2%. The distribution of the disease in Amur, when compared to that in Zabaikalye, was shown to have some specific features: it was more localized, and the prevalence among young people under 20 was twice as high (Yu. A. Yatsik, 1962).

In 1931, N.I. Damperov noted that some Chinese and Koreans who came to work in Zabaikalye had manifestations of Kashin–Beck disease. A survey of medical literature showed an occurrence of a similar disease in Manchuria and in the north-west of Korea. A Japanese doctor, M. Aiiso (1932–1937), while on military service in Manchuria, examined several patients, living in villages, who had a specific pathology of the joints. He found endemic manifestations in the province of Tochendo

numbering some 3 million people; in some settlements the morbidity was as high as 60%.

In the north western part of Korea, the disease was studied by the Japanese doctors, Okana, Nakamura, and Kane (1919), and the Bulgarian researcher workers B. Zografsky, G. Terziyev, I. Tolev and M. Belchev (1957).

Single cases of Kashin-Beck disease occur in different parts of the USSR and abroad. For instance, M.I. Schwarzmann (1926, 1935) and V.G. Shchipachev (1931, 1936) reported cases of Kashin-Beck disease among the inhabitants of the Lena and Vikhor river, in the Angara and over the eastern shore of lake Baikal. N.I. Vulpe (1926) described several cases in the town of Kirensk, and P. Nikiforov and G. Goldstein (1931) reported cases in the Mari ASSR. S.I. Verzhbitsky (1934) diagnosed Kashin-Beck disease in Balci in the Chita region among the newcomers from the Vologda region, and D.G. Rokhlin (1938) reported some cases in Pskov, Kiev, and Leningrad. He diagnosed the disease in a patient from Holland who came to consult him. S.S. Irincheyeva (1965) examined Kashin-Beck patients in the Buryat ASSR. O.I. Shershevskaya (1956) reported one case in the settlement of Tumanovka in the Altai region. In 1960, L.F. Karavchenko and V.S. Sidorov examined Kashin-Beck patients in the village of Zharcha in the Chita region, which is far from the endemic area. Kashin-Beck patients were also examined by A.A. Shtuss and S.G. Aliyeva in Baku. A similar disease was reported by S. Ribbing in 1983, in the family of a woodcutter in Sweden.

Thus, Kashin-Beck disease is endemic in : eastern areas of the Chita region (Nerchinsky Zavod, Kalga, Shelopugino, Shilka, Balci, and Sretensk); southern areas of the Amur region; northern areas of China; and north western area of Korea. Single cases occur sporadically in different parts of the USSR and abroad. The distribution of the disease has a mosaic character with prevalence ranging from 5 to 80%.

Concerning the dynamic picture of the disease, F.P. Sergievsky (1941) stated that there had been a sharp reduction in the number of severe forms of the disease. Recent data showed that 2nd and 3rd-stage cases were rare in young children. At that time, no universally accepted valid criteria exist for diagnosing the early stage of the disease. X-ray examination plays a decisive role. N.I. Kashin (1868), E.V. Beck (1906) and N.L. Sakovich (1928) did not use this method at all, while N.I. Damperov (1931-1932), V.G. Shchipachev (1931) and F.P. Sergievsky (1941) applied it only partially. An increased morbidity rate reported by Yu. A. Petrov in 1969 may be accounted for by a greater number of early cases diagnosed by this method.

To elucidate the dynamic picture of Kashin-Beck disease among the population of the Chita region over 60 years, we studied the archive data, collected by the Problem Research Laboratory attached to the Chita Medical Institute, covering the period 1924-81 (V.N. Ivanov, 1983; A.V. Voshchenko and E.E. Ustinova, 1983, 1984). The survey covered 50,691 cases. The archive data and the findings ob-

tained through the check-up examinations carried out over 1980-83 reflect the prevalence of the disease in a single district of the Chita region. Data from settlements (Poperechny and Bolshoi Zerentui) where the rate of morbidity still remains very high (about 20.4%) indicate that the structure of morbidity is according to age and the severity of Kashin-Beck disease.

In the endemic district, 246 cases of Kashin-Beck disease were reported from 3907 persons examined.

The comparison of the data reveals a reduction tendency in the prevalence of the disease. For instance, in 1924 the prevalence was $31.0 \pm 0.4\%$ while, in 1983, it was $6.3 \pm 0.4\%$. Although the incidence of the disease remained as high in 1940 as in 1930 ($29.6 \pm 0.4\%$ and $26.4 \pm 0.6\%$, respectively), if the difference between these values was statistically significant ($P < 0.02$). The prevalence fell considerably to $7.8 \pm 0.5\%$ in 1959. In 1961, examination of 2891 people in the endemic district revealed 280 patients with endemic osteoarthritis, i.e., $9.7 \pm 0.5\%$. This slight increase in the rate of morbidity in the population is statistically significant ($P < 0.01$), but the general tendency to a reduced prevalence in the sixties remains evident. Moreover, the difference between the prevalence levels in 1961 and 1957 is still significant ($P < 0.001$). The 1980-83 examination of 3907 cases in the endemic district revealed the lowest prevalence of Kashin-Beck disease ($6.3 \pm 0.4\%$).

Table 1 shows the prevalence of Kashin-Beck disease among the population over the period of 1924-83.

At present, new cases of Kashin-Beck disease in the mildly endemic settlements of the Chita region are quite rare; endemic osteoarthritis occurs only in middle and old age. That is why an analysis of the prevalence of Kashin-Beck disease was made on the basis of data from the more seriously affected settlements, where the disease still occurs in both children and adults, the prevalence amounting to $23.4 \pm 1.6\%$ (Table 2).

Table 1. Prevalence (%) of Kashin-Beck disease among the population of the endemic district

Period of examination	Number of population examined	Number of cases	
		Absolute number	Prevalence \pm S.E. %
1924	14079	4367	31.0 \pm 0.4
1930-31	14005	4141	29.6 \pm 0.6
1939-40	5707	1507	26.4 \pm 0.4
1949-50	7627	1403	18.4 \pm 0.4
1956-57	4347	696	16.0 \pm 0.6
1959	2481	193	7.8 \pm 0.5
1961	2891	280	9.7 \pm 0.5
1980-83	3907	246	6.3 \pm 0.4

Table 2. The prevalence of Kashin-Beck disease in more seriously affected settlements according to age

Age (Years)	6-10	11-20	21-30	31-40	41-50	51-60	over 60	Total
Number of population examined	113	159	122	51	87	83	64	679
Number of cases	18	39	6	7	18	36	35	159
Prevalence (%) \pm s.e.	15.9 \pm 3.4	24.5 \pm 3.4	4.9 \pm 1.9	13.7 \pm 4.8	20.7 \pm 4.3	43.4 \pm 5.4	54.7 \pm 6.2	23.4 \pm 1.6

According to Table 2, the prevalence in children under 10 years of age is high (15.9 \pm 3.4%). This group includes school children (aged 6-10 years) with the initial stage of the disease. In the 11 to 20 year group, the prevalence is higher though difference between these two groups is not statistically significant ($P > 0.05$). In the 21-30 year age group, Kashin-Beck disease was only diagnosed in 6 out of 122 people examined, which gave the lowest level of morbidity for all age groups (4.9 \pm 1.9%). The prevalence is significantly different from those for 6-10 year and 11-20 year age groups ($P < 0.01$ and $P < 0.001$, respectively).

The morbidity of Kashin-Beck disease increases with age. For instance, in the 31-40 year age group, the prevalence is 13.7 \pm 4.8%, in 51-60 year age group, 43.4 \pm 5.4%, and in the age group over

60 years, $54.7 \pm 6.2\%$. Though the differences in the prevalences of endemic osteoarthritis between 21–30 year age group and 31–40 year age group, and between 31–40 and 41–50 year age groups are not significant ($P > 0.05$ and $P > 0.10$, respectively), the difference is significant between 21–30 and 41–50 year age groups ($P < 0.01$). It is also significant between 41–50 and 51–60 year age groups ($P < 0.01$). The difference in prevalence between the age groups 51–60 and over 60 years is not statistically significant. At the same time, it should be noted that the morbidity in the working population (aged 20–50) is lower.

The prevalence of the disease in seriously affected settlements is presented according to age and stage of severity. In Table 3. It can be seen that 12 patients in the 6–10 year age group showed the initial stage of the disease and 6 patients, the 1st stage, out of 113 children examined, i.e. $10.6 \pm 2.9\%$ and $5.3 \pm 4.4\%$ respectively. Though the number with the initial stage in this group is nearly twice as high as that with the 1st stage, the difference is not statistically significant ($P > 0.05$).

The 11–20 year age group includes 14 patients with the initial stage, 24 patients with the 1st stage, and 1 patient with the 2nd stage, out of 159 people examined, i.e., $8.8 \pm 2.2\%$, $15.1 \pm 2.8\%$, and $0.6 \pm 0.6\%$, respectively. This age group shows a certain prevalence of 1st stage patients, though the difference between the 1st and the initial stages of endemic osteoarthritis is not significantly different ($P > 0.05$). The initial stage was found only in 6– to 20-year-old patients, the prevalence being practically the same in both groups.

The 1st stage of the disease is much more frequent in the 11–20 year age group; in all the subsequent age groups, the prevalence of the 1st stage is significantly lower, while in patients of 34–40 year and those over 60 years old, the 1st stage has not been registered. Only 6 cases of Kashin–Beck disease occurred in the 21–30 year age group, out of 122 people examined. This included 5 patients with the 1st stage and 1 patient with the 2nd stage, i.e. $4.1 \pm 1.8\%$ and $0.8 \pm 0.8\%$, respectively. In the 31–40 year age group, 7 out of 51 people examined presented the 2nd stage, which is equal to $13.7 \pm 4.8\%$. In the subsequent age groups, the prevalence of the 2nd stage disease increases. In the 41–50 year age group, the number of patients with 2nd stage amounts to $16.1 \pm 3.9\%$ and $4.6 \pm 2.2\%$ with the 1st stage. Third stage patients with endemic osteoarthritis have only been reported among people over 50 years of age. For instance, the 51–60 year age group includes 10 patients with 3rd stage, i.e. $12.1 \pm 3.6\%$. As in other groups, patients with 2nd stage are most common. In the oldest age group, 64 people were examined and only patients with 2nd and 3rd stage were found ($32.8 \pm 5.9\%$ and $21.9 \pm 5.0\%$, respectively). The differences between these values are not statistically significant ($P > 0.10$). It is noteworthy that the prevalence of 3rd stage patients in the age group, over 60 years, is higher than that in the age group 51–60 years, but the difference between these two values is not statistically significant ($P > 0.10$); this is also true for the numbers of 2nd stage patients in the same age groups.

Table 3. Distribution of Kashin-Beck cases in the more seriously affected settlements according to age and severity

Age (years)	Number of people examined	Severity of the disease							
		Initial stage		First stage		Second stage		Third stage	
		No. of cases	Prevalence (%) \pm s.e.	No. of cases	Prevalence (%) \pm s.e.	No. of cases	Prevalence (%) \pm s.e.	No. of cases	Prevalence (%) \pm s.e.
6-10	113	12	10.6 \pm 2.9	6	5.3 \pm 4.4				
11-20	159	14	8.8 \pm 2.2	24	15.1 \pm 2.8	1	0.6 \pm 0.6		
21-30	122			5	4.1 \pm 1.8	1	0.8 \pm 0.8		
31-40	51					7	13.7 \pm 4.8		
41-50	87			4	4.6 \pm 2.2	14	16.1 \pm 3.9		
51-60	83			3	3.6 \pm 2.0	23	27.7 \pm 4.9	10	12.1 \pm 3.6
over 60	64					21	32.8 \pm 5.9	14	21.9 \pm 5.0
Total	679	26	3.8 \pm 0.73	42	6.2 \pm 0.92	67	9.9 \pm 1.1	24	3.5 \pm 0.7

Table 4. Distribution of Kashin-Beck disease in the more seriously affected settlements according to severity

	Initial stage		First stage		Second stage		Third stage	
	Male	Female	Male	Female	Male	Female	Male	Female
Absolute number	14	12	19	23	36	31	15	9
Total	26		42		67		24	
Prevalence (%) \pm s.e.	16.4 \pm 2.9		26.4 \pm 3.5		42.1 \pm 3.9		15.1 \pm 2.8	

Data on the case distribution according to the stage of severity are presented in Table 4.

As mentioned above, beginning with the age of 30 years the 2nd stage becomes predominant among Kashin-Beck patients, as shown in Table 4, where the greatest number of patients belong to the 2nd stage (42.1 \pm 3.9%). The 1st stage cases (26.4 \pm 3.5%) rank second. The number of 3rd and initial-stage patients are practically equal, amounting to 15.1 \pm 2.8% and 16.4 \pm 2.9%, respectively.

In order to obtain a dynamic picture of age variation among patients living in the more seriously affected settlements, we have compared our findings with the archive data of 1958 (Table 5).

Table 5 shows that the total number of cases in the more seriously affected settlements over a 25-year period has not changed much (167 patients in 1958 and 159 patients in 1983). The two initial groups (age 6-10 and 11-20 years) do not show differences in the numbers of patients in 1958 and 1983 ($P > 0.1$). But the number of patients in the 11-20 year age group is almost twice than that in the 6-10 year group, in both examination periods. There are nearly 2.5 times fewer cases in the 21-30 year age group in 1983 compared with those in 1958. The next age group (31-40 years) also includes a significantly reduced number of cases in 1983. Though the numbers of patients in 41-50 and 51-60 year age groups in 1958 and 1983 differ, the difference is not statistically significant ($P > 0.10$ and $P > 0.05$, respectively). The number of patients over 60 year old in 1983 was nearly 3.5 times more than that in 1958.

The greater number of patients now in the 45-50 year age group may be accounted for by patients who were 11-20 year old in 1958.

The dynamic picture of the prevalence in patients of different degrees of severity of Kashin-Beck disease is illustrated in Table 6.

