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THE FIBROUS ZEOLITE "ERIONITE":

MEDICAL IMPORTANCE AND EXPERIMENTAL FINDINGS AND SOME STUDIES ON IMMUNOLOGICAL EFFECTS OF MINERAL FIBRES.

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MUSTAFA ÖZESMI

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ABSTRACT

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Among the 575 inhabitants of village of Karain, Turkey, there were 18 deaths during 1975/76, therof 11 due to malignant pleural mesothelioma. In neighbouring village no mesothellomas were found. No asbestos occurs in the village. The bedrock is volcanic zeolite and contains a fibrous component called erionite.

Rocks from Karain containing erionite were grinded into dust. This dust was injected into the peritoneum of mice. In 321 mice which died spontaneously between 8 and 32 months after injection, malignant mesothelioma was seen in 41 and malignant lymphoma in 31. In addition, 11 animals had both malignant mesothelioma and malignant lymphoma. In control animals, few mesotheliomas and lymphomas were seen.

Immigrants from Karain living in Stockholm, Sweden, were invited to a health survey including clinical and radiological investigation and blood tests. Phenotypic characterisation of peripheral blood lymphoid cells was performed in 74 of them. There was a significant decrease in the percentage of T helper/inducer cells, while suppressor/cytotoxic cells were normal. Pleural reactions (visible fissures, pleural thickening and pleural plaques) were observed on chest roentgenograms. Lung function and clinical investigation were unremarkable.

Forty-five persons occupationally exposed to asbestos and with pleural plaques, diffuse pleural fibrosis or acute benign asbestos pleurisy were next investigated. Helper/inducer T cells were significantly decreased among those with diffuse pleural fibrosis and acute pleurisy, but not among those with pleural plaques only.

Thus, erionite is highly carcinogenic as seen from epidemiological and experimental studies. the mineral also affects the immune system and causes abnormal blood levels of various immunregulatory T-cell subsets. Whether these immunological changes are only secondary epiphenomena or if they have any pathogenetic importance should be further investigated.

Key words: Erionite, mineral fibre, asbestos, mesothelioma, Immune system, lymphoid cells, lymphoma.

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to Çiğdem, Uygar and Deniz

THIS THESIS IS BASED ON THE PAPERS LISTED BELOW:

Paper I. An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Ürgüp in Anatolia. Baris Y I, Sahin A A, Özesmi M, Kerse I, Ozen E, Kolacan B, Altinörs M, Göktepeli A. Thorax 1978; 33:181-192.

Paper II. Peritoneal mesothelioma and malignant lymphoma in mice caused by fibrous zeolite. Özesmi M, Patiroglu T E, Hillerdal G, Özesmi C. Brit J Ind Med. 1985; 42:746-749.

Paper III. Phenotypic characterisation of peripheral blood lymphoid cells in people exposed to fibrous zeolite. Özesmi M, Karlsson-Parra A, Hillerdal G, Forsum U. Brit J Ind Med. 1986; 46:830-833.

Paper IV. Prospective clinical and radiological study of zeolite-exposed Turkish immigrants in Sweden. Özesmi M, Hillerdal G, Svane B and Widström O. ~~In preparation.~~ Respiration 1990; 57: 325-328

Paper V. Phenotypic characterisation of peripheral blood lymphoid cells in patients with asbestos-related changes. Özesmi M, Hillerdal G, Karlsson-Parra A, Forsum U. Submitted for publication.

CONTENTS

LITERATURE REVIEW

INTRODUCTION

The term "endemic pleural plaques" was first coined by Kiviluoto in 1960. He described endemic occurrence of pleural calcification in the general population in a part of Finland where the environment was polluted by anthophyllite asbestos (39). Other such areas in Europe have since been reported. In the 1970s, investigations in Turkey revealed that endemic plaques existed in that country as well.

The first epidemiological survey on asbestos-related diseases in Turkey was carried out by The Department of Lung Medicine of the Haccettepe University School of Medicine. It was conducted in Mihaliççik Town in the region of Eskisehir where asbestos occurs in the surroundings and where there are many small asbestos mills (29) (Map, figure 1).
A subsequent study on the prevalence of pleural calcifications was done in Mihaliççik region (49). Similar field studies on endemic pleural calcification and mesothelioma were done by Yazicoglu in southeast Turkey (70,71). These environmental studies revealed that there are both chrysotile and amphibole types of asbestos in these districts.

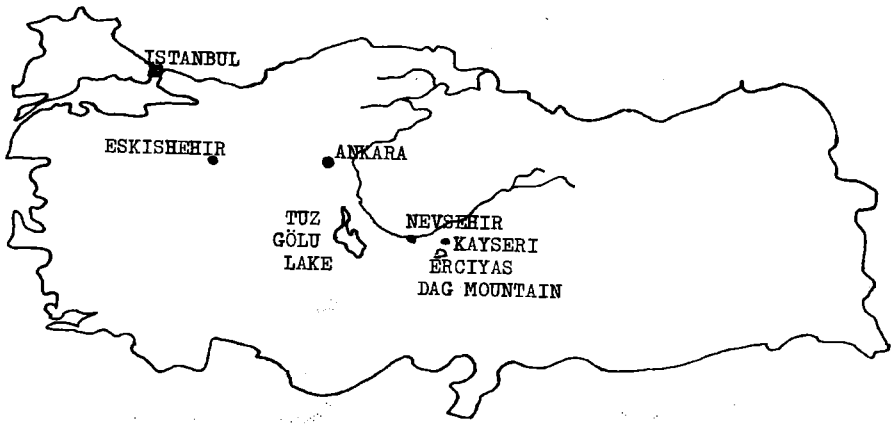

FIGURE 1. MAP OF TURKEY SHOWING SOME OF THE PLACES MENTIONED IN THE TEXT.

