

Research on organic matters in the drinking water of Kaschin-Beck disease area

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Abstract—Organic matters in drinking water of Kaschin-Beck disease areas were extracted. Then analyses and characterization were performed by means of multiply chemical and physical methods. The results did not show the obvious difference in the frame structure of humic substances and the structure of micromolecular compounds in the drinking water of disease and non-disease areas, but the difference in the contents of some micromolecular compounds and radicals. The investigation also includes the preliminary research on the photoreaction of drinking water from disease and non-disease areas and the accumulation of natural organic matter in the bone of tested animals.

Keywords: organic compounds; humic substances; drinking water; Kaschin-Beck disease; photoreaction.

In the survey of Kachin-Beck disease the relation between the incidence and the content of humic substances in drinking water was found (Lin, 1984). One of the efficient methods for the prevention of the disease was to improve the quality of drinking water in the disease region. Several methods could decrease the content of organic matter in the water. For instance, drinking water in disease area could be irradiated by UV-light (Wu, 1985) and water could be passed through the adsorbed bed of active carbon.

It is known the organic compounds from drinking water of disease regions damage the chondrocytes (Feng, 1990). The animal test in our laboratory showed glutathione peroxidase (GSH-Px) was decreased and lipid peroxide (LPO) increased in the livers and bloods of the animals by humic substances. Two questions are not clear yet whether there are any specific compounds and whether there is any difference in the structure of organic matter in drinking water of disease and non-disease regions.

In order to clarify the action of organic matter in pathology of Kaschin-Beck disease, this research includes two parts: one is the analysis and the characterization of simple organic compounds and humic substances in the environment of the disease areas by means of multiphysical and chemical spectra, another is the studies on radicals reaction in drinking water from non-disease and disease regions.

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EXPERIMENTAL METHOD

1. Physical and chemical characters of the humic substances of soil and water

Collection and extract of samples

Samples were collected in June-August of 1987 in different places with and without disease (Table 1). Extraction of humic substances followed Peng *et al.*(1981).

Characterization of humic substances

Infra red spectra (IR), Ultra-violet spectra (UV), Fluorescence spectra (FL) and the quotient of optical density at 465 nm and 665 nm (E_4 / E_6) were recorded (Peng, 1981).

2. Distribution of molecular weight

1000 ppm of HA and FA in phosphate buffer were injected into Waters-210 Gel Chromatograph with U-Bindagel E-linear Analysis Column.

3. Identification of the organic compounds in the drinking water of the disease regions

Samples were collected from the disease regions Wujiawan, Hejiawan, Peijiashuang, Liangdang in Gansu Province, Fengning in Hebei Province, and from the non-disease region: Liujiaye in Gansu Province in 1987-1990. Organic compounds were treated by GDX-102 resin and CH_2Cl_2 . Then concentrated samples are analysed by GC / MS, Finnigen.

Table 1 The sampling places of humic substances

Places	Samples	Incidence	Abbr. in the article
Shangzhi in H. P. **	Soil	Heavy	SZSHA, SZSFA
Shangzhi in H. P.	Drinking water	Heavy	SZWFA
Suiling in H. P.	Soil	Heavy	SLSHA, SLSFA
Suiling in H. P.	Drinking water	Heavy	SLWFA
Qingzhou in SD. P. *	Sediment of well	Heavy	QZSHA, QZSFA
Qingzhou in SD. P.	Drinking water	Heavy	QZWFA
Gaojiayao in G. P.	Soil	Medial	GJSHA, GJSFA
Renjiabao in G. P.	Soil	Light	RJSHA, RJSFA
Shuiqing in G. P.	Soil	Medial	SQSHA, SQSFA
Shuiqing in G. P.	Drinking water	Medial	SQWFA
Guanguangou in S. P.	Stone	Heavy	GGTFA
Luotuogou in HB. P.†	Soil	Heavy	LTSHA
Jiangjiashan in S. P.	Drinking water	No	JJWFA
Jiangjiashan in S. P. **	Soil	No	JJSFA

+ + H. P. means Heilongjiang Province * SD. P. means Shandong Province. G.P. means Gansu Province.