Table 5. Distribution of Kashin-Beck disease according to age in 1958 and 1983 (more seriously affected settlements)

Age	1958		1983	
	Absolute no.	Prevalence(%) ± s.e.	Absolute no.	Prevalence (%) ± s.e.
6-10	10	11.4 ± 2.5	18	11.3 ± 2.5
11-20	48	28.7 ± 3.3	39	24.5 ± 3.4
21-30	19	11.4 ± 2.5	6	3.8 ± 1.5
31-40	21	12.6 ± 2.6	7	4.4 ± 1.6
41-50	25	15.0 ± 2.8	18	11.3 ± 2.5
51-60	24	14.4 ± 1.9	36	22.6 ± 3.3
over 60	11	6.6 ± 1.9	36	22.0 ± 3.3
Total	167		159	

As the initial stage of the disease was not usually diagnosed in previous years, this group has been excluded from the analysis.

The data analysis reveals that the dynamic picture of the prevalence in the more seriously affected settlements is correlated with reduced endemic osteoarthritis morbidity in the whole district.

Examination of 629 people in the highly endemic settlements revealed 308 Kashin-Beck patients in 1931 (48.9 ± 2.0% cases). Ten years later, examination of 610 people in the same settlements revealed only 182 patients (29.8 ± 1.9%). This value is significantly lower than the previous one ($P < 0.001$). In 1949-50, only 142 Kashin-Beck cases were diagnosed out of 661 people examined. The prevalence was 23.2 ± 1.7%, significantly lower than that of 1940. A somewhat greater occurrence of endemic osteoarthritis was registered in 1956. The disease was diagnosed in 196 out of 739 people examined, i.e., 26.5 ± 1.6%. However, this rise is not significant compared with the value of the previous examination ($P > 0.10$).

In 1958, the prevalence of endemic osteoarthritis was 17.4 ± 1.3%, much lower than that in 1956 ($P < 0.01$).

In 1983, we found that 138 out of 679 people living in these settlements were suffering from the 1st, 2nd, or 3rd stages of Kashin-Beck disease, equalling 16.6 ± 1.4%. The significance of the difference between the prevalences of Kashin-Beck disease in the more seriously affected settlements over the 25-year period has not been stated.

Table 6. Dynamic picture of the severity of Kashin-Beck disease in the more seriously affected settlements over the period 1930-83

Examination Period	Severity of the disease						Total No. of people examined	
	First stage		Second stage		Third stage			
	No. of cases	Prevalence (%) ± s.e.	No. of cases	Prevalence (%) ± s.e.	No. of cases	Prevalence (%) ± s.e.		
1930-31	248	39.4 ± 1.9	53	8.4 ± 1.1	7	1.1 ± 0.42	629	48.9 ± 2.0
1939-40	82	13.4 ± 1.3	78	12.8 ± 1.3	22	3.6 ± 0.75	610	29.8 ± 1.9
1949-50	56	9.2 ± 1.2	75	12.3 ± 1.3	11	1.8 ± 0.74	611	23.2 ± 1.7
1956	104	14.1 ± 1.3	78	10.6 ± 1.1	14	1.9 ± 0.50	739	26.5 ± 1.6
1958	88	9.6 ± 0.97	51	5.6 ± 0.76	20	2.2 ± 0.49	914	17.4 ± 1.3
1983	42	6.2 ± 0.92	67	9.9 ± 1.1	24	3.5 ± 0.7	679	16.1 ± 1.4

Analysis of data on the severity of the disease in different periods reveals that the greatest number of 1st-stage patients was diagnosed in 1930, amounting to $39.4 \pm 1.9\%$ of all the people examined. The number of patients with 1st stage endemic osteoarthritis registered among the population of these settlements reduced to $6.2 \pm 0.92\%$ in 1983. The prevalence of 2nd-stage endemic osteoarthritis during the 50-year period remained nearly the same, with the exception of 1958 when only 51 patients with the 2nd stage of the disease were found out of 914 people examined. The prevalence was equal to $5.6 \pm 0.76\%$, which was significantly lower than those of other periods. In 1930-31, only 7 out of 629 people examined were diagnosed as having the 3rd stage of Kashin-Beck disease ($1.1 \pm 0.42\%$). In the subsequent years, a greater number of cases with this severe stage of the disease were revealed. However, the difference was significant only between the prevalences of 1930 and 1940, and 1930 and 1983 ($P < 0.05$ and $P < 0.01$, respectively). A certain stabilization of the prevalence over the last 25 years may be due to a greater number of patients with the 2nd and 3rd stages of the disease and a reduced total number of patients.

Table 7 illustrates the distribution of cases according to the severity of the disease in highly endemic settlements over a 50-year period.

Table 7. Distribution of cases according to the severity of Kashin-Beck disease in the more seriously affected settlements during different periods of examination

Examination period	First stage		Second stage		Third stage		Total
	Absolute number	Prevalence (%) \pm s.e.	Absolute number	Prevalence (%) \pm s.e.	Absolute number	Prevalence (%) \pm s.e.	
1930-31	248	80.5 ± 2.3	53	17.2 ± 2.2	7	2.3 ± 0.85	308
1939-40	82	43.1 ± 3.7	78	42.9 ± 3.7	22	12.1 ± 2.4	182
1949-50	56	39.4 ± 4.1	75	52.8 ± 4.2	11	7.7 ± 2.2	142
1956	104	53.1 ± 3.6	78	39.8 ± 3.5	14	7.1 ± 1.83	196
1958	88	55.3 ± 3.9	51	32.1 ± 3.7	20	12.6 ± 2.6	159
1983	42	31.6 ± 4.0	67	50.4 ± 4.3	24	18.0 ± 3.3	133

As mentioned above, the majority of cases in 1930 were patients with 1st stage of the disease ($80.5 \pm 3.2\%$). By 1950, the number of 1st stage cases had fallen to $39.4 \pm 4.1\%$.

In the 1950s, the number of mild stage patients increased to nearly half of the total number. In 1983, a reduction in the 1st stage cases ($31.6 \pm 4.0\%$) was registered. In 1930, the number of 2nd stage cases was only $17.2 \pm 2.2\%$ of the total patients. In the succeeding periods, a reverse proportion was observed in the correla-

tion between the 1st and 2nd stage patients and by the fifties there had been a significant increase in the number of 2nd stage cases ($52.8 \pm 4.2\%$). During the fifties, there was a significant reduction in the number of 2nd stage cases and an increase in the number of 1st stage patients.

The Etiology of Kashin-Beck Disease

Diverse hypotheses have been suggested concerning the etiology of Kashin-Beck disease.

Resident of Zabaikalye associated it with "bad" drinking water. There was a notion that the disease could be passed down from generation to generation in "diseased" families.

In northern parts of Korea, manifestations of disease were attributed to specific types of soil and water.

N.I. Kashin (1861, 1868) tried to associate the causes of this disease with some unfavourable factors in local environment, such as frequent fogs, narrow valleys, and bogging up. Later on, the role of these factors, either alone or combined, was rejected. It stands to reason that, in many climatic areas similar to Zabaikalye, these factors do not necessarily result in endemic osteoarthritis morbidity.

The first half of the 20th century witnessed several hypotheses explaining the etiology of Kashin-Beck disease including radiation effects, vitamin deficiency, endocrine, and alimentary effects and others.

I.A. Bagashev (1911, 1925), who studied the radioactivity of mineral sources in the endemic region came to the conclusion that the cause of Kashin-Beck disease was the elevated radioactivity of mineral water sources. Some time later, he rejected this idea since no clear correlation was found between morbidity and the level of radiation.

In 1934-35, Ya.K. Magnushevsky and Ya.D. Reichbaum undertook another study on the radioactivity of water sources in the endemic district. The results were again negative. Moreover, Ya.D. Reichbaum (1935) came to the conclusion that an elevated level of radioactivity associated with the high mineralization of water sources attenuated the course of the endemic.

V.G. Shchipachev (1925, 1931) and K.K. Platonov (1931, 1936) associated the causes of Kashin-Beck disease with vitamin deficiency. The manifestations of the disease were treated as polyavitaminosis. However, a series of studies did not prove the validity of this theory (F.P. Sergievsky, 1948, 1952; L.F. Kravchenko, 1959, 1968). V.A. Tikhonov (1961), in the course of a thorough check-up carried out in the Nerchinsk-Zavod, Shelopugino, Alexander-Zavod districts, showed that the prevalence of hypovitaminosis C in Kashin-Beck patients did not exceed that in healthy people. Vitamin consumption cannot be considered as a decisive factor in the development of Kashin-Beck disease, though it may affect the course of the disease.

There were other assumptions that Kashin-Beck disease was of infectious origin (V. Barykin and S. Klyukhin, 1926). These views were also rejected. Any infectious process is based on inflammation and this is

not characteristic of Kashin-Beck disease (N.F. Gamaleya, 1939). Besides, infectious etiology is contradictory to the endemic character of this disease.

At the beginning of the 20th century, which was marked by intense study of endocrine secretion, the endocrine theory emerged, according to which, the disease was treated as polyglandular pathology causing osteodystrophy (V.G. Shchipachev, 1927, 1936). In 1950, the author of these views modified this theory stating Kashin-Beck disease to be an advanced form of hypothyrosis. M.I. Dobrovolsky (1926) treated delayed bone growth as resulting from atrophy of the anterior lobe of the hypophysis, and convulsions and muscle hyperexcitability, from deficiency of the parathyroid glands.

According to S.I. Banaitis (1935), the causes of the disease lay in the pathology of the hypophysis and the thyroid gland, and according to V.P. Gratsiansky and N.S. Markelov (1935), in the impairment of the thymus, thyroid, and the anterior lobe of the hypophysis.

Popular as it was, the endocrine theory has a number of factors that appear to be contradictory. In the first place, regions with endemic Kashin-Beck disease and endemic goitre do not overlap each other. Furthermore, neither enlargement of the thyroid nor impairment of the basal metabolism were revealed through medical examinations carried out in districts of North Korea endemic for Kashin-Beck disease (B. Zagrafsky, G. Terziev, I. Tolev and M. Belchev, 1957). Besides which, it should be noted that the endocrine theory concerns the pathogenesis rather than the etiology of Kashin-Beck disease.

F.P. Sergievsky (1932, 1935, 1937), associated its causes with bogging up of the district. However, he changed his views later in favour of the alimentary-toxic fungus theory. It was based on an assumption that residents of the endemic district used wheat infected with *Fusarium sporotrichiella*. The latter exerted a toxic effect on bone growth zones causing aseptic necrosis, and osteolysis degeneration.

This hypothesis found support at the Institute of Nutrition attached to the USSR Academy of Medical Sciences (Yu.I. Rubinstein, 1949, 1953; N.V. Perkel, 1956, 1957; D.S. Papazyan, 1958) and at the Central Institute of Medicine attached to the Public Health Ministry of the People's Republic of China (Shen Chi-Zeng, 1956). However, the validity of the theory was rendered doubtful by A.M. Nesterova who failed to produce an animal model of this disease. In addition, *Fusarium sporotrichiella* is known to spread in different geographical zones of the USSR, which is contradictory to the endemic character of Kashin-Beck disease.

Many researchers tried to associate the causes of Kashin-Beck disease with some toxic factor in the environment through drinking-water. M.I. Dobrovolsky (1926) treated manifestations of the disease as chronic poisoning and recommended the use of boiled water. N.L. Sakovich (1933) who followed up several affected families recommended that they should use only rain water, melted snow or distilled water to prevent the consumption of trace elements with water. The results were very encouraging. Children with Kashin-Beck disease got on so well that they ceased to complain of pains in the upper and lower

extremities.

E.V. Beck (1906), one of the first to investigate the pathology, attributed the cause of the disease to some mineral dissolved in water, acting on the body directly or causing some "miasm".

Later on, many assumptions were made based on deficient or excessive consumption of some minerals. N.L. Sakovich (1927, 1937), I.M. Morozov, E.A. Borodin (1935) suggested an excessive content of lead in water; M. Aiiso, N. Hayashi (1932, 1937)— an excessive iron content, while N.S. Dombrovskaya (1929), A.P. Vinogradov (1939, 1949), A.P. Georgievsky (1952), and V.V. Kovalsky (1959) believed the pathology to be caused by calcium deficiency.

Some authors spoke of phosphorus deficiency (A.V. Cherkasova, 1954) or excess of sulfur (Tanaka, 1936). Ya.I. Gamsulov (1931, 1932). A.K. Chepurov, A.V. Cherkasova, N.M. Akulov, N.M. Ostrovsky, D.F. Martynyuk (1955) associated the development of the disease with general mineral deficiency, primarily calcium deficiency.

A.P. Vinogradov (1949, 1963) advanced a biogeochemical hypothesis according to which the cause of Kashin-Beck disease lay in the imbalance of trace elements in the environment of the endemic district, i.e. in the soil, water, and plants. This assumption found support in studies made by V.V. Kovalsky, I.M. Samarina (1960), V.V. Levoshina, G.M. Isayeva, and M.I. Kutz (1967, 1968, 1970), and V.N. Ivanov and V.S. Bukto (1971, 1972, 1973) who found elevated levels of strontium and barium and reduced levels of calcium, phosphorus, potassium, and sodium in the environment of the endemic district. Unfortunately, their findings differed considerably.

N.I. Muchkin (1967, 1968) revealed a deficiency of calcium, iodine, nickel, copper, iron, sulfur, beryllium, fluorine and an excess of strontium and barium. A.A. Florensov (1958), who investigated the character of deep frozen soil layers in the Urov endemic region, came to the conclusion that their thickness was much greater in settlements in which residents were more affected by the disease. According to him frozen ground, being a powerful cold factor, affects specific geophysical properties in the endemic territory, including the trace element structure of the water and the soil. It also affects the body by causing reflex vascular impairment.

V.S. Butko (1971, 1973) investigated the soil, water, and plants and revealed elevated levels of lead, zinc, silver, strontium, and manganese accompanied by a reduced level of calcium. The food ration of people living in the endemic districts showed a similar imbalance. Bones of Urov disease patients showed excess strontium, lead, and manganese accompanied by calcium deficiency. A regular relationship was established between endemic districts and an imbalance of trace elements in water sources.