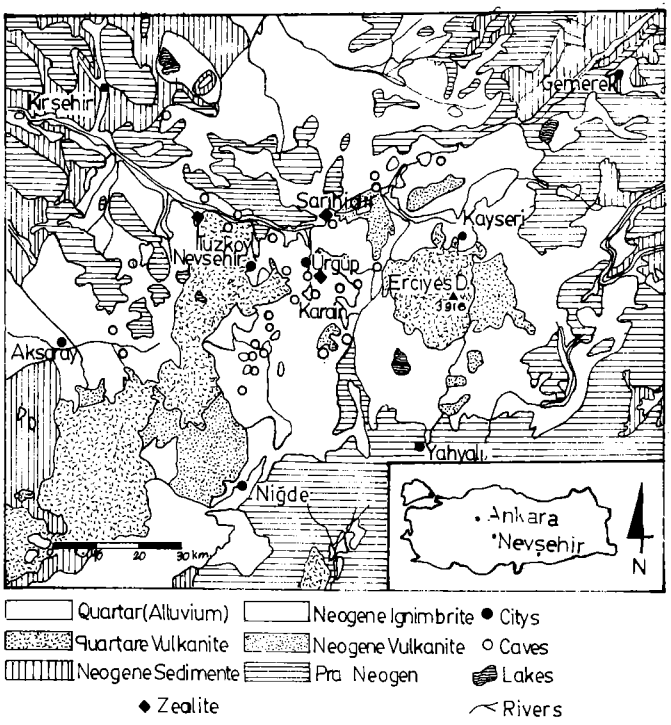

FIGURE 2. THE GEOLOGY OF THE CAPPADOCIA AREA. MODIFIED FROM THE OFFICIAL GEOLOGIC MAP OF TURKEY.

Occupational, paraoccupational and environmental exposure to asbestos is common, especially in southeastern Turkey where white stucco containing asbestos is used to paint the house walls (8,10,70).

Knowing of these studies, the head-man of Karain village in the district of Nevsehir in Cappadocia informed us at the Department that there were many cases of cancer in his village and invited us to study this. We therefore performed a survey in Karain (7). There were many persons with malignant mesotheliomas and pleural calcifications there, and consequently we expected to find asbestos in the area.

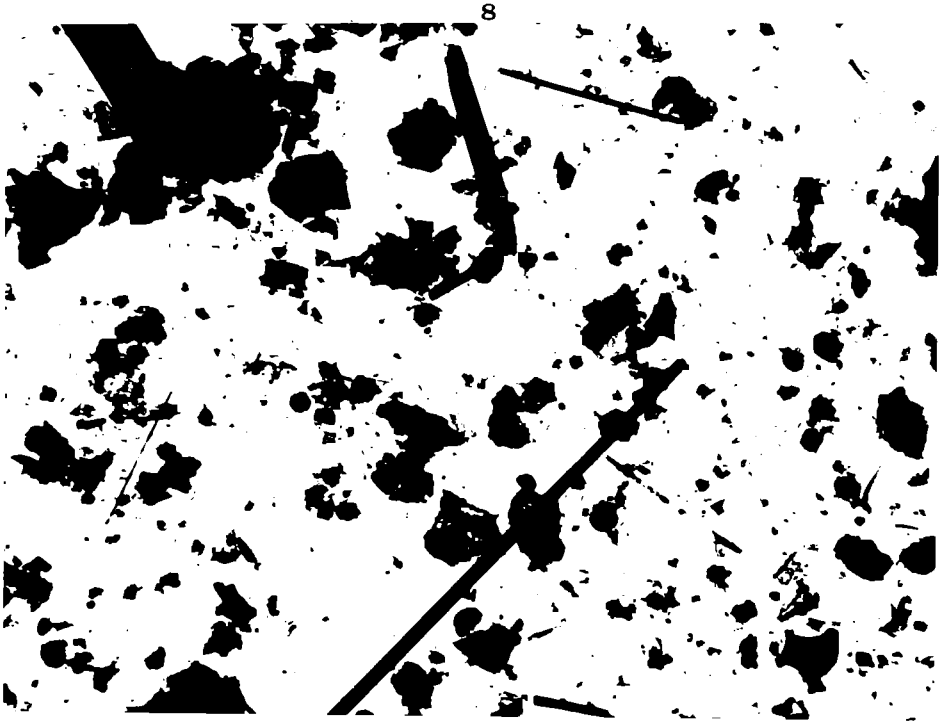

FIGURE 3. ERIONITE FIBRES IN DUST FROM KARAIN. Magnification, x7750.

However, the geological maps of Cappadocia region did not show any deposits of asbestos (Figure 2) and further research confirmed that the dust and rock samples from this region did not contain asbestos. On the bais of this data the International Agency for Research on Cancer (IARC) agreed on urgent research studies and these were started in 1978. They were conducted by The Department of Lung Medicine of Hacettepe Medical School, the MRC (British Medical Research Council), the Pneumoconiosis Unit and Department of Mineral Exploitation of the University College of Cardiff, UK, and the Unit of Epidemiology and Biostatistics of IARC. Interest was soon focused on the occurrence of a fibrous zeolite, erionite, which was found in the bedrock (Figure 3). Experimental evidence demonstrated that this fibrous zeolite (erionite) was cytotoxic. It is now known that erionite fibres are found only in three Turkish villages: Karain, Tuzköy and Sarihidir (47).

HEALTH RISKS IN RELATION TO SIZE OF MINERAL FIBRES

The fibrogenicity and carcinogenicity of asbestos fibres has long been recognized. Increasing attention is currently being addressed to the biological effects of mineral fibres other than asbestos. This interest was generated by the work of Stanton and co-workers. His group published the results of experimental works in rats with 17 samples of fibrous glass with different dimensional characteristics. They concluded that mineral fibres less then 1.5 micron in diameter and greater than 8 micron in length yielded the highest probability of pleural mesothelioma (61). They later extended their work with further experiments, confirming their earlier findings (62). Thus, it seems that the fibre diameter, length and shape are more important than the chemical composition of the fibre. Fibres of the same small sizes are also more toxic to cells in *in vitro* studies (18). That the fibrogenicity of fibres is related to the same factors is also becoming increasingly clear. The smaller fibres are phagocytosed and cleared by the macrophages and are therefore less toxic (1,2). The fact that a non-asbestos fibre could cause disease in man generated much interest since this seemed to confirm these experimental findings.