** S. P. means Shaanxi Province + HB. P. means Hebei Province

4. Radicals in drinking water

Determination of radicals in drinking water followed Peng *et al.* (1988). Determination of the radicals in photoreaction of the waters followed Haag *et al.* (1984).

5. ESR spectra for the bone of tested rat

Character of ESR spectra for the organic matter of water was described in the work by Peng *et al.* (1988).

RESULTS AND DISCUSSION

Physical and chemical characterization for HA and FA in the disease regions

The IR, UV, FL and E_4 / E_6 of VIS spectra of the HA and FA from the disease regions are shown in Fig. 1, Fig. 2, Fig. 3 and in Table 2, respectively.

In the IR spectra of Fig. 1, the $-OH$ group with hydrogen bond located at about 3400 cm^{-1} . There are some differences at 2920 cm^{-1} for the lipid link. The absorption here in QZSHA sample is not obvious, indicating the rather high aromatic content. All samples show high absorption at about 1700 cm^{-1} of $-COOH$ and 1400 cm^{-1} of $-COO^-$, meaning the rather high content of acidic groups. But comparing the samples from non-disease and disease

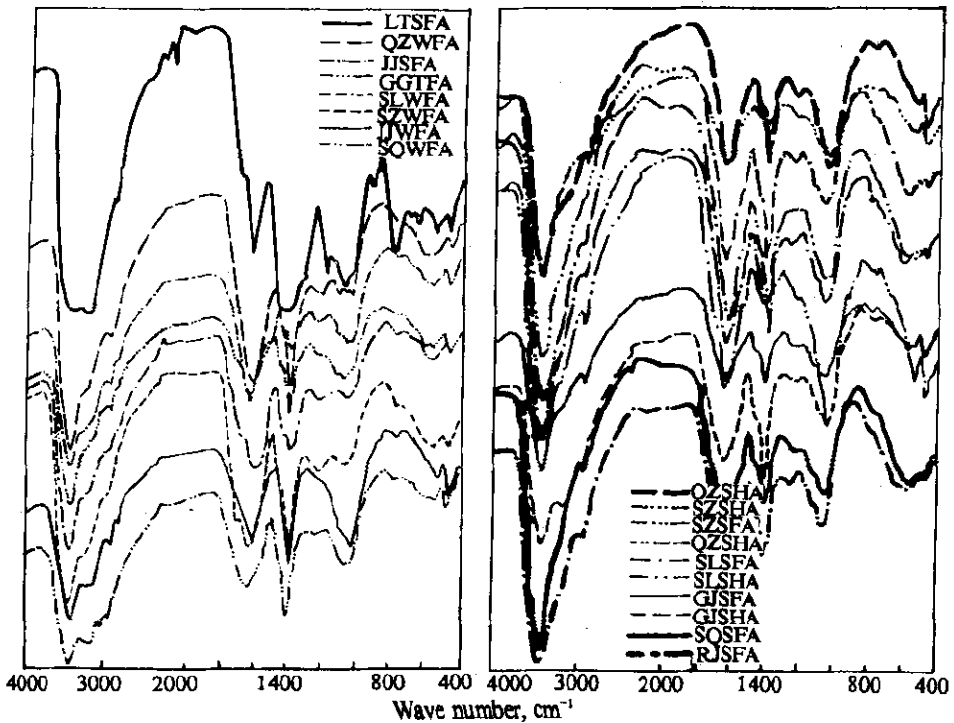


Fig. 1 IR spectra of humic substances

regions the significant distinction are not observed at the IR spectra of the humic matter samples. Compared with the IR spectra of the humic substances from rivers there are not marked differences (Peng, 1981). The character of UV spectra (Fig. 2) can not be distinguished between non-disease and disease samples as well. The spectra are monotonous rise and no specific absorption in 400–200 nm. Corresponding to no absorption of IR at 2900 cm^{-1} there is absorption at 270 nm of UV spectra in QZSHA, meaning rather strong aromaticity.