The Chinese researcher Yang Tong Shu (1985) suggested a theory explaining the etiology of Kashin-Beck disease based on selenium deficiency. In our view, it has a number of draw-backs. Guangqi Yang et al. (1984) pointed out that selenium deficiency is typical of Keshan disease. It is characterized by

megacardiopathy, while such characteristic symptoms of Kashin-Beck disease as deforming osteoarthritis are absent. Selenium deficiency in Keshan disease is more pronounced. Furthermore, Kashin-Beck disease does not have any of the manifestations of Keshan disease, which makes it almost impossible that their etiology is the same. Finally, the territories endemic for the diseases in China do not coincide.

According to our data, the Chita region includes a province with selenium deficiency (the Ulyoty district), where Kashin-Beck disease has not been registered. Animals of this province suffer from the so-called "white muscle" disease caused by selenium deficiency (L.A. Minita, 1984). The pathology is controlled by the addition of selenium to the animal feed.

A thorough study of the selenium contents in the soil, plants, and local food products of the Kashin-Beck endemic territory has shown that they are not much lower than control levels.

The most important conclusion we have arrived at in studying the geochemistry of the endemic district is a peculiar accumulation of elements in the soil and resulting from bogging-up and the presence of deep-frozen ground.

A complex study of ground rocks in the endemic district consisting of 46 elements and compared with controls (1520 samples) revealed an elevated content of only 2 elements: phosphorus (0.302%) and manganese (0.212%). The same tendency was found in the soil (Table 8).

Accumulation of manganese and phosphorus in endemic soils results from the ground being deep-frozen, which prevents run-off of the soil colloids in the upper layers. A district inverse correlation was found between the severity of the endemicity and the thickness of the deep-frozen layers of soil (Table 9).

From these studies, it can be concluded that soils in endemic districts, in which accumulation of elements takes place, contain very high concentrations of both phosphorus and manganese (Table 8).

Earlier hypotheses on the etiology of Kashin-Beck disease were based on calcium deficiency in the biogeochemical chain associated with trace element imbalance. According to our studies, the amount of calcium in ground rocks, soil, and water in the endemic regions is practically the same as that in the control district.

Polyphosphates are known to play a great role in the mineral nutrition of plants (I.S. Kulayev, 1975; M.P. Surgucheva, 1980). Modern literature contains evidence of their toxic effects on animals leading to their delayed growth (Harmful Substances..., 1977).

Table 8. Concentrations of some macro- and trace elements in soils in an endemic district (mg / 100 g)

Elements	Endemic district = 840	control district = 213
1. Lead	1.44	1.26
2. Zinc	4.28	3.77
3. Arsenic	2.17	2.81
4. Copper	1.86	1.51
5. Silver	0.18	0.16
6. Molybdenum	0.09	0.07
7. Chromium	1.65	1.27
8. Nickel	2.26	2.11
9. Cobalt	0.63	0.52
10. Vanadium	4.92	5.18
11. Gallium	2.47	2.06
12. Strontium	2.81	2.45
13. Beryllium	0.25	0.18
14. Selenium	0.84	0.62
15. Aluminium	8276	8433
16. Manganese	328	104
17. Calcium	8324	7119
18. Phosphorus	393	127
19. Magnesium	3218	3429
20. Fluorine	49.2	43.8
21. Lithium	3.65	3.02
22. Iron	4187	3864
23. Barium	97.22	88.13
24. Boron	1.38	1.25

Bearing this in mind, we made an attempt to determine the contents of polyphosphates in the soils of endemic Kashin-Beck districts compared with those of non-endemic districts.

Unlike orthophosphates, linear polyphosphates are polymer combinations in which residues of orthophosphates are linked with each other in linear chains. The general formula of linear phosphates is $nM + nP_2O_3 + n_1$, where n is the number of phosphorus atoms in the chain, and M is a polyvalent cation. The simplest representative of polyphosphates is $n-2-$ pyrophosphate.

The role of polyphosphates in the soil cycle manifests itself in the involvement of poorly soluble

salts in the biological turnover of phosphorus as follows (A. Yu. Kudayarova, 1983). Firstly, the biosynthesis of polyphosphates increases in the presence of hard soluble orthophosphorus combinations, and secondly, polyphosphates inhibit the sedimentation of hard soluble phosphoric acid salts. Plants assimilate phosphorus, not only in the form of orthophosphate produced through polyphosphate hydrolysis, but also in the form of the pyrophosphate ion, without hydrolysis (I.M. Chumachenko, 1970).

Table 9. Depth of the deep-frozen layer in the endemic Kashin-Beck district

Territory according to severity of the endemicity	Number of trials	Thickness of the seasonal active layer (m)
Mildly endemic	128	1.33 ± 0.06
Medium endemic	146	0.94 ± 0.07
Highly endemic	63	0.72 ± 0.05

Such important chemical properties of polyphosphates as chelation and complexing account for their ability to form complexes with cations of manganese, zinc, calcium, magnesium, iron, and other polyvalent cations. A comparative study of the action of ammonium ortho- and polyphosphate on combination of calcium, magnesium, manganese, zinc, and iron in soil revealed a strong solubilizing effect of polyphosphates on the above elements (A.Yu. Kudayarova, 1983). Introduction of polyphosphorus fertilizers into soils improves the trace element assimilation of plants (F.V. Yanishevsky, 1978).

Analysis of data concerning the levels of polyphosphates in the soil in endemic Kashin-Beck settlements compared with those in control soil samples from the non-endemic Ulyoty district, together with data in the literature (11-25 mg P_2O_5 per kg soil, A.Yu. Kudayarova, 1983) showed a high concentration of polyphosphates in endemic soils (Table 10). There was a direct correlation between the soil levels of polyphosphates and the severity of the endemicity (Table 11).

Table 10. Concentrations of polyphosphates in soils of settlements in Nerchinsky Zavod, Gazimur Zavod and Ulyotz districts

Settlement	Mean concentration of polyphosphates M ± mg P ₂ O ₅ / 100 gr soil
1. Poperechny Zerentui	24.20 ± 2.13 ^a
2. Patrino	34.4 ± 2.43
3. Bogdat	16.61 ± 1.72
4. Bolshoy Zerentui	16.25 ± 1.95
5. Zeren	3.31 ± 1.95
6. Ivanovka	0.49 ± 0.04
7. Ulyoty (control)	2.36 ± 0.25

a. Mean concentration ± standard error.

Table 11. Difference in values of polyphosphates in the endemic Kashin-Beck soils

Soil samples compared	Actual difference	P < 0.05	Trend in values
Highly endemic Medium endemic	2.75	2.04	+
Medium endemic Mildly endemic	6.44	2.04	+
Mildly endemic Non-endemic	5.64	2.04	+

A thorough study was made of the mineral composition of local food products, such as potatoes, meat, milk, cabbage, and carrots. The analysis was carried out using chemical and spectrographic methods. The level of common phosphorus in food products of plant and animal origin was determined by photometry (Standard 9794-74). Manganese levels in the same products were determined by the periodate method. Acid-soluble phosphorus combinations were determined according to A.M. Ermakov (1972). Analysis of the data obtained on the levels of elements in local food products and grass in the endemic and the non-endemic control districts led to the conclusion that the levels of most elements were practically the same in both districts, but that the levels of phosphorus and manganese

were much higher in the endemic districts. For instance, wheat showed phosphorus levels of 5470 ± 72.7 mg/kg in the endemic districts compared with 3324 ± 79.8 mg/kg in the control, potatoes contained 882.3 ± 6.3 mg/kg in the endemic districts compared with 380.0 ± 6.02 mg/kg in the control. The same tendency was seen in all local products of plant and animal origin.

The levels of manganese in food products and plants in endemic districts significantly exceeded those in control districts. For example, the manganese level in wheat in endemic districts was 72.4 ± 1.0 mg/kg compared with 35.3 ± 1.6 mg/kg in the control district, and levels in potatoes were 6.9 ± 0.09 mg/kg and 1.3 ± 0.1 mg/kg, respectively. The findings agree well with the data on the levels of mobile phosphorus and manganese in the soil and water sources in endemic districts. It is noteworthy that there is a direct correlation between the contents of phosphorus and manganese in the biochemical chain and the severity of the endemicity (Table 12).

Table 12. Contents of phosphates mg/kg and manganese mg/kg in the soil and water of endemic and control districts

Elements	Highly endemic districts 107	Medium endemic districts 84	Mildly endemic districts 201	Mean values Endemic districts 392	Control districts 188
Phosphates in soil	802.50 ± 40.50^b ($P < 0.001$)	699.32 ± 34.90 ($P < 0.001$)	622.5 ± 31.3^a	54.34 ± 2.93	54.34 ± 2.93
Manganese in soil	118.33 ± 7.60 ($P < 0.05$)	108.24 ± 5.61 ($P < 0.005$)	87.32 ± 4.72 ($P < 0.001$)	104.63 ± 5.97 ($P < 0.001$)	13.96 ± 0.75
Phosphates in water	2.01 ± 0.10 ($P < 0.001$)	1.43 ± 0.07 ($P < 0.001$)	1.01 ± 0.05 ($P < 0.001$)	1.48 ± 0.01	0.12 ± 0.02
Manganese in water	0.05 ± 0.06 ($P < 0.02$)	0.50 ± 0.03 ($P < 0.001$)	1.34 ± 0.02 ($P < 0.001$)	0.56 ± 0.04	0.24 ± 0.01

a. Mean value \pm standard error.

b. Compared with controls.

In our study of the levels of trace and macroelements in the food products of the endemic districts, increased levels were only found common for phosphorus and manganese (Table 13). Variations in the contents of other trace and macroelements were statistically insignificant.

Table 13. Contents of macro and trace elements in local food products in endemic and control districts (mg / kg, thermally untreated)

Element	Wheat	Potatoes	Cabbage	Carrots	Pork	Cow's milk
Endemic ^a	70	307	100	307	180	100
Non-endemic ^b	60	267	100	257	200	100
Calcium						
Endemic	490.5 ± 11.6 ^c	107.0 ± 1.7	473.3 ± 6.2	580.0 ± 13.8	109.3 ± 0.6	1047.6 ± 24.1
Non-endemic	510.2 ± 10.6	97.5 ± 1.6	468.1 ± 5.3	490.4 ± 4.3	112.2 ± 0.7	60.7 ± 28.07
Phosphorus						
Endemic	5470.1 ± 72.1	882.3 ± 6.3	504.4 ± 6.9	691.0 ± 3.9	2309.2 ± 10.1	1121.8 ± 16.7
Non-endemic	3324.0 ± 798	380.0 ± 6.1	272.0 ± 12.3	535.0 ± 3.2	1640.1 ± 28.3	870.9 ± 12.1
Sodium						
Endemic	1094.0 ± 26.5	284.0 ± 6.5	132.0 ± 5.2	210.0 ± 8.9	650.0 ± 5.5	500.0 ± 10.8
Non-endemic	1074.2 ± 26.1	280.0 ± 6.1	123.0 ± 4.3	201.0 ± 6.2	638.0 ± 6.5	486.3 ± 16.3
Potassium						
Endemic	3753.0 ± 75.2	5600.0 ± 67.0	1900.3 ± 53.3	1986.5 ± 25.5	3162.7 ± 40.4	1483.1 ± 23.6
Non-endemic	3700.0 ± 70.9	5680.1 ± 68.4	1850.2 ± 60.3	2000.1 ± 23.1	3060.3 ± 37.9	1470.0 ± 24.7
Magnesium						
Endemic	1271.0 ± 28.1	224.0 ± 6.4	170.0 ± 3.9	386.0 ± 7.9	21.0 ± 1.6	124.0 ± 7.1
Non-endemic	1140.0 ± 27.9	280.0 ± 3.8	162.3 ± 9.2	388.7 ± 4.7	220.0 ± 1.1	150.0 ± 6.8
Selenium						
Endemic	0.283 ± 0.019	0.087 ± 0.003	0.062 ± 0.001	0.109 ± 0.006	0.328 ± 0.015	19.3 ± 0.9
Non-endemic	0.241 ± 0.017	0.002 ± 0.004	0.059 ± 0.002	0.098 ± 0.007	0.3346 ± 0.018	21.4 ± 1.6
Sulfur						
Endemic	107.3 ± 3.8	323.5 ± 9.1	360.0 ± 12.6	70.2 ± 2.4	165.3 ± 4.3	36.5 ± 0.8
Non-endemic	105.6 ± 3.0	315.2 ± 8.6	330.0 ± 10.9	70.0 ± 1.9	145.0 ± 3.6	38.0 ± 1.2

Table 13. (contd).

Element	Wheat	Potatoes	Cabbage	Carrots	Pork	Cow's milk
Endemic ^a	70	307	100	307	180	100
Non-endemic ^b	60	267	100	257	200	100
<u>Manganese</u>						
Endemic	72.40 ± 3.53	6.90 ± 0.09	6.40 ± 0.15	4.90 ± 0.17	1.40 ± 0.01	0.11 ± 0.01
Non-endemic	35.30 ± 1.60	1.30 ± 0.10	1.10 ± 0.08	2.20 ± 0.11	0.29 ± 0.03	0.06 ± 0.002
<u>Iron</u>						
Endemic	67.0 ± 1.4	9.0 ± 0.4	6.3 ± 0.5	6.3 ± 0.2	23.6 ± 1.9	0.65 ± 0.04
Non-endemic	69.5 ± 1.8	9.1 ± 0.4	6.8 ± 0.3	6.2 ± 0.1	25.1 ± 1.2	0.76 ± 0.02
<u>Zinc</u>						
Endemic	33.40 ± 0.60	3.70 ± 0.14	3.60 ± 0.15	5.20 ± 0.27	22.60 ± 1.50	4.31 ± 0.26
Non-endemic	31.20 ± 0.40	3.60 ± 0.25	5.40 ± 0.20	4.90 ± 0.12	21.40 ± 0.40	4.07 ± 0.12
<u>Copper</u>						
Endemic	4.90 ± 0.30	0.50 ± 0.004	0.60 ± 0.04	0.50 ± 0.003	1.2 ± 0.07	0.09 ± 0.005
Non-endemic	5.70 ± 0.27	1.60 ± 0.008	0.65 ± 0.05	0.60 ± 0.02	0.68 ± 0.05	0.10 ± 0.005
<u>Cobalt</u>						
Endemic	0.065 ± 0.0018	0.05 ± 0.0002	0.062 ± 0.002	0.02 ± 0.0002	0.07 ± 0.005	0.008 ± 0.0004
Non-endemic	0.60 ± 0.002	0.05 ± 0.0002	0.06 ± 0.0024	0.02 ± 0.0001	0.07 ± 0.0045	0.004 ± 0.0003
<u>Fluorine</u>						
Endemic	0.60 ± 0.003	0.24 ± 0.014	0.06 ± 0.003	0.64 ± 0.002	0.69 ± 0.05	0.14 ± 0.009
Non-endemic	0.50 ± 0.003	0.28 ± 0.013	0.08 ± 0.006	0.60 ± 0.003	0.69 ± 0.05	0.10 ± 0.005

a. No. of samples (endemic).

b. No. of samples (non-endemic).

c. Mean value ± Standard error.