Crocidolite is the type of asbestos fibre which is recognized to be the most potent cause of mesotheliomas in man (30, 33). Apart from a small mine in Bolivia, South America, today it is only mined in South Africa. In the area of the mine in North West Cape Province the prevalence of malignant mesothelioma is very high. In the other important crocidolite mine, in the Transvaal, the incidence of mesothelima is much lower. Timbrell has shown that the airborne fibres of the North West Cape Province mine have a 0.06 µm median diameter with 99% less than 0.2µg. For Transvaal crocidolite and amosite fibres, the median diameters are 0.2 µm with 99% less than 0.5µg (66, 67). It has been suggested that these differences in diameter are the main reasons for the different biological effects.

Thus, the longer the fibre and the smaller the diameter, the more dangerous is the fibre. The diameter and length of erionite are similar to those of the most dangerous types of crocidolite (13,

65, 68), which agrees with the epidemiological findings of an extremely high incidence of mesothelioma in the "erionite villages".

GEOLOGY, OCCURRENCE AND OCCUPATIONAL USE OF ZEOLITES

For an explanation of some geological terms, see table 1.

Zeolite is a generic term for various crystalline aluminosilicates of alkali and alkaline earth cations having a three-dimensional structure. They are characterized by the ability to lose or gain water and to exchange cations without major damage to the structure (44). The structure is a network of channels leading into sizeable cavities, enabling the adsorbtion of water and molecules. Zeolite was first discovered by Cronsted, a Swedish mineralogist, with the discovery of stilbite in 1756. The word zeolite comes from the Greek words meaning To Boil-"Zein" an A Stone-"Lithos", thus "boiling stone" because of the loss of water that occurs when the rock is heated in the mineralogist's blowpipe (48).

Erionite is one of the over 40 species of natural zeolites (Table 2). Zeolites are among the most common authigenic silicate minerals that occur in sedimentary rocks of diverse age. Only nine zeolites commonly make up the major part of zeolitic rocks. These are analcime, chabazite, clinipliolite, erionite, ferrierite, leulandite, laumontite, mordenite and phillipsite. Numerous zeolites have also been synthetized. Linde A, Linde X, and Linde Y are some examples (17,46).

Though zeolite rocks have been used as a building material for more than 2000 years its first description appeared in 1756. Zeolites were only exhibited in museums until the 1950s. During the middle 1950s, when Union Carbide Corp. and other groups were developing processes and markets for their fledgling

TABLE 1. EXPLANATION OF SOME GEOLOGICAL TERMS IN THE TEXT.

ALKALI: Igneous rocks with silicate minerals containing mainly sodium and/or potassium.

ASBESTOS: Collective commercial name for certain distinct silicate minerals which are fibrous. They are divided into serpentine asbestos or chrysotile and amphibole asbestos, the most important types being crocidolite, tremolite, amosite and anthophyllite.

AUTHIGENIC: Rock which has developed in place during or after deposition.

DIATOMITES: Sediment consisting of the remains of microscopic plants. Consists mainly of silica.

IGNEOUS ROCKS: Very basic rocks, the primary rocks of the earth's surface. Most are the results of melted silicates.

PYROCLASTIC MATERIAL: Material blown into the atmosphere by an active volcano.

SEDIMENTARY ROCKS: Formed from materials from pre-existing rocks by erosion and transportation.

TUFF: Consolidated ask from a volcanic eruption.

ULTRA-METAMORHIC ROCKS: Rocks which are profoundly metamorphized from heat and pressure without having melted.

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synthetic zeolite businesses, natural zeolites were discovered as major constituents of numerous volcanic tuffs in saline-lake deposits of western USA and of massive marine deposits in Japan and Italy. Since that time more than 1000 occurrences have been reported from sedimentary rocks of volcanic origin in more than 40 countries of the world, and nowadays it is of great economic importance and extensively used (Table 3) (44, 45, 60).

Natural and synthetic zeolites may occur in granular and fibrous forms. Most of the zeolite species are non-fibrous, but are least two of the natural zeolites, mordenite and erionite, are fibrous. The short description of erionite is given in Table 4 (28).

Erionite was first described and named by Eakle in 1898 from Oregon (22). Erionite stems from a Greek word meaning wool. There are erionite deposits in some parts of the world. In the USA erionite is found in Oregon, South Dakota, Nevada, Arizona, California and Wyoming. There are also some deposits in Japan, Yugoslavia, Kenya, Tanzania, New Zealand, Mexico and Turkey (15,22,44).

The percentage of erionite in zeolitic tuffs differs. In Nevada and Japan some zeolite deposits contain large amounts. In the USA two of six mineable deposits have been mined. Pure erionite deposits are no longer mined for commercial use (15).

ZEOLITES IN TURKEY: GEOLOGICAL BACKGROUND

Turkey is geologically a most suitable country for the formation of zeolites. According to the studies carried out by The Department of Earth Sciences of Hacettepe University, zeolites are abundant in many regions, among those Cappadocia (3). (Fig. 2).

The Cappadocia region is underlain by about 1000 to 1500 m. of interbedded volcanic rocks. These volcanic rocks consist mainly of salt flows, airfall tuffs, and ash-flow tuffs. Interbedded are sedimentary rocks, some of which have been completely transformed to zeolites (28,47).

