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中国科学院生态环境研究中心
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（1980-2002）

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彭安研究员简介

彭安研究员，1930年6月出生于安徽省安庆市；1950-1953 年在复旦大学化学系学习，获学士学位；1958-1962年在前苏联莫斯科大学化学系学习，获副博士学位；1953-1956年中国科学院应用化学研究所，研究实习员；1962-1978年中国科学院应用化学研究所，助理研究员；1978-1986 年中国科学院环境化学研究所，副研究员；1987中国科学院生态环境研究中心，研究员；1989年评为博士生导师。

曾任中国科学院生态环境研究中心学术委员会主任，国家科委成果奖励委员会环境组委员，《环境科学》副主编，兼上海交通大学资源环境工程学院、中国农业大学资源环境学院客座教授。

从事科研工作五十载，先后参加了“钨矿全分析的研究”，“核燃料后处理工艺研究”，“稀土萃取分离的研究”工作，主持负责中科院重点课题“水体汞污染化学规律的研究”、“硒的环境化学行为及其在大骨节病中的作用”，七五国家科技攻关专题“微生物毒素及其他有机物致大骨节病作用研究”，八五国家科技攻关专题“饮水中有机物致大骨节病作用研究”，中德合作项目“不同硒化合物对软骨细胞及软骨保护作用的分子生物学机制”，国家自然科学基金委重点课题“具有生物活性的含硒化合物的分离、合成及生物效应研究”，国家自然科学基金委重大项目“典型化学污染物在环境中的变化和生物效应”中的专题“典型环境中硒的形态、转化及生态意义”及“稀土农用的环境化学行为及生态、毒理效应”中的课题“农用稀土元素的环境化学行为的研究”。发现硒抑制饮用水中腐殖质和粮食真菌毒素毒性以及由此引发的自由基反应机制，揭示了硒在大骨节病中的重要作用，提出新的大骨节病病因机制，提出了腐殖酸可使软骨基质胶原蛋白受损的分子机理，阐明了大骨节病自由基损伤发生在软骨/骨等靶部位的病因，也进一步肯定了硒在大骨节病中的保护作用。

先后获全国科学大会奖两项，主持的课题先后获中国科学院科技进步一、二、三等奖，“八五”攻关优势成果奖。自1980年以来发表论文150余篇，出版、合作出版著作共7册。

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六．专著(章节)及会议论文目录

四、硒与地方病大骨节病病因

(自然科学基金重点课题,国家“七五、八五”攻关课题)

原书空白页

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THE EFFECTS OF HUMIC ACID ON THE CHEMICAL AND BIOLOGICAL PROPERTIES OF SELENIUM IN THE ENVIRONMENT

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ABSTRACT

To shed light on the causes of Kaschin-Beck disease, which can be prevented by supplementation of the diet with sodium selenite, the interactions between inorganic selenium compounds (selenite and selenate) and humic/fulvic acid were investigated. Selenate was found to be slowly reduced to selenite by humic acid in acidic solution. Selenite was adsorbed on manganese dioxide and iron(III) oxide from solution to a much greater degree than on kaolin, humic acid, Yongshu soil, or silicon dioxide. Feeding mice a diet supplemented with sodium selenite increased the selenium concentration in the kidney, liver, spleen, lung, heart and blood. A diet containing sodium selenite and fulvic acid caused the selenium concentrations in the organs, with the exception of the spleen, to be lower than observed with the selenite-only diet. Selenium and fulvic acid increase the activity of glutathione peroxidase. Sodium selenite and fulvic acid injected in combination into the abdominal cavity of mice and rats were less toxic than either substance alone. Selenite and fulvic acid applied separately enhanced the luminosity of photobacterium phosphoreum T-3 at low concentrations but depressed the luminosity at higher concentrations. Selenite and fulvic acid in combination caused a larger enhancement and a smaller depression of the luminosity than observed with either substance alone. The hypothesis is formulated that Kaschin-Beck disease is caused by selenium levels insufficient to prevent the toxic effects of the organic compounds present in the drinking water of the regions in which the disease is endemic.

INTRODUCTION

People in a region stretching from northeast to southwest China are known to suffer from the endemic Kaschin-Beck's disease [1]. Environmental investigations and medical studies carried out during the past three decades identified selenium deficiencies [2,3], high concentrations of natural organic materials such as humic acids in drinking water [4], and fungal toxins present in grain as the causes of this disease. Removing any one of the three causes (adding extra selenium to the food, reducing the concentration of organic matter in drinking water, or providing toxin-free grain) reduces the incidence of the disease [6,7,8]. This observation suggests that selenium, organic matter, and fungal toxin, act synergystically.

This paper reports the results of an investigation of the effects of humic acid, the main component of the organic material in the drinking water of the region affected by Kaschin-Beck's disease, on the chemical behavior of selenium in the

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environment and on the biological properties of selenium given to mice and rats in their food.