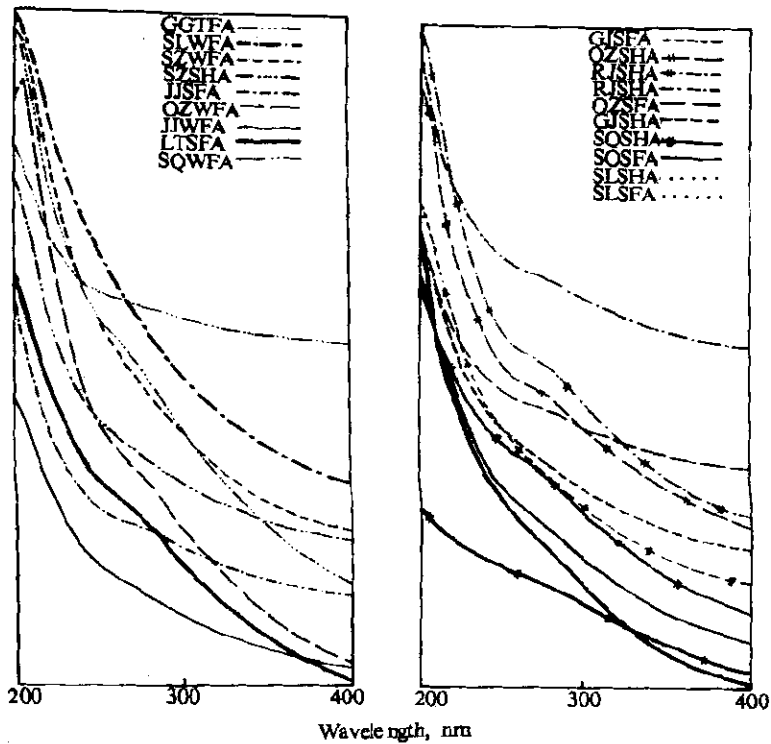


Fig. 2 UV spectra of humic substances

Evaluating semi-quantitatively the fluorescence of HA (Fig. 3) is stronger than FA from the same source. The FL spectra of water samples are simple but the spectra of soil samples are rather complicated, especially for HA. The latter shows relatively strong aromaticity because of the absorption peaks shifted behind. The distinction of FL spectra between the fractions of humic matter are consistent with the different quotients of E_4 / E_6 (Table 2). It could be concluded from the FL that the molecular weight and the aromaticity of HA are higher than those of FA from the identical source. But the regular differences of samples between the non-disease and disease areas are not found from the FL spectra and E_4 / E_6 .