Important data were obtained from the evaluation of the daily food ration according to the severity of the endemicity. A relevant correlation was found only for manganese and phosphorus (Table 14). For instance, the daily food ration in the more seriously affected settlements contained 2984.7 mg phosphorus, and those medium endemic and mildly endemic settlements, 3086 mg and 2780 mg respectively. The contents of manganese in the daily food ration were 11.1 mg in highly endemic settlements, 10.08 mg in medium endemic settlements, and 8.5 mg in mildly endemic settlements. Levels present in the daily ration in the highly, medium and mildly endemic settlements, were: 1364 mg, 1606 mg, and 1187 mg respectively; copper, 2.3 mg, 2.0 mg, and 2.1 mg; and zinc, 18.08 mg, 20.56 mg, and 17.19 mg. It should be noted that importation of food to the endemic regions considerably normalized mineral consumption.

From the levels of trace and macroelements in local food products, it is possible to calculate the daily food ration of people, some 30–40 years ago. It is noteworthy that there was significant consumption of both manganese (20.8 mg) and phosphorus (3988.7 mg). On the basis of the above mentioned data, A.V. Voshchenko, V.N. Ivanov, E.E. Ustinova, L.V. Zaiko, and N.N. Druzhokova (1981, 1983) suggested a phosphate–manganese hypothesis on the etiology of Kashin–Beck disease, its causes lying in excessive consumption of phosphates and manganese salts in the endemic districts.

The Clinical Picture of Kashin–Beck Disease

The disease usually starts in children. At the moment, there is no unanimous opinion as to the time of appearance of the initial symptoms of the disease. This is because the disease starts slowly and patients hardly ever notice the first manifestations (E.V. Beck, 1906; N.I. Damperov, 1939; L.B. Mayun, N.I. Smolenkov, Yu.A. Petrov 1969; E.P. Chetvertakova, 1962, 1965). Moreover, in some cases, there are no subjective or objective symptoms, in spite of the presence of characteristic radiological changes (F.P. Sergievsky, 1934, 1952; N.I. Damperov, 1935, 1939; V.G. Shchipachev, 1931, 1936; L.B. Mayun, N.I. Smolenkov, 1969; V.N. Chugaev, 1971).

F.P. Sergievsky considered that 4 years was the earliest possible age for the onset of the disease (1941, 1943, 1952). However, L.B. Mayun, E.I. Smolenkov, and Yu.A. Petrov (1969) diagnosed Kashin–Beck disease in patients 3–7 years old, and A.M. Popov (1935) reported a 2-year old patient. E.V. Beck (1906) described a 1-year-old patient and several patients only 2–5 years of age. V.G. Shchipachev (1931, 1936, 1950) found the disease in some very young babies. In fact, according to him, some children were born with deformed joints. At embryonal autopsy, he found degenerative changes typical of Kashin–Beck disease in the embryo.

Table 14. Contents (mg) of phosphorous, and manganese in the daily food ration of people in endemic and control districts

Elements	Highly endemic districts	Medium endemic districts	Mildly endemic districts	Mean values	
				Endemic districts	Control districts
Phosphorus	2985 ± 36.0 P < 0.05	3086.4 ± 44.8 P < 0.001	2780.4 ± 31.2 P < 0.001	2950.8 ± 37.3	2191.2 ± 26.8
calcium	1364.5 ± 24.0 P < 0.001	1606.4 ± 25.1 P < 0.001	1187.4 ± 31.3 P < 0.05	1366.1 ± 26.8	6.5 ± 0.2
Manganese	11.1 ± 0.3	10.0 ± 0.4	8.5 ± 0.5	9.9 ± 0.4	6.5 ± 0.2
Calcium: phophorus ratio	1:2.3	1:1.9	1:2.3	1:2.2	1:1.7

The views on the peak age of morbidity are controversial. E.V. Beck suggested that it was from 8 to 16 years, L.O. Dobrovolsky, (1925, 1926), from 9 to 17 years; A.M. Popov (1934, 1935), from 13 to 17 years; N.I. Damperov (1939), from 8 to 19 years; Z.V. Bazilevskaya (1951, 1954), from 7 to 13 years; L.F. Kravchenko (1961, 1965), from 10 to 16 years; and L.B. Mayun and Yu.A. Petrov (1969), from 8 to 14 years. There may be several reasons for such a diversity of views, including different criteria for classification, or the irregular course of the endemicity.

Many authors note that, after 17 years of age, morbidity falls and that after skeleton formation is complete, fresh cases of the disease are only rarely found. The morbidity in persons over 20 years of age is 10% according to E.V. Beck (1906), 4.5% according to M. Aiiso, N. Hayashi (1932, 1937), 14% according to N.I. Damperov (1932, 1938, 1939), and 29% according to N.Z. Mochalin (1934, 1939). F.P. Sergievsky held the view that, in persons over 15 years of age, the disease progressed in those already affected, but there were no new victims.

The first detailed description of the clinical picture of the disease was given by E.V. Beck (1960) who wrote: "The onset of the disease is latent, without general malaise of fever or any changes in the internal organs, without any subjective complaints of pain. Only the very keen and observant patients note early fatigue in some joints when at work. Upon close examination one can see a slight thickening of the joint ends, more often interphalangeal joints of both wrists. Very often the same is noted for secondary interphalangeal joints. This is one of the earliest symptoms". E.V. Beck also referred to limited movement in affected joints, accompanied by crepitation, as being another early symptom.

The same author distinguished 3 stages of the disease according to its severity, i.e. mild, medium, and severe. This classification still holds good.

Later on, this classification was further modified. I.A. Toporkov (1934) and O.V. Nikolayeva (1935, 1936) added the initial, or prodromal, period. A detailed description of the initial period was given by N.I. Damperov (1938, 1939). He noted characteristic neurological and vasomotor symptoms in the initial stage: early fatigue, paraesthesia, muscle convulsions, and aching and temporary crepitation in the joints. The behaviour of children, such as crying, refusal to play, etc, may indicate the initial stage.

In describing the first, or mild stage, E.V. Beck (1960) referred to cases that were characterized by deformation and thickening of the interphalangeal joints and slightly limited movement of both interphalangeal and bigger joints. N.I. Damperov, in discussing the 1st stage, referred only to cases with deformation of the 2nd and 3rd or 4th fingers. In addition to the symptoms described by E.V. Beck, other research workers reported pain in the affected joints, which appeared in the morning or on physical exertion (N.L. Sakovich, 1927, 1928, 1932; V.G. Shchipachev, 1929, 1931; N.I. Damperov, 1939; F.P. Sergievsky, 1949, 1952; P.F. Kotrkhov, 1953; L.F. Kravchenko, 1961). These authors be-

lieved that subjective symptoms of paraesthesia, and constrained movement are characteristic of the 1st stage. In addition, N.I. Damperov (1939), F.P. Sergievsky (1952), and L.F. Kravchenko (1961) listed limited movement in the ulnar, knee, and talocrural joints, with characteristic fine crepitation among the 1st stage symptoms. They noted that local manifestations in the affected joints, such as swelling, erythema, exudate, and local hyperthermia were absent; patients were considered as practically healthy persons (N.I. Damperov, F.P. Sergievsky).

In describing the 2nd stage of the disease, E.V. Beck (1906) added involvement of one or two bigger joints to the symptoms of the 1st stage. N.I. Damperov (1939) demonstrated deformation and thickening of interphalangeal joints of all the fingers of the upper extremities and the ulnar, knee, and talocrural and radiocarpal joints. An important factor in diagnosing the 2nd stage of the disease was limited movement of the wrist and, especially, extension of the fingers (N.I. Damperov, 1939). Brachydactyly, one of the most characteristic symptoms of the 2nd stage, was noted by many authors (M.P. Kireyev, 1927; L.F. Sakovich, 1927, 1928; N.I. Damperov, 1939; L.F. Kravchenko, 1961). The second stage is characterized by the presence of intrajoint bodies that tend to pinch, thus causing joint blockage and sharp unbearable pain (F.P. Sergievsky, 1925; L.F. Kravchenko, 1961). The deformation of the bone joints is also accompanied by muscle atrophy in the lower and upper extremities (V.G. Shchipachev, 1931, 1950; N.I. Damperov, 1939; F.P. Sergievsky, 1962; L.F. Kravchenko, 1961).

The 3rd stage of the disease manifests itself in distinct brachydactyly combined with marked deformation and thickening of interphalangeal joints (E.V. Beck, 1906; N.I. Damperov, 1939). The patient is unable to make a fist, and movement in the bigger joints is limited, even as far as their complete fixation. This leads to the development of a peculiar gait accompanied by the turning of the pelvis — the so called "binding" gait (E.V. Beck, 1906; N.I. Damperov, 1939). The vertebral column is moderately deformed with a marked compensatory lordosis of the lumbar region (E.V. Beck, 1906; A.I. Kasantsev, 1934, 1954; N.I. Damperov, 1939; F.P. Sergievsky, 1952). Partial or complete platipodia (splay foot) is combined with shortening and deformation of the foot sole (F.P. Sergievsky, 1952). Unlike 1st or 2nd stage patients, 3rd stage patients are considered to be disabled (N.I. Damperov, 1939; F.P. Sergievsky, 1952).

The course of the disease was classified by L.F. Kravchenko and E.P. Chetvertakova as fast-progressing or slow-progressing. In the fast-progressing course, the pain syndrome is more pronounced, contracture and deformation of the joints develop faster, and the disease progresses rapidly to the next stage.

L.O. Dobrovolsky (1926), V. Barykin and S. Klyukin (1926), V.G. Shchipachev (1936, 1950), and N.Z. Mochalin all reported a sudden onset of the disease, in some cases. In contrast, E.V. Beck (1906), A.K. Belyavsky (1926), M.I. Schwarzmann (1936), N.I. Damperov (1939), and P.F. Kotrekhov (1953)

rejected the idea of an acute onset of the disease.

All the research workers admit that Kashin-Beck disease affects not only the bone joints but also other organs and systems. P.M. Mikhailov and V.N. Zhinkin (1935), and E.N. Kalinovskaya (1958) believed it is necessary to treat the disease as involving the whole organism, primarily affecting the endocrine system and the autonomic nervous system.

In examining the upper respiratory tract, I.V. Goldfarb V.A. Donskov (1934), and E.N. Manuilov (1935, 1949, 1952) revealed atrophy of the mucus of the nose and pharynx and the involution of the lymphoid tissues of the throat ring.

N.Z. Mochalin (1934) and P.F. Kotrekhov (1953) often diagnosed bronchitis and pulmonary emphysema in Kashin-Beck patients.

S.F. Baranov, M.Z. Levinson (1935), S.N. Lvinson (1957) and G.I. Frolova (1967) noted a more frequency of maxillo dental deformation in Kashin-Beck patients than in healthy people. S.N. Levinson stated that, in Kashin-Beck disease, changes in hard dental tissue resemble those in mineral metabolism impairment, in particular that of calcium metabolism.

Various functional shifts were found in the cardiovascular system. E.V. Beck (1906), L.N. Sakovich (1927), A.V. Belkovsky (1927, 1928), N.Z. Mochalin (1939), N.I. Damperov (1939), F.P. Sergievsky (1952), and L.F. Kravchenko (1961) noted dullness of the heart sounds of different intensity. E.N. Manuilov (1951) explained their origin by myasthenia or myocarditis in severe cases. L.F. Kravchenko attributed cardiac symptoms to myocardiodystrophy.

There are controversial views on the state of the arterial pressure. F.P. Sergievsky (1952) reported hypotension, while N.Z. Mochalin (1939) and V.S. Sidorov (1961) found a tendency to hypertension in Kashin-Beck patients compared with healthy people. It should be noted that the normal values of arterial pressure in healthy people in Zabaikalye have only been determined in recent years (N.A. Nikolayeva, 1970, 1971). This is why the problem still has to be solved.

ECG studies made by L.F. Kravchenko (1959, 1960) showed functional impairment of the conductive system of the heart and diffuse dystrophic changes in the myocardium. P wave voltage was reduced and T wave voltage was 25% negative.

Pathomorphological studies carried out by V.G. Shchipachev (1936) revealed changes in the structure of the liver; considerable growth of connective tissue, accumulation of lymphoid elements around vessels, and lobes of an unequal size. M. Aiiso and N. Hayashi (1936) indicated an increased content of glycogen and an irregular deposit of iron-containing pigment in the liver tissue. I.A. Leontyev (1935) and V.P. Skipetrov (1961, 1962) reported an elevated level of bilirubin in the patient's blood. Significant changes in the blood-protein formula, i.e., a reduced albumin content and an elevated level of globulin were reported by A.S. Krylova (1962), T.M. Isayeva and V.V. Levoshin (1962). All this is evi-

dence of the functional insufficiency of the liver.