During the last 100 million years in Cappadocia there was repeated formation of saltstone. West from Aksaray there are more than 1000 meters thick deposits of saltstone. Tuzköy region contains a distinctive saltstone in the upper part. It consist largely of salts, carbonate rocks and diatomites. Near Cappadocia is the inactive vulcano Erciyes Dagi (fig. 4). When it was active, ash and lava were spewn out 60-80 km away. When this lava came into contact with the alcalic salt lakes and saltsone, zeolite was formed. The combination of highly reactive pyroclastic material and saline, alkaline solutions is apparently ideal for the crystallization of zeolites (28).

TABLE 2. Some natural zeolites and their formulas (15,21,48)

Analcime
$NaAlSi_2O_6.H_2O$

Chabazite
$(Ca,Na2)Al_2Si_4O_{12}.6H_2O$

Clinoptilolite
$(Na_2K_2Ca)_3Al_6Si_{30}O_{72}.24H_2O$

Epistilbite
$(Ca, Na_2)_3Al_6Si_{18}O_{48}.16H_2O$

Erionite
$(Na2, K2, Ca)_{4.5}Al_9Si_{27}O_{72}.27H_2O$

Faujasite
$(Na2, Ca)_{1.75}Al_{3.5}Si_{8.5}O_{24}.16H_2O$

Ferrierite
$(K, Na)_2(Mg, Ca)_2Al_6Si_{30}O_{72}.18H_2O$

Gismondine
$(Ca, Na_2, K_2)_4Al_8Si_8O_{32}.16H_2O$

Gonardite
$Na2CaAl_4Si_6O_{20}.5H_2O$

Harmotome
$(Ba, Na_2)_2Al_4Si_{12}O_{32}.12H_2O$

Heulandite
$(Ca, Na_2)Al_8Si_{28}O_{72}.24H_2O$

Laumonite
$Ca_4Al_8Si_{16}O_{48}.6H_2O$

Mordenite
$(Na_2, K_2, Ca)Al_2Si_{10}O_{24}.7H_2O$

Natrolite
$Na_4, Al_4, Si_6, O_{20}.4H_2O$

Phillipsite
$(K_2, Na_2, Ca)_2Al_4Si_{12}O_{32}.12H_2O$

Scolecite
$Ca_2Al_4Si_6O_{20}.6H_2O$

Stilbite
$(Ca, Na_2)_4Al_8Si_{28}O_{72}.28H_2O$

Thomsonite
$NaCa_2Al_5Si_5O_{20}.6H_2O$

Wairakite
$CaAl_2Si_4O_{12}.2H_2O$

Yugawaralite
$CaAl_2Si_4O_{12}.2H_2O$

Mesolite
$Na_2Ca_2(Al_2Si_3O_{10})_3.8H_2O$

Edingtonite
$Ba(Al_2Si_3O_{10}).4H_2O$

Dachiardite
$(Ca, K_2, Na_2)_3Al_4Si_{18}O_{45}.14H_2O$

Gmelinite
$(Na_2, Ca)Al_2Si_4O_{12}.6H_2O$

Levyne
$Ca(Al_2Si_4O_{12}).6H_2O$

Ashcroftine
$KNaCa(Al_4Si_5O_{18}).8H_2O$

TABLE 3. Medical and industrial uses of zeolites (15,46):

1.**Oxygen enrichment**: The use of O_2-concentrators makes long term O_2-therapy at home easier and less expensive. Nitrogen gas is adsorbed from air by zeolite yielding a gas containing up to 95% oxygen. The useful zeolites for oxygen are mordenite, clinoptilolites and chabazites.

2. **Polishing agent** in toothpaste and for separation of ammoniac nitrogen from hemodialysis liquids. Clinoptilolites are used for this.

3. **Radioactive waste disposal, sewage effluent treatment, agricultural waste water treatment, stack-gas cleanup and oil spill cleanup** because of the ion-exchange and absorption properties.

4. **Agricultural uses**: Carriers of herbicides and pesticides; use as soil conditioners and to control the moisture content of soils. Also used as a dietary supplement in animal husbandry and as sand for cats.

5. **Industrial use** : Coal gasification; petroleum production; solar energy plants; natural gas purification; paper production; construction; in mining and metallurgy.

To recognize zeolized tuff macroscopically is difficult. Therefore zeolites were not described until 1978 in Cappadocia. After the reports of endemic mesothelioma in the Cappadocia region Ataman surveyed the region and found chabazite, mordenite and erionite types of zeolites, mainly in Tuzköy, Karain and Sarihidir villages (5).

Most chrysotile fibres are found in metamorphosed ultramorphic rocks of igneous origin that were altered to serpentine by hydrothermal action. Then changes of pressure in the earth's crust caused an irregular network of cracks in the rocks, and hot ground waters were forced into the cracks under pressure. This was

TABLE 4. Mineralogical properties of erionite (12,23):

Origin of name	Greek, comes from the word "wool"
Chemical formula	$(Na_2, K_2, Ca)_{4.5}Al_9Si_{27}O_{72}.27H_2O$
Crystal system needle,	Hexagonal Habitus: fine line fibrous, fibre
Dispersion constant	a_0 13.26 C_0 15.12
d-values	11.4 6.61 4.32 3.86
Refraction	n_E 1.474 n_0 1.471
Specific gravity	2.07
Length of fiber	8 to 40 microns
Diameter of fiber	0.1 to 2 microns
Use	Ion exchange, production of high purity gases, gas absorption and catalyst

followed by a slow process of dissolution from the walls, and recrystallization in the form of a close-packed assembly of fibers generally running across the veins (57).

Due to these very diferent backgrounds, it is unlikely that these two types of mineral fibers (erionite and asbestos) can occur together in nature.