EXPERIMENTAL

Humic acids and fulvic acids were extracted as described earlier [9] from the drinking water of Yongping village and soils collected in Yongshou County, Shaanxi Province, an area in which Kaschin-Beck's disease is endemic. Fulvic acid was extracted from humic acid and then purified [9]. Humic acid samples were similarly obtained from peat provided by the Institute of Chemistry, Academia Sinica, Beijing.

To prepare soil water, 100 g of air-dried soil from Yongping village, Yongshou County, was thoroughly mixed with 10 l of distilled water. The clear supernatant (pH 6.47, E_h + 464 mV) was used for the experiments.

Standard solutions of Se(IV) and Se(VI) were prepared from A.R. grade sodium selenite, selenium dioxide, or sodium selenate supplied by Beijing Chemical Plant. Se(IV) and Se(VI) were determined by gas chromotography (Lunan Chemical Industry Instrument Factory Model SP-501 GC) with an electron-capture detector or spectrophotometrically [15] (RF-520 Shimadzu Spectrofluorophotometer) using 1,2-diamino-4-nitrobenzene as the selenium-specific reagent [10]. The adsorption of selenium on various materials was studied with the γ-active Se-75 isotope (Beijing 261 Factory Model FH-408 Gamma Counter). The glutathione peroxidase activity in the blood of mice was determined according to Hafeman et al. [16].

Effect of humic acid on the Se(VI)/Se(IV) ratio

Sodium selenate and sodium selenite were added to soil water to obtain solutions containing 100 mg l^{-1} Se(VI) and 100 mg l^{-1} Se(IV). Humic acid (extracted from drinking water) was then added to aliquots of the Se(VI)/Se(IV) solutions to achieve concentrations of humic acid in the range of 0–500 mg l^{-1}. Each of the humic acid solutions was divided into four parts, each of which was mixed with acid or base to achieve pH values of 3, 5, 7, or 9. The redox potentials of these solutions were measured at 25°C using a pHS-3 research pH meter equipped with a Pt-electrode.

Reduction of selenate by humic acid

Humic acid was dissolved in soil water to obtain solutions with 5.0 mg humic acid per liter. Aliquots of this solution were adjusted with hydrochloric acid or with sodium hydroxide to a pH of 3.24, 7.95, or 9.01. Sufficient sodium selenate solution was then added to achieve a Se(IV) concentration of 0.097 mg l^{-1}. Se(IV) was determined as the selenazole.

Adsorption of selenite

Humic acid (1000 mg) was added to 1 l of a selenite solution in soil water [0.5 mg Se(IV) l^{-1}] spiked with Se-75 selenite. Aliquots of these solutions were adjusted to pH values in the range 2–12 using hydrochloric acid or sodium hydroxide. Quartz test tubes (20 ml) with ground-joint caps were charged with 10–50 mg of adsorbent [manganese dioxide iron(III) oxide, kaolin, humic acid, soil from Yongshou County, or silicon dioxide] and 10 ml of one of the Se(IV) solutions. The tubes were sealed, and the mixture shaken for 10 h at 25°C. The phases were then separated in a centrifuge at 10 000 r.p.m. The supernatant was analyzed for dissolved selenium with a γ-ray counter.

Effect of sodium selenite and fulvic acid on mice

Groups of 12 Kunming male white mice provided by the Beijing Medical College were fed a normal diet of maize for 35 days. The first group then received the normal diet for 10 days, but the drinking water contained sufficient sodium selenite to assure that each mouse obtained 5 μg Se per day. The diet of the second group had the same amount of selenium in the drinking water as the first group, but received in addition 3 mg of fulvic acid via drinking water. The third group, fed a normal diet without selenium and fulvic acid, served as control. The mice were sacrificed after 10 days. The selenium concentrations were determined in their main internal organs, in their blood, and in their bones. The glutathione peroxidase activity was determined in the blood.

Selenium toxicity in mice and rats

Female white rats and mice were reared on a normal diet. Solutions (pH 7) (0.5 ml) of selenium dioxide in water (1–5 μg Se/g body weight), solutions of fulvic acid (20–100 μg/g body weight), or solutions of selenium dioxide and fulvic acid were injected into the abdominal cavity of the animals. Their survival or death was noted.

Effect of sodium selenite and fulvic acid on the luminosity of bacteria

The photobacterium phosphoreum T-3 isolated from the Australian fish *Tripterophysics intemedus* was provided by Dr Y.T. Tchen [14] of the Nanjing Soil Institute. The bacterial cultures were diluted with 3% aqueous sodium chloride solution [17]. Aliquots of the diluted culture were placed into colorimeter cuvettes, and the selenium/fulvic acid solutions were added (culture/Se, FA solution 1:3 to 1:9 v/v). Half an hour after mixing, the luminosity was determined. The luminosity of a control obtained by diluting the bacterial culture with 3% sodium chloride was also determined.