Changes were also found in the functioning of the gastrointestinal tract. M. Aiiso and N. Hayashi (1936) found infiltration of the mucus and submucus by lymphocytes and eosinophil cells, and impairment of the evacuator function in Kashin-Beck patients was reported by V.M. Agenkova (1958). A.E. Myaki (1967) reported that hypacidity and impairment of the secretory and excretory functions of the stomach were correlated with the severity of the disease.

The first research workers (N.I. Damperov, 1939, F.P. Sergievsky, 1952) to observe shifts in the peripheral blood reported mild hypochromic anaemia, and reduced haemoglobin and colour indicator accompanied by monocytosis lymphocytosis. Similar changes were described by T.A. Kikinskaya (1954) and the Japanese research workers M. Aiiso and Say (1936).

In a thorough study, V.P. Skipetrov (1959, 1960, 1962) showed that the haemopoietic function of bone marrow was much impaired in Kashin-Beck disease. This was manifested as reduced numbers of oxyphil erythroblasts, and a shift to the right of the bone marrow index of neutrophils etc. These changes became more pronounced as the severity of the disease increased. At the same time, there were changes in the peripheral blood, which returned to normal after administration of the proper treatment (V.P. Skipetrov, 1958, 1959, 1962, 1963).

As the mineral hypothesis of the etiology of Kashin-Beck disease was highly popular, many research workers studied the contents of different trace elements in blood-serum. However, the results obtained were controversial. S.V. Oparin (1939) and E.N. Asmolova (1954) reported reduced contents of calcium and phosphorus in patients with Kashin-Beck disease. On the other hand, V.P. Gratsiansky, N.S. Markelov (1935), V.P. Tyutyunnikov (1958, 1959), T. Takamori (1939), and T.M. Isayeva and V.V. Levoshin (1962), who compared the calcium contents in the blood of Kashin-Beck patients and of healthy people living in the endemic districts did not find deviations from the commonly accepted norms. K. Hyieda (1937) considered an elevated level of iron in the patient's blood to be an etiological factor, but these findings were not confirmed by other research workers.

The introduction of X-ray examination became a reliable objective method of diagnosing Kashin-Beck disease in its initial stages, when the course is either asymptomatic or lacking clinical manifestations (G.I. Turner, 1932; F.T. Smirnov, 1933; F.P. Sergievsky, 1934; M.V. Kopylov, 1935, 1936; D.G. Rokhlin, F.P. Sergievsky, 1938; V.A. Tilhonov, 1958, 1965).

According to the X-ray picture, 3 phases are distinguished (F.P. Sergievsky and D.G. Rikhlin, 1934, 1938). The first metaphyseal stage is characterized by sclerotic concavity, zigzag and wavy contours of the metaphysis, and the formation of pits.

In the second metaphyseal phase, additional changes include fragmentation, immersion of the epiphysis into deeper tissue structures, thinning of the epiphyseal plate, and partial or total necrosis of

the epiphysis.

In the third epiphyseal phase, metaphyseal changes are insignificant while the epiphysis is deformed, with osteolytic processes taking place in it. The joint surface becomes uneven, and sclerotic, with exostoses at its margins.

V.A. Tikhonov (1961, 1962) distinguished 2 stages in the X-ray picture of Kashin-Beck disease, one passing into the other. The 1st stage is characterized by broadening of the roentgenologic joint fissure and formation of pits in the metaphysis. In the 2nd stage, the cartilage zone narrows and the epiphysis becomes fused with the metaphysis.

L.G. Ryumkina and B.N. Yerofyeyev (1969), and V.P. Puzyryov (1969) noted delayed development of the secondary nuclei of ossification.

L.F. Kravchenko, who had been studying the disease over a long period, came to the conclusion that most characteristic manifestations were neurological and vasomotor symptoms rather than those of the bones and joints. A similar view had been expressed earlier by A.A. Savelyev (1933), who pointed out a considerable involvement of the central nervous system (muscle atrophy, early fatigue, and a number of accompanying processes). Many research workers who studied Kashin-Beck disease, attributed the greatest importance to the pathology of the nervous system. For instance, E.V. Beck (1906) observed that the causes of the disease might lie in a peculiar influence of "harmful substances" on the corresponding parts of the brain, with the disease manifesting itself mainly in trophic impairments. The same view was shared by I.A. Toporkov (1934) and N.Z. Mochalin (1934, 1939). They considered that the endemic factor acted primarily on the autonomic nervous system and that its impairment led to the symptomatology of Kashin-Beck disease.

Histological studies carried out by V.G. Shchipachev (1932, 1950) and V.G. Konskov (1935, 1940, 1949) revealed osteo- and chondrodystrophy, based on neurotrophic and vascular impairment. In their turn, P.A. Velyaminov (1924), G.I. Turner (1932), V.P. Gratsiansky (1935), and N.I. Damperov (1939) classified Kashin-Beck disease as trophoneurosis resulting from toxic effects on the nervous system. A similar conclusion was arrived at by A.A. Florensov (1941, 1951, 1958) who found impairment of blood vessels when studying the pathomorphology of joints, cartilages, and synovial membranes in Kashin-Beck disease.

In studying Kashin-Beck disease in animals, K.P. Chepurov (1955) concluded that neurotrophic disorders led to the development of symptomatology of Kashin-Beck disease and were associated with mineral deficiency. A number of authors treated the disease as a syndrome of peripheral impairment of the nervous system. For example L.F. Sakovich (1927, 1928) believed it to be a manifestation of lead polyneuritis, L.O. Dobrovolsky (1925, 1926, 1929) thought it was based on toxic polyneuritis, and V.G. Shchipachev (1931, 1925, 1927, 1928), polyavitaminosis polyneuritis. Yet neurological studies carried

out by N.I. Fyodorov (1933, 1935) and I.A. Toporkov (1934) did not reveal symptoms characteristic of polyneuritis.

Taking into consideration the diversity of symptoms and the involvement of many organs and systems, E.P. Chetvertakova suggested that, under the influence of exogenous and endogenous factors, a number of pathological processes occur in the organism, which modify the functions of the central nervous system. Disturbance of chondrogenesis results from shifts in the cortico-visceral associations.

I.A. Toporkov (1934) associated the disease with the impairment of the trophic functions of the sympathetic cells lying in the lateral cornua of the spinal cord, their malfunction being due to overstimulation of sense receptors situated both inside and outside the joint cavity.

A.A. Galchenko (1958) shared this view, proposing lesions of the segmental autonomic centres and the diencephalon.

At the same time, some authors did not specify which parts of the central nervous system were affected by the possible toxic factor, though they were treating Kashin-Beck disease as resulting from lesions of the central nervous system (N.I. Fyodorov, 1935; A.M. Popov, 1934; N.I. Daperov, 1939; L.F. Kravchenko, 1959, 1962).

To sum-up the essential role of the nervous system in the pathogenesis of Kashin-Beck (Urov) disease is a fact accepted by all the authors.

E.N. Manuilov (1935, 1939) described reduced or absent olfaction in Kashin-Beck disease patients, its prevalence increasing with the severity of the disease. Using an autoscope, he revealed pathology of the middle and internal ears, which was also correlated with the severity of the disease. He associated impaired hearing with the pathology of the sound-receiving apparatus. In contrast, I.V. Goldfarb and V.G. Donskov (1934) attributed hearing impairment to lesions of the sound-conducting apparatus. In Kashin-Beck patients, they often diagnosed catarrhal otitis, atrophic processes of the mucosa of the upper respiratory tract (15% in the 1st stage, 60% in the 2nd stage, 90% in the 3rd stage). Impairment of hearing was registered by F.P. Sergievsky (1952) and L.F. Kravchenko (1961). For instance, F.P. Sergievsky associated reduced hearing with presbycusis, while L.F. Kravchenko (1961) described it as a characteristic symptom of the 3rd stage of the disease.

Pathological changes were revealed in the functioning of the visual nerve. Having examined 2000 patients living in the endemic district, S.I. Volkhonsky (1934) noted frequent impairment of the vascular and corneal membranes in patients with Kashin-Beck disease. Pathology of the blind spot was revealed by N.M. Savushkina and A. Smelovsky, (1960), and impairment of darkness adaptation, by T.G. Uglova and S.K. Shkolnikova (1970).

Studies by S.V. Babenkova, E.A. Zhirmunskaya, and M.E. Syroechkovskaya, M.B. Tsuker, Yu.S. Yusevich (1955) revealed considerable malfunctioning of the cranio-cerebral nerves including:

anisocoria, nystagmoid, irregular pupils, limited morbidity of the eyeballs, and asymmetry of the nasolabial folds. M.P. Mikhailov and V.N. Zhinkin (1935) had also noted anisocoria, impaired accommodation, heterotropia, and reduced pharyngeal reflex. The observation was confirmed by K.K. Sergeyev (1958).

As can be seen from the above data, Kashin-Beck disease is manifested through different symptoms indicating impairment of the cerebro-cranial nerves, some of which are considered pathognomonic of the disease.

As far as the locomotory system is concerned, the most common symptoms are reduction of muscle force, increased muscle fatigue, and muscle atrophy (E.V. Beck, 1906; L.O. Dobrovolsky, 1925; N.L. Sakovich, 1927; V.G. Shchipachev, 1929, 1931, A.A. Savelyev, 1933; A.M. Popov, 1934; I.A. Toporkov, 1934; N.I. Fyodorov, 1935; F.P. Sergievsky, 1937, 1952; N.I. Damperov, 1939; P.F. Kortrekhov, 1953; L.F. Kravchenko, 1961; E.P. Chetvertakova, 1967). Two assumptions were made concerning the cause of muscle atrophy. Their arthrogenous nature was indicated by M.I. Schwarzman (1935, 1936), I.A. Toporkov (1934), Z.V. Basilevskaya G.M. Pogodayev (1954), and S.V. Babenkova (1955). B. Zagafsky (1957), and Dang Dyg San (1971) believed the muscle atrophy to be of neurogenic origin. L.O. Dobrovolsky (1925) proposed a convincing argument in favour of his view, that the severity of muscle atrophy was not necessarily correlated with the degree of joint lesion. F.P. Sergievsky (1933) and E.P. Chetvertakova (1967) came to the same conclusion.

G.I. Turner (1932), E.V. Beck (1906), A.E. Norenberg-Charkviani (1925), A.M. Popov (1934), and I.A. Toporkov (1934) found overactive tendon reflex and anisoreflexia in Kashin-Beck patients. However, according to other authors, several patients showed reduced tendon reflex (N.I. Fyodorov, 1933; A.A. Podshivadov, 1945.)

Most authors reported muscular hypotension (N.I. Fyodorov, 1935; F.P. Serfievsky, 1937; D.Z. Komissaruk, 1957), but A.M. Popov, (1934); observed muscular hypertension in some children. E.V. Beck (1906) and N.I. Fyodorov (1935) noted muscle hyperexcitability in Kashin-Beck patients, while A.V. Belkovsky (1928), when studying the working capacity of a 4-kg loaded muscle, reported a 40% reduction. Local muscle spasms were seen by L.O. Dobrovolsky (1925, 1926), V.G. Shchipachev (1929, 1950), and N.I. Fyodorov (1933).

Lesions of the sensory system are manifested in parasthesia in the form of prickling, creeping, and numbness (L.O. Dobrovolsky, 1935; V.G. Shchipachev, 1931; I.A. Toporkov, 1934; N.I. Fyodorov, 1935; E.A. Zhirmunskaya, M.E. Syroechkovskaya, M.B. Tsuken, and Yu.S. Yusevich, 1955). A.M. Popov (1934), I.A. Toporkov (1936), and N.I. Fyodorov (1935) reported reduced vibration sensitivity resulting from change in bone structure.

Abnormal superficial sensitivity, either hyper- or hyposensitivity, was noted by N.I. Fyodorov

(1935), I.A. Toporkov (1934), and A.M. Popov (1934). S.V. Babenkova and colleagues (1955) observed "bracelet-like" sensitivity around affected joints, while E.P. Chetvertakova (1967) observed localized spots of impaired sensitivity in Kashin-Beck patients. In general, the authors were unanimous in reporting a peripheral type of impaired sensitivity, it being either hypoaesthesia or hyperaesthesia.

To sum up, the most characteristic symptoms concerning impairment of the locomotory and sensory systems are paraesthesia, reduction of muscle force, muscle atrophy, and reduced vibration sensitivity. In spite of the muscular hypotension (which has been reported by most research workers) the majority of patients suffer from overactive tendon reflexes. Apparently, in view of slight insufficiency of the pyramid, there are lesions in the cerebellum. It should be noted that, though such symptoms as abnormal gait, clumsy movements, and instability in Romberg posture were listed by many authors (E.V. Beck, 1906; N.I. Damperov, 1938), they were explained by deforming arthrosis.

Autonomic malfunction has been described by many research workers. This includes excessive sweating of the palms and feet, acrocyanosis, coldness of the distal parts of the extremities, coarse skin, and altered dermatography (I.A. Toporkov, 1934; L.I. Yudovich, 1934; N.I. Fyodorov, 1935; N.I. Damperov, 1939; C.V. Babenkova, 1955; L.F. Kravchenko, 1959, 1962; V.M. Shushpanovskiy, 1959; E.P. Chetvertakova, 1962). The clinical picture was later supplemented through the use of special methods of examination (capillaroscopy, response to pilocarpine and adrenalin, Ushner reflex, determination of the thermoregulator reflex). Unfortunately, the data obtained were controversial in quality. For instance, capillaroscopy (L.F. Kravchenko, 1961) showed angiospasm and angiodystonia (E.P. Kravchenko, 1961, 1971). In 1934, I.A. Toporkov reported enhanced response to adrenalin and diminished response to pilocarpine, while, in 1936, the results were quite the opposite. In many cases, symptoms were interpreted differently. N.Y. Fyodorov (1935) was in favour of sympathicotonia, while A.M. Popov (1934), K.P. Chepurov (1955) and V.M. Shushpanovskiy (1959, 1961) were in favour of vagotonia. Moreover, sometimes the authors adopted opposing views at different times. All this is proof that the problem needs further investigation.