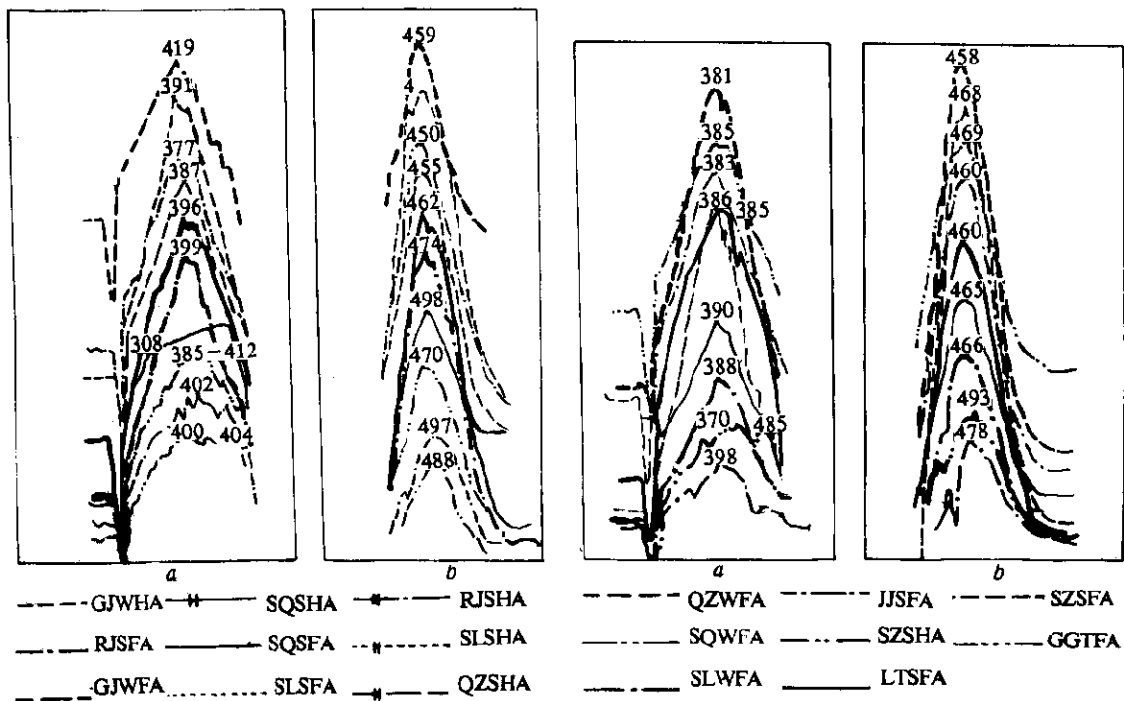


Fig. 3 FL spectra of humic substances

a . EX spectra; b. EM spectra

Distribution of molecular weights

Distribution of molecular weight of humic substances is in Table 3. The gel-chromatograph spectra are shown in Fig. 4. The regular of the fractions are found. Their number average molecular weights of the samples from waters, except QZSHA, are $(4-8) \times 10^3$. The lowest one is < 1000 . The number average molecular weights of samples from soil are more than 10^5 . The highest one is $> 10^6$. The results are consistent with the character by the spectra and quotient of E_4 / E_6 , which shows the high aromaticity of polymer. The tendency of difference among the fractions is FA from water $<$ FA from soil $<$ HA from soil in number average molecular weight. But the relativity between the distribution and the incidence of the disease is not found

Conclusively, with the common methods of the physical and chemical characterization for humic substances the differences between the fractions of the samples are found instead of the distinction between non-disease and disease regions. This means that the main skeleton structures of humic matters for non-disease and disease areas are undistinguished. The obvious difference of the humic substances between non-disease and disease regions presents in their quantity

rather than in their quality (Wu, 1987).

Table 2 The E_4 / E_6 of VIS spectra of humic substances

Samples	D_{465nm}	D_{665nm}	E_4 / E_6
SZSHA	0.267	0.052	5.13
SZWFA	0.047	0.008	5.88
SLSHA	0.191	0.039	4.90
SLSFA	0.055	0.004	13.75
QZSHA	0.150	0.029	5.17
QZSFA	0.027	0.004	6.75
QZWFA	0.154	0.017	9.06
GJSHA	0.057	0.018	3.17
GJSFA	0.062	0.011	5.64
RJSHA	0.077	0.010	7.79
RJSFA	0.018	0.002	9.00
SQSHA	0.160	0.036	4.44
SQSFA	0.070	0.008	8.75
SQWFA	0.065	0.012	5.42
JJSFA	0.047	0.008	5.88
FFWFA	0.041	0.006	6.83
GGTFA	0.024	0.006	4.00
LTSFA	0.020	0.003	6.67