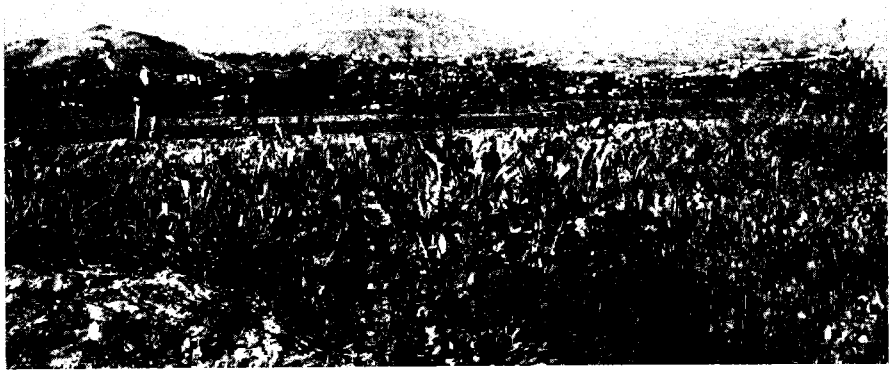

FIGURE 4. THE NOW INACTIVE VOLCANO ERCIYES DAGI

HISTORICAL BACKGROUND

The Cappadocia region of central Turkey has long been recognized as an area of outstanding scenic and cultural value. Centered around Nevsehir, about 250 kg southeast of Ankara, and bounded by the city Kayseri on the east and the saline lake Tuz Gölü on the west, the region abounds with valleys which have been cut into plateaus of soft, volcanic tuff spewn out during neogene times by the predecessors of the now inactive volcano Eriyes Dagi (3916m) (Fig. 1 and 2).

The area has seen many different cultures through the centuries, although the date of the first settlement is not exactly known. Mazaka (Kayseri) had been the capital of Cappadocia when the region was ruled by Persians. The Persian domination was put to an end by Alexander the Great, and the area was then ruled by Macedonia until his death.

The Cappadocia Kingdom gained its freedom during the period when Ariarathes III was ruling the country. Cappadocia became a province of Rome in the first century B.C. In this time the volcano Erciyes Dagi was still active (28). Between the 4th and 14th centuries Christian communities settled in Cappadocia, hewed the tuff into lightweight dimension stone and cut dwellings and worship chambers into the soft rock itself. More than 300 monastic caves and some underground cities have been found in the tuff cones (Figure 5). Some of the churches are the Elmali Kilise, the smallest and newest of the group, and the Yilanli Kilise which has fascinating frescoes of the damned in the coil of serpents.

FIGURE 5. VOLCANIC TUFF CONES WITH CUT DWELLINGS.

Kaymakli and Derinkuyu are underground cities with 7 flats and a depth of 85 m. These intriguing underground cities of rooms interconnected by tunnels were used in Byzantine times as refuges from Arab raids. For a thousand years a Christian civilization prospered in Cappadocia, becoming troglodytic during times of Roman persecution and Arab border raids, but emerging to live in harmony with the Seljuk Turks who settled in the area in the 11th century. The population exchanges between Greece and Turkey in 1924 greatly diminished the Christian community, and most of the underground chapels were abandoned. Many, however, were set aside by the Turkish government as archeological antiquities and a flourishing tourist trade exists today in the area.

FIGURE 6. SCENE FROM KARAIN VILLAGE.

There are many villages in the region. Three of them (Karain, Tuzköy and Sarihidir) are the only villages built upon and into erionite-rich zeolite rocks (Figure 6). Today Sarihidir has been moved across the river Kizilirmak to where the rocks are free from erionite,and no more pleuropulmonary diseases appear in the young generation. Unfortunately, the villagers in the other two villages have refused to move, in spite of the goverment's offer to help for the resettlement.

Many inhabitants of Karain village have emigrated to Sweden in search of work. They settled in Stockholm.

EXPOSURE TO FIBROUS ZEOLITE

There are three known areas where erionite with fibres within the respirable size has been found: in Oregon and Nevada in the USA, in Turkey, and in the north Island of New Zealand.

The erionite findings in the USA are situated in desert areas where the population density is very low and no active mining or other disturbances occur. The early immigrants occassionally used rocks containing erionite for buildings like stables and pony express stations (22,44). Reports of health risks from this area are rare. A case control study of mesothelioma has been reported. Seventeen cases and 12 controls had lived for 20-40 years before death within 20 miles of zeolite deposits containing erionite in western USA. A paired analysis gave a relative risk of 1.60 after adjusting for occupational exposure to asbestos (43). One patient with extensive pulmonary and pleural fibrosis probably due to environmental exposure to erionite has been reported. He was an operator of heavy equipment for years in this hot and dusty area of Nevada. Investigation revealed extensive pulmonary deposition of erionite (19).

The Turkish "erionite areas" of Nevsehir in Anatolia are moderately densely inhabited and are very dry and dusty in summer. The local villagers use caves carved out of volcanic tuff for storage. The tuff is also used for building houses (Figure 5 and 6). The soil itself is largely composed of eroded tuff and contains various fibrous and nonfibrous zeolites mixed with volcanic glass and pumice (68). Airborne fibre levels in the villages have been shown to be generally low (11,13).

FIGURE 7. Chest roentgenogram from a patient from the village of Tuzköy with diffuse pulmonary fibrosis.

HEALTH EFFECTS OF EXPOSURE TO ERIONITE

PULMONARY FIBROSIS ("ERIONITOSIS"). Clinical and radiological surveys done in Karain and Tuzköy villages have revealed that diffuse lung fibrosis with or without other radiological abnormalities is present in a high percentage of subjects (3,4,11,14) (Figure 7). Fibrous zeolite has been recovered from sputum samples (59) and in the lungs (58).
Lung fibrosis due to fibrous zeolite inhalation has also been reported from Nevada (19). Extensive fibrosis has been seen with exposure to erionite in mice (64).

MALIGNANT DISEASES. Malignant pleural and peritoneal mesotheliomas are rare tumours and the incidence is estimated to be 1-2 per million in the general population (33,42,63). In Turkey the incidence of pleural mesothelioma in the erionite area is high.