Different lesions of the higher cortical functions have always been reported by Kashin-Beck research workers. N.I. Kashin (1861) found significant psychic abnormalities. By falsely associating the Urov disease with thyroid pathology, he considered all patients with psychic problems to be cretins. In some patients, he described "absence of any intellectual faculties". For instance, in a family of 3 brothers and 3 sisters, he registered vacant stare, limited vocabulary, absence of purposeful actions. Although he numbered only 10 such families, patients with more or less marked symptoms of abnormal psychic symptoms were rather common.

V. Barykin and S. Klyuhin (1926) expressed the view that Kashin-Beck disease resulted in weakened memory and limited intellect.

Mental backwardness as an accompanying symptom in Kashin-Beck disease was indicated by A.V. Belkovsky (1927, 1928) who registered delayed and flaccid movements, reluctant speech, interrupted by long pauses and accompanied by a dull sidelong glance.

N.I. Fyodorov, who shared the view of an early onset of the disease, observed delayed physical and mental development in children with Kashin-Beck disease. The children were late to start walking and speaking, and voluntary movements were also delayed. Children over 3 years of age had pathological foot sign, enuresis, and sometimes, spasmophilic diathesis.

P.F. Kotrekhov (1958) treated psychic flaccidity as a symptom of Kashin-Beck disease. He observed derangement of memory in 8 out of 645 patients examined.

E.P. Chetvertakova (1962) came to a similar conclusion registering emotional instability, slow adaptation to new surroundings, and absent-mindedness in Kashin-Beck children. In 62% of patients, she noted weakened memory and 42% complained of headaches.

N. Ozeretsky, O. Plotnikova and S. Khotina (1935), and T.P. Svistunova (1984) registered mental backwardness, limited vocabulary, psychic flaccidity, and low intellect in children with the Urov disease.

Interesting data were presented in studies by K.P. Chepurov (1955) and A.V. Cherkasova (1956). After thorough examination of animals suffering from Kashin-Beck disease, they concluded that the nervous system was the first to be affected by the pathological process. In the acute stage of the disease, newly born calves exhibited trembling, convulsions, and tic. General fits occurred but only seldom. Adult animals with Kashin-Beck disease were sleepy, and had flaccid movements and reduced reactions to stimulation. Tendon and surface reflexes were reduced. Colts were born underdeveloped, and were unable to suck properly. Adult animals demonstrated atrophied muscle and excessive sweating.

Involvement of the nervous system in the pathological process was clearly indicated in the morphological studies of V.G. Chchipachev (1927, 1928, 1931). Histological examination revealed perivascular space dilatation, moderate oedema of the brain, and cell infiltration of the grey substance of the brain and spinal cord. On autopsy of animals with Kashin-Beck disease, K.P. Chepurov (1955) found nerve degeneration (change in form, lighter cytoplasm, nuclear shift), and lymphocytic infiltration with brain congestion.

In studying the contents of some bioelements, we found significant elevation of inorganic phosphorus levels in the serum and manganese levels in the whole blood of patients ill with endemic osteoarthritis (Tables 15, 16, 17). The calcium contents of the blood serum were within the norm, though much lower than those in the controls. Thus, it could be regarded as reduced in patients. Excretion of inorganic phosphorus and manganese in the urine was elevated. As far as calcium is concerned, its excretion in urine was lower in patients than in healthy people in Chita, but somewhat higher than

that of healthy people in the endemic region. The latter might be accounted for by the action of a compensatory mechanism regulating calcium homeostasis. Elevated contents of manganese in the blood and urine of these people might be attributed to its excessive consumption with food. The severity of Kashin-Beck disease was shown to be inversely proportional to the levels of calcium in the blood and urine; levels were elevated in blood serum and reduced in urine.

Table 15. Contents of some elements in the blood of Kashin-Beck patients and in healthy people of Chita and the endemic district

Element	Blood levels in ^a		Blood levels in		Blood levels in	
	healthy people in Chita (mg%)	P	Kashin-Beck patients (93) (mg%)	P	healthy people in the endemic district (36) (mg%)	
Calcium	11.05 ± 0.23	<0.01	10.36 ± 0.08	<0.01	11.47 ± 0.15	
Inorganic phosphorus	3.80 ± 0.14	<0.001	6.34 ± 0.06	<0.001	3.75 ± 0.09	
Manganese	0.018 ± 0.0015	<0.001	0.053 ± 0.0014	<0.001	0.034 ± 0.002	

a. Mean ± standard error.

Table 16. Contents of some elements in the blood of patients ill with endemic osteoarthritis of different degrees of severity

Element	Blood levels in ^a		Blood levels in		Blood levels in	
	first stage patients (32) (mg%)	P	second stage patients (37) (mg%)	P	third stage patients (24) (mg%)	
Calcium	9.42 ± 0.11	<0.02	10.38 ± 0.09	<0.05	11.26 ± 0.14	
Inorganic phosphorus	5.42 ± 0.08	<0.001	6.82 ± 0.06	<0.10	6.76 ± 0.10	
Manganese	0.043 ± 0.0016	<0.01	0.051 ± 0.0022	<0.01	0.064 ± 0.0032	

a. Mean ± standard error.

Table 17. Contents of some elements in the urine of patients with different stages of Kashin-Beck disease

Element	Urine concentration ^a		Urine concentration		Urine concentration
	in first stage patients (32) (mg%)	P	in second stage patients (37) (mg%)	P	in third stage patients (24) (mg%)
Calcium (mg%)	10.3 ± 0.21	<0.001	8.86 ± 0.13	<0.05	8.39 ± 0.18
Inorganic phosphorus (g/day)	1.22 ± 0.07	<0.14	1.14 ± 0.05	<0.01	1.37 ± 0.06
Manganese (mg/day)	1.92 ± 0.17	<0.001	3.77 ± 0.16	<0.05	4.38 ± 0.21

a. Mean concentration ± standard error.

Levels of inorganic phosphorus in the blood and urine of patients at all stages of the disease were above the norm, but there was no strict correlation with the severity of the pathological process. The level of inorganic phosphorus in the blood correlated with the level of excretion. For instance, in the 2nd stage of Kashin-Beck disease, a higher level of inorganic phosphorus in the blood might be accounted for by its reduced excretion. The level of inorganic phosphorus in the blood in the 3rd stage of the disease did not differ from that in the 2nd stage, though its excretion with urine was much greater. This may be due to the compensatory mechanism contributing to a somewhat reduced level of inorganic phosphorus in the blood. The levels of manganese in the blood and urine were directly proportional to the severity of the disease.

On the basis of the biogeochemical hypothesis concerning the etiology of endemic osteoarthritis, it was possible to determine excessive contents of phosphates and manganese salts in biological media in the endemic environment (A.V. Voshchenko, V.N. Ivanov, N.E. Shiber, V.N. Chugayev, E.E. Ustinova and colleagues, 1983). Accumulation of these elements resulted from specific characteristics of the locality (presence of deep-frozen layers of soil, bogging-up) and the high contents of the elements in mountain rocks. The daily ration of people in the endemic district was found to contain 2-3 times more phosphates and manganese than normal. An excessive level of manganese was found in the whole blood of 8 patients. Levels in the blood of healthy people living in the endemic district were also somewhat higher. Excretion of manganese in urine was high in both cases.

Elevated contents of inorganic phosphorus were found in the blood serum and urine of patients with endemic osteoarthritis, but not in healthy people in the endemic district. This fact might be due to a greater

biological control over the absorption and excretion of phosphorus compared with manganese.

A.P. Venchikov and colleagues believed (1983) that trace elements had toxic effects only when significantly large doses passed through the physiological barriers of the body. Since the sensitivity of the regulatory systems in different individuals to the chemical factors in the environment differ, simultaneous malfunctioning never occurs in all the individuals in an endemic area (V.V. Kovalsky, 1974). This might also explain the different manifestations and stages of the disease.

It is noteworthy that an elevated level of inorganic phosphorus in the blood serum of Kashin-Beck patients has been reported more than once (S.I. Banaitis, 1935; S.V. Oparin, 1939; E.N. Asmolova, 1954; M.P. Soshnyanina and colleagues, 1981). Unfortunately, the results obtained were interpreted differently and never as resulting from excessive consumption of phosphorus by the patient. From the literature survey, it is clear that an excessive consumption of phosphates always has a toxic effect on the body. A number of studies carried out by a group of research workers from the Institute of Nutrition showed that rats fed a phosphate-rich diet developed hyperphosphaturia, hyperphosphataemia, hypocalcaemia, and osteoporosis. The toxic effects were found to be more pronounced at an early age.

Changes in the blood and urine of patients with endemic osteoarthritis, which were observed in our studies, were similar to those found by N.V. Blazhevich and colleagues (1974). According to them, hyperparathyroidism, which resulted from excessive consumption of phosphates, had its own specific features, i.e., reduced calcium in the blood and urine and elevated phosphorus levels in the blood. Similar features were typical for Kashin-Beck disease. The parathormone acting on the bone enhances the metabolic processes in osteoclasts, thus increasing their number, which leads to osteoporosis (B.S. Kasavina, V.P. Torbenko, 1975). This is why an elevated content of acid phosphatase in the blood of Kashin-Beck patients found by V.N. Ivanov and colleagues (1983), is evidence of a greater activity of osteoclasts, which might be due to the above effect.

Foreign literature also contains references to osteoporosis developing in people following excessive consumption of phosphates (G. Ellestad-Sayed et al. 1975; H.H. Draper and C.A. Seythes, 1981).

When studying the correlation between the levels of calcium and phosphorus in the blood and urine of patients and the severity of Kashin Beck disease, we found a significant increase in calcium levels in the blood serum and a reduction in calcium levels in the urine, according to the stage of severity of the disease. Inorganic phosphorus levels in the blood serum and urine were elevated.

Assumption that the severity of the disease is correlated with a greater consumption of phosphorus and manganese leads to the conclusion that a considerable dose of phosphates will tend to reduce the calcium ions in the blood. This in turn, will stimulate parathormone secretion with a pronounced effect on the bone. Accumulation of calcium in the blood will manifest itself as marked osteoporosis, which is known to characterize severe forms of Kashin-Beck disease. Hypodynamia in patients with extensive degenerative-destructive

tive processes in the joints is also assumed to contribute to the development of osteoporosis (E.A. Kovalenko, N.N. Gurovsky, 1980). Furthermore, prolonged calcium deficiency in the body has been shown to result in the impaired metabolism of glycosaminoglycans and glycoproteins with their accumulation in the blood and excretion in the urine (R.V. Merkur'yeva, Yu.A. Rakhmanina, Z.I. Koganova, N.A. Koganova, N.A. Nazarova, 1978).

A similar phenomenon in Kashin-Beck patients was reported by L.P. Nikitina, M.P. Soshnyanina, V.N. Ivanov and colleagues (1981), and I.V. Rosin (1983).

Studies by G.I. Beloskurskaya, Yu.V. Krylov, and D.D. Djanabayev (1979), G.I. Beloskurskaya, M.T. Berdykhodjin, and B.N. Aitbembetov and colleagues (1983) on the clinical manifestations of chronic occupational intoxication with phosphates revealed an astheno-neurotic syndrome and, in some cases, encephalopathy. The findings indicate dystrophic processes in the myocardium, neurocirculatory dystonia of a hypertensive type, with obligatory hypocyde or normocyde gastritis and liver pathology. The last finding manifests itself as chronic hepatitis, which is thought to follow a benign course. The blood of patients suffering from chronic occupational phosphate intoxication contains high levels of inorganic phosphorus. Bone-joint pathology in the form of bone pain and limited movement in the joint is noted. X-rays show exostoses.

The clinical picture of chronic phosphate intoxication appears to include a number of manifestations characteristic of Kashin-Beck disease.

The involvement of manganese in the pathogenesis of Kashin-Beck disease was suggested by V.V. Kovalsky in 1974. Our study has shown a distinct correlation between the level of manganese in the blood and the urine of patients and the severity of the disease. Since the contents of manganese in the blood tend to be constant (A.O. Voinar, 1953, P.R. Nozdryukhina, 1977), its abnormal elevation in the blood of patients with endemic osteoarthritis should be given a proper explanation.

I.K. Musabayev and T.I. Metskan (1976) reported a significant elevation of manganese in the blood at the peak of viral hepatitis; the values returned to normal when the process died down. Excretion of manganese with the urine was increased when the hepatitis was at its peak. This was due to impairment of the excretory function of the liver and necrotic processes in its parenchyma. Unpronounced pathology of the hepato-biliary system in Kashin-Beck disease and enhanced excretion of manganese with urine would very soon exhaust the body stores of this element, in the case of its normal consumption. The theory that manganese levels are elevated because of its re-distribution, as seen in chronic dysentery (M.N. Nazarmukhamedova, cited by G.A. Babenko, L.P. Reshetkina, 1971) or typhoid (A.I. Kortev, A.P. Lyasheva, and G.I. Dontsova, 1969, 1972), is without foundation. Evidently, the manganese increase in the blood and urine of patients with endemic osteoarthritis is the result of its constant consumption with food (L.V. Zaiko and colleagues, 1981). Occupational elevation of manganese in the blood and urine of workers

was observed by Z. Ya. Shcherbitskaya (1962).

Experimental studies on animals fed with a diet rich in manganese salts showed elevation of the manganese content in the blood and bone tissues (A.I. Voinar, 1960; L.I. Kulikova, 1961; E. Tal Guggenheim, 1965). V.S. Butko (1972) also found 1.6 times more manganese in the bones of patients with endemic osteoarthritis, believed to be due to its excessive consumption with food.

Many authors (H. Fore, K.A. Morton, 1952; L.S. Hertey and G.W. Asling, 1963) noted a close relationship between manganese and mineral metabolism, stating its prevalence over other elements in osteogenesis. Thus, there is every reason to believe that an excessive content of manganese in bone tissue exerts a certain effect on osteogenesis.

Excessive consumption of this element led to impaired calcification, osteoporosis, and delayed bone growth (E. Tal, K. Guggenheim, 1965). Similar changes are observed in patients with Kashin-Beck disease. L.I. Kulikova (1963) revealed that experimental animals had the highest possible accumulation of manganese in the central nervous system (7.7 times), a somewhat lower content in the liver (4.6 times) and 2-3 times higher content in bone tissue, hence, the resulting impairment of these organs.