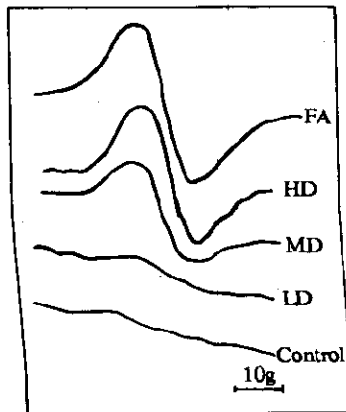


Fig. 4 ESR spectra of bones injected with FA

Table 3 The distribution of molecular weight of humic substances

Samples	NAMW ⁺	WAMW ⁺⁺	Distribution	Dispersion
SLSHA	26789	65542	2056-768034	2.45
SLSFA	37360	49110	6546-77496	1.31
SLWFA	7335	37831	411-121971	5.18
SZSHA	26383	68560	2148-123703	2.03
SZSFA	37983	176384	1734-358553	4.64
SZWFA	8992	25067	3083-1584040	2.79
QZSHA	11397	69594	1734-785354	6.10
QZSFA	23702	53757	1968-230283	2.27
QZWFA	22743	36169	5321-1092110	1.59
LTSHA	-	53311	22855-88245	-
LTSFA	41426	61604	5701-165493	1.49
SQWFA	4270	25610	480-71396	6.00
SQSFA	40015	51894	6966-82902	1.30
GJWFA	8162	25230	1588-160207	3.09
RJSHA	50086	57483	28706-95720	1.15
RJSF	26772	276094	1161-1209200	10.31

+ NAMW: number average of molecular weight ++ WAMW: weight average of molecular

Identification of organic micromolecular compounds in drinking water of the disease regions

The attempt to search some compounds which caused Kaschin-Beck disease has been for a long time. A Japanese geological chemist Noguchi proposed ferrous and p-hydrocinnamic acids as causative agents of Kaschin-Beck disease in 1967. Attention has also been given to other phenolic compounds (Lin, 1981).

There are some relatively simple compounds, for example hydrocarbon, amino-acid, protein and organic acid that the molecular is small and the structure is fixed in the environment. By means of GC-MS the compounds from the drinking water in the disease areas were detected and compared with those in the non-disease areas. The main part of the compounds from the spectra are saturated and unsaturated lipid hydrocarbon. Table 4 and 5 present the analytical results of the compounds with oxygen, sulfur and nitrogen in the samples collected in 1988 and 1989.

The compounds with oxygen, nitrogen and sulfur are important in the research because of their physiological activities. It can be seen from the tables that benzothiazole compounds exist repeatedly in both the disease villages (Wujiawan and Fengning). On the contrary, this kind of compounds was detected only in the samples of 1989. The phenolic compounds containing oxygen are identified in both disease and non-disease villages. But they are in different contents. Comparing semi-quantitatively, the content in non-disease village Liujiache is lower

than that in disease villages Wujiawan and Fengning.

The investigation above indicates that the frame structure of polymolecular humic substances in water of both non-disease and disease regions are similar. The specific organic compounds for the disease are not found from the drinking water of the disease area. Therefore, it is necessary to study the difference of the organic matter in specific function groups or their biological activities between disease and non-disease regions.

Table 4 Identification of partial compounds with O, S and N by GC-MS (Samples in 1988)