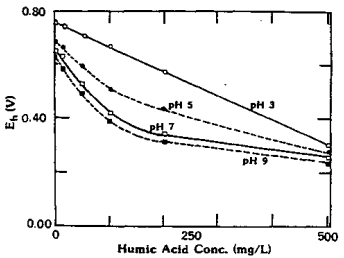

Fig. 1. The effect of the humic acid concentration and the pH on the E_h values for Se(VI)/Se(IV).

RESULTS AND DISCUSSION

Selenium and humic/fulvic acid

Aqueous solutions containing sodium selenate (97 μg Se l^{-1}) and humic acid at pH 3.24, 7.95, or 9.01 were kept for 7 days. Determinations of the oxidation state of selenium in these solutions showed that humic acid reduced selenate to a small degree in the acidic solution but not at all in the neutral and basic solutions (Fig. 1).

Sodium selenite and sodium selenate were dissolved in soil water. Humic acid was added to provide humic acid concentrations in the range 0–500 mg l^{-1}. Measurement of E_h [Se(VI)/Se(IV)] of these solutions indicated that their reducing ability increased with increasing humic acid concentration (Fig. 2). The concentration of selenite increases gradually. The dependence of E_h on the pH of the humic acid-containing solutions is the same as for the humic acid-free solutions [11].

Adsorption of selenite

The adsorption of selenite was determined by shaking aqueous solutions of sodium selenite (pH 2–11) with metal oxides, humic acid, and a soil sample from Yongshou County for 10 h. Under these conditions, manganese dioxide and iron(III) oxide adsorbed much more selenite than did kaolin, humic acid, Yongshou soil, and silicon dioxide. A similar sequence has previously been reported [12]. The absorbability of selenite on these materials decreased with increasing pH, with a particularly drastic decrease between pH 9 and 11 (Fig. 3). The adsorption of selenite on the hydrous oxides of iron and manganese at

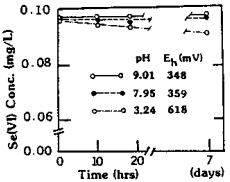

Fig. 2. The time-dependence of the reduction of selenate by humic acid in acidic and basic aqueous media.

low pH can affect the bioavailability of selenium. However, the low adsorbability of Yongshou soil for selenium makes it unlikely that the low selenium levels in this region are caused by adsorption of selenium on soil.

Distribution of selenium in mice

Selenium is an essential element that becomes toxic at elevated levels. Selenium is associated with several diseases, inhibits the activity of some carcinogens, and acts as an antagonist for elements such as Cd, Hg, Ag and As. Selenium is present in organs and other parts of the body. The concentration of selenium in the organs of mice fed a diet without selenium supplementation is in the range 0.1–0.7 $\mu g\,g^{-1}$ (Table 1). A diet supplemented with sodium

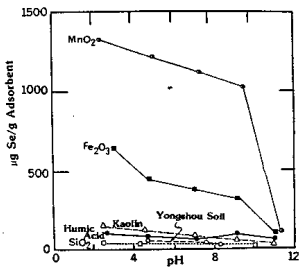

Fig. 3. The adsorption of selenite by Yongshou soil, humic acid, kaolin, silicon dioxide, iron(III) oxide, and manganese dioxide from aqueous solutions of pH 2–12.

TABLE 1

The distribution of selenium in mice fed diets containing sodium selenite or sodium selenite/fulvic acid

Diet	Selenium concentration* ($\mu g\,g^{-1}$)						
	Kidney	Liver	Spleen	Lung	Heart	Blood	Bone
Control	0.72	0.38	0.44	0.36	0.29	0.38	0.16
With Na_2SeO_3	1.36	1.37	0.61	0.60	0.45	0.71	0.14
With $NaSeO_3$ and fulvic acid	1.33	0.91	0.89	0.54	0.32	0.71	0.15

*The organs of 12 mice in each group were combined and digested. The concentrations are expressed on a wet weight basis.

selenite to provide a dose of 5 μg Se per day increased the selenium concentration in the kidney, liver, spleen, lung, heart and blood. The largest increase (3.6-fold) occurred in the liver. Such increases have been observed earlier [19]. When the mice were fed a diet supplemented with sodium selenite and fulvic acid, the selenium concentrations in the organs were found to be higher than those of the control group, but lower than those for animals fed a diet supplemented by selenium only. The spleens of mice on the selenium/fulvic acid diet had a higher selenium concentration than the spleen of mice on the selenium diet. Fulvic acid did not affect the selenium concentration in blood. The selenium level in bone was not changed by selenium or selenium/fulvic acid (Table 1). The low and constant concentration of selenium in bone observed in this study agrees with results obtained with autoradiography [18].