The annual incidence of 216/100 000 cases of malignant mesothelima in Tuzköy is at least 942 times higher than expected (3,6,15). In a 4-year study of the three villages (Karain, Sarihidir, and Tuzköy) 115 deaths among those aged 20 years or more were seen. Of those, 29 were malignant pleural mesotheliomas, 4 malignant peritoneal mesotheliomas, 17 lung cancers, and 22 cancers of other sites or undefined site. In the village of Karain, 21 out of 40 deaths were due to malignant mesothelioma (13). Emigrants from Karain village to Sweden carry the same risk, and in the Stockholm population a number of malignant mesotheliomas has been reported (16, work IV).

Experimental studies have resulted in mesotheliomas in animals (36,41, 52,64,65,69). In one study all the animals exposed to erionite, both those inoculated intraperitoneally and those which inhaled the fibre, have developed mesothelioma (69).

FIGURE 8. ERIONITE FIBRE ISOLATED FROM THE LUNGS OF A KARAIN VILLAGER. Magnification: x7750.

FIGURE 9. CALCIFIED PLEURAL PLAQUES IN A PATIENT FROM KARAIN.

The incidence of lung cancer, gastrointestinal cancer and cancer of other sites in the population of Cappadocia with a high incidence of mesothelioma is still unknown. However, experimental studies with erionite in mice showed in addition to mesotheliomas other tumours: Plasmocytoma, histiocytoma, liposarcoma, osteosarcoma and adenocarcinoma (64, 66). In our studies (Work II) one epidermoid carcinoma and a number of lymphomas were observed and 11 of the cases of mesotheliomas were associated with lymphoma.

A genotoxic activity of fibrous erionite has been demonstrated. Erionite appears to be more active than crocidolite in this regard (38, 50).

NON-MALIGNANT PLEURAL DISEASE. In the village of Tuzköy, persons at least 25 years old were investigated by chest roentgenogram. Seventeen percent showed pleural calcification, 10.5 percent pleural thickening, and 15 percent obscured costodiaphragmatic angle (3,4) (Figure 9). In Karain, the figures for plaques and/or chronic pleurisy has been given as 8.7 percentfor adult males and 4 percent for females (4). In 549 chest X rays from the erionite villages which were restudied in 1983, there were 14.2 percent with pleural calcification and 10 percent with obscured costophrenic angle (31).

The 564 photofluorograms taken by the mobile team of the Tuberculous Control Unit in Karain in 1971 were reviewed and there were calcified pleural plaques in 2 persons. In 1975 of 452 films there were two with calcified pleural plaques, three with pleural thickening, and three with benign pleural effusion. Another five patients with benign pleural effusion and pleural thickening were seen during a follow-up over 5 years (13).

FERRUGINOUS BODIES - "ZEOLITE BODIES". "Ferruginous" or "asbestos" bodies in the lungs of persons who have inhaled asbestos were first described in 1914 (26). Such bodies consists of a central fibre coated with an ironcontaining proteinous material and can be formed around several kinds of fibres. In industrialized countries ferruginous bodies isolated from the general population almost always form around a central amphibole asbestos fiber (20). Animal experiments have shown that fibrous aluminium silicate, silicon carbide whiskers, cosmetic talc and glass fibers can also produce ferruginous bodies. Not surprisingly, erionite ferruginous bodies have been found in sputum and in the lungs of inhabitants of Karain and Tuzköy (58,59). The occurence of ferruginous bodies in sputum is related to the length of exposure to fibrous dust (25, 55). The occurence of zeolite bodies in the sputum thus reflects considerable fiber accumulation in the lungs.

Some authors stress that all or a very high portion of erionite fibres in the human lung are uncovered. Since the formation of ferruginous bodies around a fibre is considered to be a protective measure, this low occurrence of bodies might be one factor of importance for the effect of the fibres.

IMMUNOLOGICAL EFFECTS. The mineralogic characteristics of erionite and deleterious effects on human health are similar to those of asbestos. In asbestosis several immunological stigmata, such as hypergammaglobulinemia, *in vitro* cellular immune dysfunction and abnormal peripheral blood levels of various immunoregularatory T-cell subsets have been reported (23,24,37, 51). Our experimental study suggested that erionite can cause tumours of the lymphatic system (Work II), and there are indications that asbestos can also cause such tumours (37). Further investigations in the area of interaction of the immune system and mineral fibres and their relation, if any, to tumours, in particular mesothelioma, are therefore needed.

SUMMARY OF OWN INVESTIGATIONS

AIMS

PAPER I. The first study was intended to confirm the increased risk of pleural mesothelioma in the village of Karain and to study the occurrence of other radiological changes there. It had for a long time been known locally that the inhabitants of the village died of cancer. Having confirmed this increased incidence, the next step was of course to identify the responsible agent, a task which, however, necessitated the participation of many international experts in various fields.

PAPER II is an experimental study, the aim of which was to verify that the dust from Karain, containing the fibrous zeolite erionite, was carcinogenic.

PAPERS III AND IV are the results of a follow-up study of persons from Karain village, who reside in Stockholm, Sweden. The aims of were to investigate whether any clinical and/or radiological changes were evident and also to see if any more mesotheliomas had occurred in this group. The findings in paper II indicated that the immune system was affected in exposure to erionite, and another aim was therefore to investigate whether any effects on the peripheral blood could be seen in this group.

PAPER V compares a group of people occupationally exposed to asbestos with various radiological lesions in the pleura to see whether any similarities with the erionite group were found.

MATERIAL AND METHODS

PAPER I. The 575 inhabitants of the village of Karain were investigated. Information on medical problems and diseases were collected for the time up to 1975 from interviews with elderly residents and especially with the village headman, the "muhtar". As controls were used neighbouring villages. In addition, retrospective studies of medical information from the Health Institute in Karain and neighbouring hospitals were made. A radiographic survey from 1971 was restudied and information of all deaths between 1970 and 1974 was reviewed. A prospective study was made of all patients developing respiratory symptoms during 1975 and 1976 and a new chest radiograph survey was performed. In addition, geological and chemical tests were done on airborne dust, water supplies, soil and rock, etc.