Places	MW	MF	Name
Fengning	135	C ₇ H ₅ NS	Benzothiazole
	148	C ₁₀ H ₁₂ O	1-(2,4-dimethylphenyl)-Ethanone
	278	C ₁₆ H ₂₂ O ₄	Di-n-butylphthalate
	270	C ₁₈ H ₃₈ O	5,7,7-trimethyl-2-(1,3,3-trimethyl butyl)-1-octanol
	390	C ₂₆ H ₃₈ O ₄	Di-iso-octylphthalate
Wujiawan	139	C ₇ H ₅ NS	1-ethyl-2-pyridithione
	135	C ₇ H ₅ NS	1,2-Benzisothiazole
	220	C ₁₅ H ₂₄ O	3-methyl-4,6-di- <i>t</i> -butylphenol
	220	C ₁₅ H ₂₄ O	4-methyl-2,6-di- <i>t</i> -butylphenol
	170	C ₁₁ H ₂₂ O	Nor-hexyl-nor-propylketone
	232	C ₁₇ H ₁₄ Cl ₂ O	1-chloro-2-(chloromethyl)-3-methoxypropyl benzene
	278	C ₁₆ H ₂₂ O ₄	N-butyl-isobutyl-phthalate
Liujiabe	184	C ₁₁ H ₂₀ O ₂	3,5,5-trimethylhexyl-2-enylacetate
	220	C ₁₅ H ₂₄ O	2,6-di- <i>tert</i> -butyl-4-methylphenol
	226	C ₁₅ H ₁₆ S	3-phenyl-3-phenylthio-propene
	234	C ₁₃ H ₂₂ O ₂	3,5-di- <i>t</i> -butyl-4-hydroxy-benzaldehyde
	278	C ₁₆ H ₂₂ O ₄	N-butyl-isobutyl-phthalate
	270	C ₁₈ H ₃₈ O	5,7,7-trimethyl-2-(1,3,3-trimethyl butyl)-1-octanol

Radicals in drinking water of disease regions

From the research of clinical pathology for Kaschin-Beck disease, it is known that cell membrane is decayed by superoxydation. That gives us an inspiration to search the possibility of free radicals in drinking water of disease regions. Senisi *et al.* (1977) indicated that the phenolic, quinone compounds and humic matter occur the signals of stable radicals in the ESR spectra. The value of *g* factor in ESR implicated the presence of semiquinone radicals in the materials above. The semiquinone radicals are able to lead the result of the active oxygen

Table 5 Identification of partial compounds with O, S and N by GC-MS(Samples in 1989)

Places	MW	MF	Name
Wujiawan	278	$C_{16}H_{22}O_4$	Isobutyl phthalate
	336	$C_{18}H_{24}O_6$	Butyl carbutoxymethyl phthalate
	298	$C_{15}H_{23}OBr$	2, 6-di-t-butyl-4-bromomethyl-phenol
	135	C_7H_5NS	Benzothiazole
Liangdang	418	$C_{26}H_{42}O_4$	Dinonyl phthalate
	278	$C_{16}H_{22}O_4$	Butyl phthalate
	220	$C_{14}H_{20}O_2$	3, 5-cyclohexadiene-1, 4-dione
	167	$C_7H_5NS_2$	2-mercapto benzothiazole
	148	$C_7H_6O_3$	Triethyl orthoformate
Peijiazhuang	278	$C_{16}H_{22}O_4$	Di-n-butylphthalate
	220	$C_{15}H_{24}O$	2, 6-ditertiary butyl-p-phenol
	148	$C_7H_{16}O_3$	Triethyl orthoformate
Hejiawan	219	$C_{12}H_{13}O_3H$	3-ethyl-4-hydroxy-7-methoxy
	148	$C_{10}H_{12}O$	Ethanone, 1, (2, 4-cimethylphenyl)-
	135	C_7H_5NS	Benzothiadiazole
	134	$C_9H_{10}O$	Benzoldehyde, ethyl
	134	$C_9H_{10}O$	Dimethyl benzcnemethanol
	162	$C_8H_{18}O_2$	2-proponol, 1-(2-ethoxypropoxy)
	204	$C_{11}H_8O_4$	4-formal-7-methoxy coumarin
	214	$C_{14}H_{30}O$	2-tetradecanol
Liujiuhe	168	$C_{11}H_{20}O$	1, 3-di-t-butyl-propadieneoxide
	219	$C_{16}H_{13}N$	Phenyl alpha-naphthylamine
	298	$C_{19}H_{38}O_2$	Methyl 10,methyl heptadecanoate
	278	$C_{16}O_{22}O_4$	1, 2-benzenedicarboxylic acid dibutyl ester
Fengning	219	$C_{16}H_{13}N$	Phenyl alpha-naphthylamine
	278	$C_{16}H_{22}O_4$	1, 2-benzenedicarboxylic acid dibutyl ester
	234	$C_{15}H_{22}O_2$	3, 5-di-t-butyl-4-hydroxy-benzoldehyde
	181	C_8H_7NS	2(methylthio) benzothiazole
	236	$C_{16}H_{28}O$	1, 5-di-t-butyl-3, 3dimethyl-benzene
	220	$C_{14}H_{20}O_2$	2, 6-di-t-butyl-1, 4-benzo-quinone
	148	$C_{10}H_{12}O$	1-(2, 4-dimethylphenyl)-ethanone
	167	C_7H_5NS	Benzothiazole
	134	$C_9H_{10}O$	3, 4dimethylbenzoldehyde
	134	$C_9H_{10}O$	2-phenyl-propylaldehyde
	194	$C_{12}H_{18}O_2$	1, 4dimethoxytetramethyl-benzene
255	$C_{17}H_{37}N$	N-heptadecylamine	