Glutathione peroxidase contains selenium. The level of glutathione peroxidase activity in the blood could be a measure of selenium uptake and utilization. Mice on the selenium-supplemented diet had a glutathione peroxidase activity (10.6 U ml^{-1}) almost twice the activity in the control group (5.5 U ml^{-1}). With selenium and fulvic acid the activity increased to 13.6 U ml^{-1}. Fulvic acid appears to promote the activity of glutathione peroxidase.

Toxicity of selenium/fulvic acid

The toxicity of selenite and of fulvic acid was checked by injecting solutions of these substances into the abdominal cavity of mice and rats. The mice were alive 7 days after receiving 50 or 200 mg fulvic acid per kg body weight. A dose of 400 mg kg^{-1} fulvic acid caused death within 1 day. Rats receiving 2.94 mg Se (SeO_2) per kg body weight died within 1 day. A similar fatal dose of 3.0 mg Se kg^{-1} for rats was also found by other investigators [19,20]. When solutions containing selenium (SeO_2) and fulvic acid were injected at doses that were fatal when applied singly, the mice and rats survived. The results of these experiments are summarized in Table 2. Mixtures of selenite and fulvic

TABLE 2

Effect of solutions of selenite and fulvic acid injected into the abdominal cavity of female mice and rats

Animal	Weight (g)	Dose (mg kg^{-1} body weight)		Effect
		Se	Fulvic acid	
Mouse	30	0	50	Alive after 7 days
Mouse	30	0	200	Alive after 7 days
Mouse	30	0	400	Dead within 1 day
Mouse	28	0	535.7	Dead within 1 day
Mouse	30	0	800*	Dead within 12 h after the last injection
Mouse	30	4.7	500	Alive after 5 days
Mouse	28	5.1	535.7	Alive after 7 days
Mouse	30	4.0*	800*	Alive after 8 days
Rat	170	2.9	0	Dead within 1 day
Rat	200	3.0	132.6	Alive after 3 days
Mouse	23	5.4	338.3	Alive after 3 days

*Several injections used to reach total with 6 h between injections.

acid are clearly less toxic than selenite or fulvic acid alone. Combinations of elements such as selenium/mercury and selenium/cadmium are known to have lower toxicity than the individual components. The interactions responsible for these reductions of toxicity have been investigated [21]. However, the literature does not appear to report similar effects for mixtures of fulvic acid and selenium. The observations described here indicate that carboxylic acids

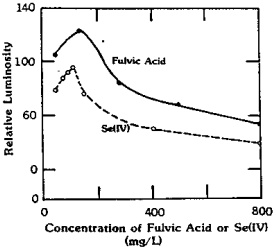

Fig. 4. The separate effects of selenite and Yongshou fulvic acid on the luminosity of Photobacterium phosphoreum T-3.

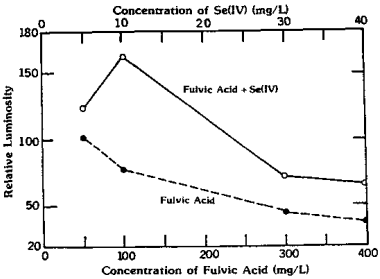

Fig. 5. The luminosity of Photobacterium phosphoreum T-3 in a medium containing selenite and fulvic acid.

might serve as detoxifying agents for various elements, and suggest that one of the functions of selenium might be the detoxification of humic and fulvic acids.

Effect of selenite and fulvic acid on the luminosity of photobacterium phosphoreum T-3

The luminosity of the photobacterium phosphoreum T-3 was used as a probe for the effects of selenite and fulvic acid on the biological processes in this organism. Selenium concentrations $< 100\,mg\,l^{-1}$ and fulvic acid concentrations $< 120\,mg\,l^{-1}$ enhance the luminosity. At higher concentrations the luminosity decreased (Fig. 4). These results are in agreement with the law that an organism functions best at a restricted range of trace element concentrations as expressed by the Bertrand diagram [22]. In a medium containing fulvic acid and selenite (at selenium concentrations one-tenth of those of fulvic acid) the luminosity of the bacteria was considerably higher than the luminosity in media containing fulvic acid alone (Fig. 5). Selenite and fulvic acid affect the bacterium less in combination than singly, as was observed with mice and rats.

Cause of Kaschin-Beck disease

Organic compounds in the drinking water of the regions in which Kaschin-Beck disease is endemic damaged the cells of cartilaginous tissues [13]. One can hypothesize that these organic compounds and other pathogenic factors cause Kaschin-Beck disease. The observation that selenium reduces the toxicity of fulvic acid suggests a cause for the disease. When selenium levels in the water are sufficiently high, the toxic effects of the organic compounds are suppressed and the disease cannot become endemic. However, at low selenium levels, insufficient selenium is available to completely interact with toxic organic