Our observation of primary dystrophic changes in these organs in Kashin-Beck disease may be the result of an excessive accumulation of manganese.

Excessive manganese is known to inhibit a number of enzymes participating in amino acid synthesis. This phenomenon has been observed in manganese intoxication in people (B. Boyadjiev, I. Denyev, L. Khalacheva, 1974) and in experimental rats (G.S. Shunkla, S.V. Shandra, 1982). It is natural to postulate the inhibitory action of manganese on the synthesis of enzymes in bone tissue. It may be demonstrated by data on a certain reduction in alkaline phosphatase activity in the blood of patients ill with endemic osteoarthritis (M.F. Kan and colleagues, 1981; V.N. Ivanov and colleagues, 1983). The reduction of chondroitin sulfate excretion, reported by I.V. Rosin (1983), may also be indicative of a reduction in its synthesis. According to B. Boyadjiev, I. Denyev and L. Khalacheva (1974), excessive manganese exerts the greatest effects on the synthesis of such amino acids as proline, glutamic acid, and methionine. Therefore, there are grounds to treat synthesis impairment as one of the mechanisms of bone-cartilage tissue degeneration in Kashin-Beck disease. This is proved by the collagen synthesis reduction found by I.V. Rosin (1983). According to V.V. Kovalsky (1974), manganese in biotic doses activates enzymes of the tricarboxylic acid cycle. Evidently, inhibition of these enzymes, in the case of excessive consumption of manganese, leads to the accumulation of pyruvic acid through depression of its oxidation. This phenomenon was revealed in patients with Kashin-Beck disease by L.P. Nikitina and colleagues (1981). The results of studies by V.V. Kovalsky and A.V. Dubinskaya (1970, 1971) show that the highest concentration of manganese in bone tissue is present in the bone growth zone during the period of intense growth. In animals consuming excessive doses of manganese, its accumulation in this zone proves to be extremely high. Evidently, this factor affects the grow-

ing child, so the first pathological manifestations are registered in the bone-growth zone.

The clinical picture of Kashin-Beck disease reveals symptoms similar to those of industrial manganotoxicosis. For instance, the astheno-neurotic syndrome discovered in our studies may be due to the toxic action of manganese. The extrapyramidal features (tremor, muscular hypertension) in Kashin-Beck disease are also characteristic of manganotoxicosis (G.C. Cotrial, 1958; V.A. Mikhailov, 1971; M.N. Ryzhkova, L.A. Tarasova, 1975). Cortical malfunction, registered by A.V. Voshchenko (1974) and T.P. Svistunova (1983), in patients with endemic osteoarthritis is also common in manganotoxicosis (A.A. Platonov, 1975).

Leukopenia and hypochromic anaemia characterizing manganese intoxication (G.C. Cotrias, 1958), which was described by N.I. Damperov (1939) and L.F. Kravchenko (1973), also occur in Kashin-Beck disease.

Although the pathogenesis of Kashin-Beck disease is too complicated to be limited to the action of manganese and phosphates only, the involvement of these elements in the pathology of endemic osteoarthritis is evident. It is proved by their direct and indirect action on osteogenesis, the central nervous system, and the internal organs, with resulting dystrophic processes characteristic of Kashin-Beck disease.

Taking this phenomenon into account, we have suggested a new diagnostic test for Kashin-Beck disease, based on the determination of possibly excessive contents of manganese and phosphorus in the blood and urine of patients. Suggested prophylactic measures should be estimated on the basis of the patients and on the clinical course of the disease.

We have suggested a number of curative measures including:

- 1) normalization of impaired metabolism;
- 2) treatment of arthrotic joints;
- 3) normalization of the functioning of the internal organs and the central nervous system.

Improvement of the metabolism and excretion of manganese and phosphorus is achieved through administration of the following drugs: cholagogues, spasmolytics, calcium and hydrochloric preparation, B-vitamins and ascorbic acid (E.E. Ustinova, 1984). Administration of calcium preparations is aimed at: a) normalization of the calcium : phosphate ratio in food; b) formation of insoluble combinations of calcium and phosphorus which may contribute to their transit passage along the digestive tract with the least absorption. Calcium phosphates formed in large amounts pick up and carry away manganese, thus also facilitating the excretion of manganese from the body. Administration of hydrochloric and ascorbic acid preparations improves absorption of calcium.

An elevated content of calcium in the blood presents the further bic acid preparations improves absorption of calcium.

An elevated content of calcium in the blood presents the further development of osteoporosis at the ex-

pense of decreased parathormone synthesis. Furthermore, it has a cholagogic effect. Enhanced bile secretion is achieved by the administration of spasmolytics and cholagogic preparations. Excessive manganese and phosphates are excreted mainly in this way.

The treatment described above administered to a group of patients with Kashin-Beck disease (36 cases) resulted in an improvement in their general state on the 11th day of treatment; the concentration of calcium in the blood had increased. Over a period of 6 months, such a treatment was carried out twice, each treatment lasting 1.5 months. It produced a considerable improvement, including diminished pain, greater mobility of joints, and greater clenching of the fists.

Treatment of arthrotic joints includes electrotherapy (electrophoresis, ultrasound), mud and paraffin applications, massage and exercise therapy. A favourable effect is achieved by the administration of novocain-hydrocortisone periarticular blockade (40-80 mg hydrocortisone and 20 ml 0.5% novocain solution) in the affected joint in a course of 3-5 injections at intervals of 2 days. In some cases, bruphen and rumalon in accepted doses are indicated.

The astheno-neurotic syndrome is relieved by neuroleptic and tonic preparations and polyvitamins.

On the basis of the phosphate-manganese theory on the etiology of Kashin-Beck disease, recommendations have been worked out to check excessive consumption of phosphates and manganese salts.

For instance, the boiling of cow's milk and water in a special pan reduces the phosphate content of the milk 4 times (from 1100 mg / litre to 340 mg / litre), and of the water, 10-12 times (from 1-2 mg / litre to 0.1-0.05 mg / litre). Washing, soaking, or thermally treating potatoes, cabbage, and other vegetables reduces the phosphate and manganese contents 3-4 times (B.E. Ustinova, L.V. Zaiko, A.V. Voshchenko, 1982). The same effect is achieved by pretreatment of meat products. When the daily food ration was calculated after the above pretreatment, the phosphorus content was reduced from 2673 mg to 1420 mg, and the manganese, from 17.4 mg to 6.8 mg, which fulfills the daily requirements of man for these elements.

Much improvement is attached to endemic prognosis for newly inhabited territories, which includes the following:

- 1) Phosphorus contents of rocks and soil exceeding 0.14% and 0.18%, respectively;
- 2) Manganese contents of rocks and soil exceeding 0.12 and 0.15%, respectively;
- 3) Phosphate-ion content of drinking-water exceeding 1 mg / litre and manganese ion content exceeding 0.15 mg / litre;
- 4) The presence of deep-frozen layers of soil;
- 5) Diagnosis of Kashin-Beck disease in people who have lived in the region for a long time.

REFERENCES

- Aliiev, S.G., Kashin-Beck disease in a lifelong resident of Baku, *Vestn. rentgenologii i radiologii*, 1968, No.5, pp. 93-94
- Aiiso, M., Kashin-Beck disease in To-Hen-Do of Manchoukuo, *J. Oriental Medicine, Dairen*, 1936, Vol. 24, No. 3, pp. 34-34
- Asmol'kova, E.N., Calcium, Phosphorus and potassium in the whole blood of patients suffering from Urov disease, In: *Vopr. travmatologii i ortopedii (Irkutsk, 1954)*, part 2, pp. 71-75
- Beck, E.V., Dissertation for the degree of Doctor of Medicine (Saint Petersburg, 1908), pp.114
- Belakovskij, M.S., Blazevic, N.V., Spiricev, V.B., Sergeev, I.N., Spicyna, N.E., The effect of excessive ingestion of phosphorus on some aspects of phosphorus and calcium metabolism and the condition of the bone tissue of rats in hypokinesia, *Kosmiceskaja biologija i aviakosmiceckaja medicina*, 1982, No. 5, pp. 70-73
- Belkovskij, A., New findings on Beck's disease, in: *Mediko-sanitarnyi sbornik (Sretensk, 1928)*, pp. 3-4
- Belokurskaja, G.I., Berdyhodkin, M.T., Ajtsembetov, B.N., Dzanabaev, D.D., Paraskevopulos Ja. G., Clinical picture of chronic, occupational phosphorus poisoning, *Gigiena truda i prof. zabol'evanij*, 1983, No. 3, pp. 19-23
- Belokurskaja, G.I., Krylov, Yu.V., Dzanabaev, D.D., Clinical picture of gastrointestinal complaints in chronic phosphorus poisoning, *Zdravoohranenie Kazahstana*, 1979, No. 9, pp. 48-50
- Blazevic, N.V., Spiricev, V.B., Pereverzeva, O.G., Tenditnaja, L.V., Fancenko, N.D., Calcium and phosphorus metabolism and provision of vitamin D under living conditions in the far north, *Vopr. pitanija*, 1983, No. 1, pp. 17-22
- Blazevic, N.V., Spiricev, V.B., Pozdnjakov, A.L., Dynamics and mechanism of disruption of mineral metabolism in dietary excess of phosphorus; *Vopr. pitanija*, 1978, No. 3, pp. 19-26
- Blazevic, N.V., Spiricev, V.B., Pozdnjakov, A.L., Isaeva, V.A., Gazdarov, A.K., Development of carcinosis of the kidneys and aorta in rats with various dietary proportions of calcium and phosphorus, *Vopr. pitanija*, 1974, No. 5, pp. 28-33
- Bogasev, I.A., The spread of endemic goitre and osteoarthritis in Zabajkal'e; *Tr. fiziko-matematicheskogo obščetva Moskovskogo universiteta za 1910 god Moskva*, 1911, pp. 47-52
- Boldyreva, I.V., Vysepan, E.D., Byckov, S.M., Phosphorus and calcium metabolism in individuals suffering from Kashin-Beck disease; *Arh. biol. nauk*, 1940, Vol. 60, Part 2, No. 11, pp. 11-15
- Butko, V.S., Changes in the proportion of calcium and strontium in the femurs of healthy individuals and of patients suffering from Kashin-Beck disease; in *Urovskaja bolezn' v Zabajkale (Irkutsk, 1974)*, part 1, pp. 82-98
- Cerkasova, A.V., Urov disease in animals; In: *Endemiceskie bolezn'i zivotnyh (Moskva, 1968)*, pp. 146-173
- Cetvertakova, E.P., The antitoxic function of the liver of children suffering from Urov disease; In: *SB. Nauc.*

po pediatrii (Cita, 1968), Part 1, pp. 75-76

Cikert, M., Pokus o ovlivneni vylucovani manganu stoloci podnim cholestyraminu; *przgl. Lek.*, 1981, V. 3: 351-355

Cotzias, C.G., Manganese in health and disease; *Physiol. Rev.*, 1958, 38: 503-532

Cotzias, C.G., Greenough, J.J., The high specificity of the manganese pathway through the body, *J. Clin. Invest.*, 1958, 37: 1298-1305

Cotzias, C.G., Miller, S.T., Edwards, J. Neutron activation analysis: the stability of manganese concentration in human blood and serum, *Clin. Med.*, 1966, 67: 838-849

Damperov, N.I., Kashin-Beck disease in foreign literature; *Klinic. medicina*, 1941, No 3, pp. 131-133

Damperov, N.I., Map of the distribution of Urov Disease in eastern Zabajkal'e, in *Urovska ja bolezn' v Zabajkale'e* (Irkutsk, 1934), part 1, pp. 15-24

Damperov, N.I., Kashin-Beck disease in foreign literature; *Klinic. medicina*, 1941, No. 3, pp. 138

Dobrovol'skij, L.I., Urov Disease (Cita, izdanie Zabajkal'skgo gubotdela zdravoohraneni ja, 1925), pp. 11

Donskov, V.A., The Histology of Urov Disease; *Medicinskij bjulleten*, Irkutsk, 1948, No 8, pp 38-45

Florensov, A.A., The morphology and physiology of articular cartilage; In: *Tez. dokl. naucnoj konferencii morfologv* (Irkutsk, 1961), pp. 326-326

Geller, G.M., Fomina L.S., Slygin, G.K., On the cases of Urov Disease; *Vopr. pitani ja*, 1954, No.4, pp. 47-52

Geller, G.M., Fomina L.S., Slygin, G.K., Description of the diet of the population of the Urov disease endemic area; *Vopr. pitani ja*, 1952, No. 5, pp. 59-266

Georgievskij, A.P., The chemical composition of water in the focus of Urov disease; *Gigiena i sanitari ja*, 1952, No. 9, pp. 27-29

Gersberg, A.L., Notes on Urov Disease; *Sov. vracebny j zurnal*, 1937, No. 3, pp. 185-190

Goldfarb, I.V., Donskov, V.A., Clinical pathological and histological studies of the upper respiratory tract in patients suffering from Urov disease; in *Urovska ja bolezn' v Zabajkal'e* (Irkutsk, 1974), part 1, pp. 140-163

Gordienko, V.P., Urov Disease in the endemic region of Amur Oblast; abstract from dissertation for the degree of Candidate of Medical Sciences (Irkutsk, 1973), pp. 15

Hiyeda, K., Aliiso, M., Hayashi, N., Iron as the causative agent of Kashin-Beck disease; *Journal of Oriental Medicine*, 1937, Vol. 36, p. 6

Isanov, V.N., Nikitina, L.P., Sosnjanina, M.P., Spirina, A.P., Stupina, O.P., The probable mechanisms of degeneration of cartilaginous substance in patients suffering from Urov disease; In: *X Evropejskij kokgress revmatologov*, Moskva, 26 ijun ja-2 jul ja 1983 g., (Moskva, 1983), p.17

Isaeva, T.M., Levosin, V.V., Aldolase and transaminase activity in the blood in Kashin-Beck disease; In: *nektorye voprosy eksperimental' noji kliniceskoj' mediciny* (Cita, 1962), pp. 82-85