radicals on organisms. Therefore the radicals in the drinking water of disease regions are carried on primarily in the research.

Table 6 gives data of the concentration of organic matters from drinking water of disease and non-disease areas, their radicals and the g value in ESR spectra. It can be seen that the difference of the concentrations of the radicals between the disease and non-disease areas is more obvious than that of the organic carbon. And the concentrations of the radicals in drinking water of the disease areas are higher than those of the non-disease areas. The g value of radicals in the drinking water is close to the data of fulvic acid (Senisi, 1977). Wang *et al.* (1989) showed the contents with notable difference including the contents with quinone structure in the drinking water. The samples were collected from the village with heavy incidence of Kaschin-Beck disease. Later the farmers moved to a new place about 3 km away from the old and changed their drinking water so that new patients are not found since then. From the results of new and old drinking water detected by GC/MS the compounds with quinone structure are rather special. The phenolic compounds are easy to change to quinone chemically. Therefore the phenolic and quinone compounds should be involved when the sources of the semi-quinone radicals are studied.

Table 6 Concentration of free radicals and g values in organic matters of drinking water

Places	TOC, ppm	Conc. radic, $\times 10^6$	g values
N. D. :			
Huairou	2.2	2.28	2.0037
Miyun	2.0	3.67	2.0030
Tanghekou	7.0	4.55	2.0043
D. :			
Zhalantun 1	8.9	16.9	2.0038
2	5.0	15.7	2.0034
3	5.6	25.3	2.0039
4	5.8	20.8	2.0030
5	5.4	13.8	2.0038
6	7.6	21.0	2.0037
Suiling	—	9.0	—
Shangzhi	—	10.4	—
Fusong	—	9.6	—
Fengning	—	9.6	—

Note: TOC : total organic carbon; N. D.: non-disease regions; D. : disease regions

Photoreaction of organic compounds in water of disease region

In earlier time local residents found that water irradiated before drinking can prevent the disease. Recent experiment concludes that organic content in drinking water of disease area decrease after irradiation (Wu, 1985). It implies that the chemical contents in the water are changed by sunlight.

From the research on photoreaction of the organic compounds in the environment humic matters are of strong light sensitivity (Zepp, 1988). It changes itself in photoreaction and it leads the change of other substances in the water. Free radicals including single oxygen, $^1\text{O}_2$, Superoxygen $\cdot\text{O}_2^-$ and $\cdot\text{ROO}^-$, as well as hydroxyl radicals $\cdot\text{OH}$ occurs in photoreactions.

The solution of FA from the soil of disease village in Fengning was irradiated by the light with more than 290 nm from high pressure mercury lamp. The agent furfuralcohol (FFA) to trap $^1\text{O}_2$ is homogeneously mixed before light. The FA and FFA concentration in the solution are detected at different time. The results are presented in Table 7 and Fig.4, respectively. The FA concentration decreases with the increase of time in Table 7. It is coincidental with the work on UV light by Wu and Ren (1985). The FFA concentration in the solution decreases with the increase of irradiation time. That implies the continuous release of the $^1\text{O}_2$ in the photoreaction. The photoreaction is accelerated in the presence of metal ions such as Fe (II) (III) and Se (IV) in the solution.