PAPER II. To verify the carcinogenicity of dust from Karain, 486 Swiss albino mice were injected intraperitoneally with erionite, talc, or physiological saline, and were observed for up to 32 months.

PAPER III. On a sub-group of 74 persons, phenotypic characterisation of lymphoid cells in the peripheral blood was performed. Commercial antisera for determining Leu-4, Leu2a, Leu3a, Leu7, Leu11b, Leu10, Leu12 and HLA-DR were used. As controls were used healthy blood donors.

PAPER IV. In 94 persons from Karain chest roengenograms, clinical investigation, and lung function with a vitalograph were performed.

PAPER V. Phenotypic characterization of peripheral blood lymphoid cells with the same methods as in paper III was made on 45 persons occupationally exposed to asbestos. Twenty persons had radiological pleural plaques alone; 15 had diffuse asbestos-related pleural fibrosis; and 10 had benign asbestos pleural effusion. The controls were 24 healthy blood donors.

RESULTS

PAPER I. During the two observation years, 18 deaths occurred in the village. Eleven of tem were due to malignant mesothelioma. No cases of mesothelioma were seen in the control villages. Twenty-five retro-spective cases of mesothelioma were identified during the previous 5 years in the village. Calcifified pleural plaques and pleural thickening were also seen. No asbestos could be found in the village, but a few unidenitifed mineral fibres were seen in ashed pleural tissue from one patient.

PAPER II. Eighty-four malignant tumours were found in the 321 mice injected with erionite which died spontaneously between 9 and 32 months after injection. There were 41 mesotheliomas, 31 lymphomas, 1 epidermoid carcinoma, and 11 mesotheliomas and lymphomas together. In the control groups, three mesotheliomas were found in the talc group (24 animals) and three mesotheliomas and one lymphoma in the saline group (46 animals).

PAPER III. In the 74 persons from Karain village, there was a significant decrease of the mean percentages of "helper/inducer" T cells, while the percentage of "suppressor/cytotoxic" T cells was normal. B cells were increased, but killer and natural killer cells showed a normal percentage.

PAPER IV. Lung functional and clinical findings in the Karainers were unremarkable, but during four years, three cases of malignant mesothelioma had occurred in this little group. Only 7.4 percent showed pleural plaques. There were signs of affection of the visceral pleura in the form of a visible minor fissure in 76 %, and in addition it was thickened in 8.5 %.

PAPER V. In the patients exposed to asbestos, a significant difference was seen among the subgroups. The group with pleural plaques only had lymphoid cell subsets not significantly different from normals, while those with benign asbestos pleurisy and diffuse pleural thickening had changes similar to those found in the Karain villagers, i e a decrease of the mean percentages of "helper/inducer" T cells, while the percentage of "suppressor/cytotoxic" T cells was normal.

DISCUSSION

The first study (Paper I) was designed to dertermine the prevalence of pleural mesothelioma and other pleural abnormalities in the Anatolian Village of Karain, where neither asbestos nor any other known environmental carcinogen had been detected. The results confimed that the villagers suffered a very high risk of mesothelioma and other pleural changes. Therefore, an experimental study was made of the carcino-genicity of Karain dust, which verified the risks.

An unexpected result from this investigation was the relatively common occurence of malignant lymphomas in the experimental animals. There are no epidemiological findings to confirm this, since no tumours other than malignant mesothelioma have been shown to have an increased incidence in the zeolite area. Tumours of the haematopoetic and lymhatic systems have been described in people exposed to asbestos (37). Since the effects of erionite are similar to those of asbestos, stimulation of the lymphatic system with the ultimate formation of lymphomas seemed a possibility.

In asbestosis several immunological stigmata such as hypergamma-globulinemia, *in vitro* cellular immune dysfunction and abnormal peripheral blood levels of various immunregulatory T-Cell subset have been reported (51). Since an intact immunoregulatory network is of central importance for an effective cancer defense, such observed immunological derangements are of interest when pathogenetic mechanisms are sought in mesothelioma development.

The aim of the Work III was to investigate whether there were any numerical lymphoid cell aberrations in the blood of Karain people, and if so, to correlate them with roentgenographic findings, family history of mesothelioma and future development of disease, particularly mesothelioma. The findings of a reduced number of Leu 3a positive cells, leading to decrease of Leu 3a/Leu 2a ratio are similar to the T cell subsets derangement found in asbestos-exposed persons (23, 24). Natural Killer (NK) cells and Killer (K) cells, which have a spontaneous cytotoxic reactivity against a variety of tumour cell lines and primary tumour cells, were found in normal proportions in the Karain people. The Leu 3a subsets includes T-cells with inducer/helper capacity, while the Leu 2a subsets mainly contains cells with supressor/cytotoxic capacity. Whether the T-cell abnormalities found in erionite-exposed people (and in asbestos-exposed people) are of aetiological importance for mesothelioma development or only represent a secondary epiphenomenon is unclear.

Asbestos and erionite have similar mineralogical characteristics. Asbestos can cause mesotheliomas and other malignant tumours, as well as pleuropulmonary changes such as plaques, pleurisy, diffuse thickening and pulmonary fibrosis. Identical manifestations have been described in the population exposed to erionite.

The carcinogenic power of erionite appears to be stronger than any other mineral fiber. Both these mineral fibres (erionite and asbestos) can also cause similar immunological changes.

The results from the asbestos group (paper V) indicate that there is a relation between immunological reactions and different asbestos-related pleural lesions. In particular, pleural plaques do

not signify any serious effect on the immune system; however, patients with diffuse pleural thickening and/or benign asbestos pleural effusion do show immunological aberrations. Comparisons with the erionite group are interesting: this group shows few clinical or radiological defects, but there is a significant aberration of the immune system - and a very much increased risk of mesothelioma.