Isaeva, T.M., Levosin, V.V., Some biochemical indicators in Kashin-Beck Disease; In: *Materialy k izuceniju*

endemicskih zbolevanij Zabajkal'ja (Cita, 1962), pp. 82–85

Jurenskiĭ, I. M., Malformation among people living on the banks of the Urov in Eastern Siberia; Tr. Imperatorskogo Vol'nogo ekonomiceskogo obscestva (Saint Petersburg, 1849), Part 2, pp. 87–91

Kan, M.F., Nikitina, L.P., Ivanov, V.N., Egorova, O.A., The activity of acid and alkaline phosphatase in Urov disease; In: Tez. dokl. k predstojascej konferencii "Problemy revmatologii i uroscskoj (Kashin-Beck) disease; bolezni" (Cita, 1981), pp. 41–42

Kan'sina, N.F., Morbid anatomy of the osteoarticular system in adults suffering from Urov (Kashin-Beck) disease; Problem geograficeskoj patologii (Moskva, 1964), pp. 76–78

Kashin, N.I., Goitre and cretinism within and beyond Russia, especially in the Lena valley and other parts of the Irkutsk province; In: Protokoly obscestva vracej Vostocnoj Sibiri (Irkutsk, 1868 / 69), pp. 1–206

Kashin, N.I., Medical and topographical description of Knjaz' -Konstantin region, Nercinskiĭ okrug; Moskovskaja medicinskaja gazeta, 1860, No. 1–7, No.10; No. 1, 1–5; No. 2, 9–14; No. 3, 17–20; No. 5, 33–38; No. 7, 53–58; No. 10, 77–82

Kashin, N.I., A description of endemic and othe diseases on the River Urov; in Protokoly fiziko-medicinskogo obscestva pri Moskovskom university, 3 janv. 1859 (Moskva, 1959), pp.26

Kashin, N.I., Information on the spread of goitre and cretinism on the territory of the Russian Empire; Moskovskaja medicinskaja gazeta, 1861, No. 39–51, 329–337; No. 40, 341–348; No. 41, 353–358; No. 42, 365–370; No. 43, 377–384; No. 44, 389–398; No. 45, 401–407; No. 46, 413–442; No. 47, 425–431; No. 48, 437–445; No. 49, 449–455; No. 50, 461–470; No. 51, 473–479

Kireev, M.P., Clinical damage caused by Beck's disease; Moskovskij med. zurnal, 1927, No. 2, pp. 45–51

Kirkinskaja, T.A., Morphological picture of the blood of patients suffering from Urov disease and undergoing multiple treatment; In: Vopr. travmatologii i ortopedii (Irkutsk, 1954), Part 3, pp. 25–29

Koval'skiĭ, V.V., The geochemical environment, health and diseases; In: Fiziologiceskaja rol' i prakticeskoe primenenie mikroelementov, (Riga, 1976), pp. 177–193

Koval'skiĭ, V.V., The Geochemical Ecology (Moskva, Nauka, I 1974). 297pp.

Kolomijceva, M.G., Vorob'eva, A.M., Radovskij, V., The influence of dietary trace elements on the activity of certain enzymes in the bone tissue of experimental animals; Vopr. pitaniĭa, 1970, No. 2, pp. 57–62

Kopylov, M.B., X-ray images of Urov disease; In: Voprosy endokrinologii (Moskva, 1936), pp. 970–975

Kotrehov, P.F., Urov (Kashin-Beck) Disease (Leningrad, Medgiz, 1953), pp. 83

Kravchenko, L.F., Kashin-Beck disease today; In: Sb. naucn. trudov (Vladivostok, 1968), Vol. 4, pp. 64–67

Kravchenko, L.F., Concerning the clinical classification of Kashin-Beck disease; In: Voprosy teoreticeskoj i kliniceskoj mediciny (Cita, 1962), pp. 203–204

Kravchenko, L.F., Clinical and electrocardiographical alterations in individuals suffering from Kashin-Beck disease; In: Materialy k izuceniju endemicskih zbolevanij Zabajkal'ja (Cita, 1962), pp. 73–79

- Krylova, A.S.**, The functional condition of the liver in Kashin-Beck disease; In: *Materialy k izuceniju endemiceskih zabojevanij Zabajkal'ja* (Cita, 1962), pp. 58-63
- Kulikova, L.I.**, The influence of manganese on phosphorus and calcium metabolism and on the morphology of certain organs in white rats; abstract from dissertation for the degree of Candidate of Medical Sciences (Moskva, 1963), 14pp.
- Levenson, S.N.**, The maxillo-dental system in individuals suffering from Kashin-Beck disease; In: *Sb. tr. Irkuskogo gosudarstvennogo medicinskogo instituta* (Irkutsk, 1957), pp. 344-35
- Levosin, V.V., Isaeva, T.M.**, Some indicators of lipid metabolism in Kashin-Beck disease; In: *Voprosy medicinskoj geografii i Kurortologii* (Cita, 1967), Prilozhenie 1, pp. 141-143
- Leont'ev, I.A.**, Changes in the morphological blood picture in Kashin-Beck disease; *Tr. Permskogo medicinskogo instituta 'Perm'*, 1935, Part 7, pp. 100-105
- Magnusevskij, L.K.**, Drinking-water radioactivity in the endemic region of Urov disease; In: *Urovskaia bolezn' v Zabajkal'e* (Irkutsk, 1934) Part 1, pp. 65-81
- Malinina, V.S.**, Data on the composition of foodstuffs in the endemic region of Urov disease; *Tr. biogeohimiceskoj laboratorii Moskva-Leningrad*, 1949, Vol. 9, pp. 55-65
- Mihajlov, V.A.**, The mechanism of the toxic activity of manganese; abstract from dissertation for the degree of Candidate of Biological Sciences (Sverdlovsk, Moskva, 1952) 11pp.
- Mocalin, N.Z.**, Clinical analysis of Urov (Kashin-Beck) disease; In: *Urovskaia bolezn' v Zabajkal'e* (Irkutsk, 1934), Part 1, pp. 82-91
- Nikitina, L.P., Sosnjagina, M.P., Ivanov, V.N., Spirina, A.P., Minzar, M.V.**, Carbohydrate metabolism in patients suffering from endemic and non-endemic arthroses; In: *Tez. dokl. k predstojascej konferencii "problem revmatologii i urovskoj (Kashina-Becka) boleznii"* (Cita, 1981), pp. 39-40
- Nikiforov, P., Godstejn, O.**, "Kashin-Beck" Disease (Cita 1931), 15pp.
- N'juman, U., N'juman, M.**, Mineral metabolism (Moskva, Izd-vo inostrannaja literatura, 1961), 270 pp.
- Nozdrjuhina, L.R., Grinkevic, N.I.**, Disruption of trace-element metabolism and how to treat it (Moskva, Nauka, 1980), 279pp.
- Oparin, S.V.**, Phosphorus and calcium metabolism in patients suffering from Urov disease; *Med. dulleten'*, Irkutsk, 1939, No. 2, pp. 81-92
- Palej, N.P.**, The calcium content of drinking water in the endemic region of Urov disease; *Tr. biogeohimiceskoj laboratorii, Moskva-Leningrad* 1949, No. 9, pp. 31-55
- Platonov, A.A.**, Experimental-psychological study of the attention span of individuals suffering from manganese poisoning; *Zdrvoohranenie Kazahstana*, 1975, No. 2, pp. 16-19
- Platonova, A.T., Ponomareva, R.I.**, Blood-serum levels of phosphorus, calcium, magnesium and protein in patients suffering from Urov disease; In: *Vopr. travmatologii i ortopedii* (Irkutsk, 1954), Part 2, pp. 68-70

- Popov, A.M., Clinical picture of Urov disease in children; In: Vsesojuz. konf. endokrinologov (Moskva, 24-29 marta 1935g.) Tez. dokl. (Moskva, 1935), pp. 56-57
- Rosin, I.V., Collagen glycosaminoglycane and glycoprotein metabolism in individuals suffering from Kashin-Beck disease; In: X Evropejskij kongress revmatologov, Moskva, 26 ijunja-2 julja 1983g., 1983, p.84
- Rosin I.V., Govorin, A.V., Some indicators for conjunctive tissue metabolism in patients suffering from Urov disease; In: Tez. dokl. k predstojascej konferencii " Problemy revmatologii i urovskoj (Kasina-Beka) bolesti" (Cita, 1981), pp. 58-60
- Rosin, I.V., Zolotuev, N.I., Collagen metabolism in individuals suffering from Urov (Kashin-Beck) disease and deforming osteoarthroses in the vicinity of the Bajkal-Amur highway; In: Formative mechanisms for adaptive reactions in extreme conditions in Zabajkal'e (Cita, 1979), pp. 72-73
- Rohlin, D.G., Sergievskij, F.P., X-ray diagnosis of Kashin-Beck disease with special reference to the initial phases of osteoarticular complaints in children and adolescents; Vestn. rentgenologii i radiologii, 1938, Vol. 20, pp. 155-165
- Rubinstejn, Yu.I., Toxic cultures of Fusarium from cereals in Zabajkal' e (towards an etiology of Kashin-Beck disease); Gigiena i sanitarija, 1949, No. 12, pp. 35-39
- Sakovic, N.L., On the etiology of Beck disease (sretensk, Izdanje Sreten skogo okrzdruva, 1927), 27pp.
- Sai Ryo, Clinical observations of Kashin-Beck disease found in Fushun, Manchoukuo; Journal of Oriental Medicine, Dairen, 1936, 24:70.
- Samarina, I.A., The Urov biogeochemical province of Amur oblast; Tr. biogeochemičeskoj laboratorii, Moskva, 1960, Vol. 11, pp. 164-167
- Sannomiya, K., Osaki, M., Kubo, K., Matsuura, T., Miki, Y., Feranisi, M., Yamanaka, T., Tsuzawa, T., Chang, F., Wei, J., Chung der Kashin-Beckchen Krankheit; The Journal of Oriental Medicine, Dairen, 1936, 25:76-77
- Sergievskij, F.P., Clinical classification of Urov disease; Kazanskij medicinskij zurnal, 1935, No. 1, pp. 58-61
- Sergievskij, F.P., Urov (Kashin-Beck) disease (Cita, Citgiz, 1948), 96pp.
- Sidorjuk, Z.A., Description of immune factors in Urov (Kashin-Beck) disease; Abstract from dissertation for the degree of Candidate of Biological Sciences (Vladivostok, 1979), pp. 18
- Skipetrov, V.P., The leukocyte formula in Urov (Kashin-Beck) disease; In: Nekotorye voprosy klimatofiziologii i kraevoj patologii (Cita, 1961), Part 2, pp. 105-114
- Skipetrov, V.P., Some special features of hemopoiesis in Urov (Kashin-Beck) disease; In: Materialy IV plenuma patofiziologov Sibiri i Dal' nego Vostoka (Tomsk, 1962), pp. 255-258
- Sosnjanina, M.P., Pikitina, L.P., Ivanov, V. N., Gajmolenko, S.G., Puskareva, O.P., The state of organic and

- mineral phosphorus compound metabolism in Urov disease; In: Tez. dokl. k predstojećej konferenciji "Problemy revmatologii i urovskoj (Kashin-Beck) bolesti" (Cita, 1981), pp. 40-41
- Svarcman, M.L., So-called "Urov disease", or endemic deforming osteoarthritis in Zabajkal'e Sov vraceb. zurnal, 1937, No. 3, pp. 182-184
- Sipacev, V.G., Kashin-Beck (Urov) disease and control thereof (Irkutsk, Irkutskoe oblastnoe gosudarstvennoe izd-vo, 1950), 24pp.
- Sipacev, V.G., The pathogenesis of osteochondros deformans in Zabajkal'e; Irkutskij med. zurnal 1925, Vol. 3, No. 1-2, pp. 126-155
- Sipacev, V.G., The etiology of Kashin-Beck disease (Osteochondrous deformans) in Zabajkal'e (Irkutsk, Izdanie Irkutskogo universiteta, 1928), 25pp.
- Tanaka, T., Studien Uber die Kashin-Beckische Krankheit, Journal of Oriental Medicine, Dairen, 1936, 25:46-47
- Tihonov, V.A., Kashin-Beck Disease (Irkutsk, Vostocno-Sibirskoe izd-vo, 1976), pp. 191
- Tjutjunnikov, V.P., Non-organic phosphorus in the blood serum of patients suffering from Kashin-Beck disease and undergoing radon carbonate bath treatment; In: Vtoraja naucnaja konferencija urovskoj stancii (Sretensk, 1959), pp. 33-35
- Vencikov, A.I., Teaching on trace elements in medicine; Klinic. Medicina, 1972, No. 9, pp. 11-14
- Vinogradov, A.P., Biogeochemical provinces and their role in organic evolution; Geohimija, 1963, No. 3, pp. 199-213
- Vinogradov, A.P., The causes of the Urov endemic; Tr. biogeohimiceskoj laboratorii, Moskva-Leningrad, Vol. 9, pp. 5-31
- Vojnar, A.I., The biological role of trace element in the organism of animals and man (Moskva, Vyssaja skola, 1960), 543pp. 89.
- Voscenko, A.V., Cugaev, V.N., Siber, N.E., Ustinova, E.E., Cercesova, T.B., Zajko L.V., Druzkova N.N. (Title of article missing) In: X Evropejskij kongress revmatologov. Moskva, 26 ijunja - 2 ijulja 1983g. (Moskva, 1983), p.41
- Zaiko, L.V., Voscenko, A.V., Ustinova, E.E., The phosphate theory in the etiology of Kashin-Beck Disease; In: Tez. dokl. predstojećej konferencii: Problemy revmatologii i urovskoj (Kashin-Beka) bolesti (Cita, 1981,) pp.42-43
- Zografskij, B., Dang Dyg Zan, The first cases of Kashin-Beck disease in the Democretic Republic of Vietnam; Klinic. Medicina, 1971, No. 10, pp.127-130

THE IPCS / WHO / PRC MEETING ON KASHIN-BECK DISEASE

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