Table 7 Concentration change of FA with increase of time

Time, h	0	0.08	0.17	0.25	0.50	1.00	1.50	2.00	4.00
Conc., ppm	9.7	8.1	6.6	6.1	4.5	3.5	3.0	2.4	1.9

Salicylic acid is a kind of single molecular organic compound distributed widely. Its solution is irradiated with the method above. The concentration single oxygen $^1\text{O}_2$ and the photoproducts are detected (Wang, 1988). The results are similar to FA. SA and FFA concentrations decrease with the increase of the irradiated time. The photoproducts include CO_2 and yellow polymeric humic-like substance. The concentration of two products increases with the decrease of SA concentration. It suggests the photodegradation and photopolymerization occur simultaneously. The single molecular compounds are humified to a humic-like substances with high molecular. Under the same condition tested the concentration of benzene monocarbonic acid (BA) solution and its UV spectra are not changed without the yellow products. This means the phenolic hydroxyl group $-\text{OH}$ plays an important role.

The $^1\text{O}_2$ concentrations in the irradiation for the drinking water of Wujiawan and Liujiahe are detected (Fig. 4). The speed of FFA consumed in the drinking water of Wujiawan is faster than that in the drinking water of Liujiahe. It indicates the drinking water

of the disease village Wujiawan is of higher ability of production of $^1\text{O}_2$.

Accumulation of FA on the bone of tested rat

The pathological change of Kaschin-Beck disease is caused at the human bone. It is questionable whether the organic compounds in the drinking water of the disease area can be attacked in the bone. FA solutions with several concentrations are injected into the tested rat for 130 days. The bones of the tested animals are studied. The ESR spectra are shown in Fig. 5. The colours of the bones are more yellow and the singles of ESR spectra are strong with the increase of FA dose. Their g values are very close to that of FA injected, showing the semi-quinone radicals. It can be deduced that the organic compounds in drinking water of the disease areas are able to arrive at and deposit on the human bone.

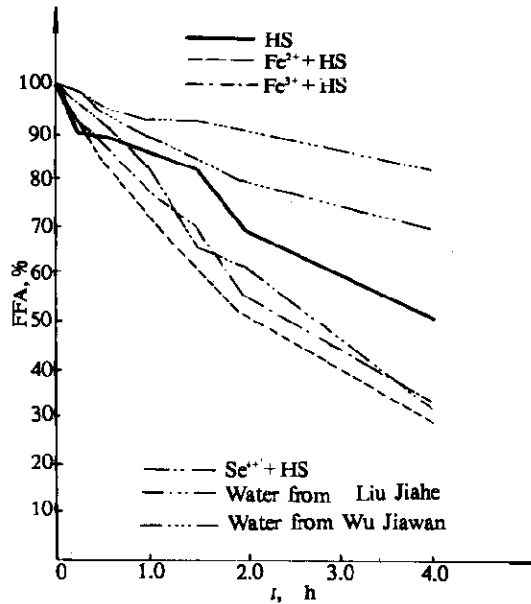


Fig. 5 FFA in different solution irradiated

With the normal analytical and characteristic methods the definitive information could not be given in the investigation. The researches on the radicals indicate: (1) the radical content in organic matter of the drinking water in disease region is more than that in the non-disease region. (2) The water from disease area is potentially of more strong ability to produce the radicals. (3) FA can arrive at the "target" of Kaschin-Beck disease, the bone. Some works have shown free radicals induce damage of chondrocytes and synthetic of abnormal matrix (Wang, 1989; Feng, 1990). It is important to pay more attention to the research on the radicals generated by organic matters from the drinking water in the investigation of the causes of

Kaschin-Beck disease.

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