Thus, we can conclude that:
a) erionite has a stronger tendency to affect the immune system than does asbestos (at least the common types of asbestos formerly used in Sweden).
b) Immunological aberrations in asbestos-exposed persons are more often seen in patients with diffuse pleural thickening than in persons with pleural plaques alone.
c) Exposure to erionite carries an extremely high risk of malignant mesothelioma.

In addition, some earlier observations should be added (Table 5). Careful study of this table reveals that in villages with a high mesothelioma risk the minor fissure is more often enlarged and rounding of the costo-phrenic angle(s) is also more frequent than in controls and in areas of endemic plaques where the mesothelioma risk is not increased. Calcified plaques, however, are even more frequent in the area with no increased mesothelioma risk.

Thus, there are a number of pieces in the jig-saw puzzle. Further investigations of the relations between radiological findings, immunological aberrations, and risk of malignancy might shed new light on their interrelationships, both on the individual and the group levels.

TABLE 5. Radiological findings in populations with endemic pleural changes (percent). References: 32 and 33.

Population	Turkey: Erionite villages	Asbestos villages	Controls	Finland Anthopyllite area	Controls
Pleural calcification	14.2	22.3	<1	29.1	0.4
Persons with pleural calcification:					
Rounded costophrenic angle					
unilateral	23.1	19.2	-	4.7	-
bilateral	9	5	-	0.9	
Enlarged minor fissure	26	15.7	-	7.9	-
Persons without pleural calcification:					
Rounded costophrenic angle					
Unilateral	7.7	6.4	1.6	7.8	6.5
Bilateral	2.0	1.3	0	2.2	0.7
Enlarged minor fissure	34.2	21.2	-	2.5	9.0
Mesothelioma risk	Extremely high	High	Not increased	Not increased	Not increased

CONCLUSIONS

Epidemiological, clinical and experimental studies have proven that erionite fibres can induce malignant pleural mesotheliomas in man and experimental animals. It is a more potent carcinogen than asbestos. In addition, there are immunological effects of the mineral, which merit further investigation.

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REFERENCES

1. Adamson, I.Y.R., Bowden,D.H.: Response of mouse lung to crocidolite asbestos. 1. Minimal fibrotic reaction to short fibres. J. Pathol. 152: 99-107, 1987.
2. Adamson, I.Y.R., Bowden,D.H.: Response of mouse lung to crocidolite asbestos. 1. Pulmonary fibrosis after long fibres. J. Pathol. 152: 109-117, 1987.
3. Artvinli, M. and Baris, Y.I.: Malignant mesotheliomas in a small village in the Anatolian region of Turkey: An epidemiologic study. JNCI 63:17-22,1979.
4. Artvinli, M., Baris, Y.I.: Environmental fiber-induced pleuro pulmonary diseases in an Anatolian village: An epidemiologic study. Arch. Environ. Health 37:177-181, 1982.
5. Ataman, G.: Les tufs zeolites de Cappadoce et leur liason probable avec certain type de cancer du poumon et de mesothelioma pleurale. C. R. Acad. Sc. Série D 287:207-210, 1978
6. Ataman, G.: Mise en évidence du role de l'érionite (zéolite) dans le mésothelioma pulmonaire. C. R. Acad. Sc. Paris, Série D 291:167-169, 1980.
7. Baris, Y.I., Özemi, M., Kerse, I., Özen, E., Sahin, A.A., Kolacan, B., Ogankulu, M.: An outbreak of pleural mesothelioma in the village of Karain/Ürgüp-Anatolia (Turkey). Kanser 5:1-14, 1975
8. Baris,Y.I., Artvinli, M., Sahin, A.A., Savas, T., Erkan, M.L.: Etude du mésothéliome pleural malin, de la pleurésie fibrosante chronique et des plaques pleurales liées a l'environment en Turquie. Rev. Fr. Mal. Resp. 7:687-694, 1979.
9. Baris, Y.I., Artvinli, M., Sahin, A.A.: Environmental mesothelioma in Turkey. Ann. N.Y. Acad. Sci. 330:423-432, 1979.
10. Baris, Y.I: The clinical and radiological aspects of 185 cases of malignant pleural mesothelioma. In: Wagner, J. C., ed. Biological Effects of Mineral Fibres . Vol:2. Lyon, International Agency Research on Cancer (IARC) Sicentific Publ. N:30, 1980. pp 937-947.
11. Baris,Y.I., Saracci, R., Simonato, L., Skidmore, J.W., Artvinli, M.: Malignant mesothelioma and radiological chest abnormalities in two villages in central Turkey. Lancet 1:984-987, 1981.
12. Baris, Y.I., Artvinli, M.: Natural mineral fiber-induced chest diseases in Turkey, Arch. Immun. Ther. Exp. 30:161-168, 1982.
13. Baris, Y.I., Simonato, L., Artvinli, M., Pooley, F., Sacci, R., Skidmore, J., Wagner, J.C.: Epidemiological and environmental evidence of the health effects of exposure to erionite fibres: A four-year study in the Cappadocian region of Turkey. Int. J. Cancer 39:10-17, 1987.

14. Baris, Y.I., Artvinli, M., Sahin, A.A., Sebastien, P., Gaudichet, A.: Diffuse lung fibrosis due to to fibrous zeolite (erionite) exposure. Eur J. Respir. Dis. 70:122-125, 1987.
15. Baris, Y.I.: Asbestos and erionite related chest diseases. Semih Ofset . Matbaacilik Ltd. Co. Ankara, Turkey 1987.
16. Boman, G., Schubert, V., Svane, B., Westerholm, P., Bolinder, E., Rohl, A.N., Fischbein, A.: Malignant mesothelioma in Turkish immigrants residing in Sweden. Scand. J. Work Environ. Health 8:108-112, 1982.
17. Breck, D.W.: Synthetic zeolites: Properties and applications.Industrial Minerals and Rocks, 4th ed. Lefon, S.J. (editor in chief). AIME publications, New York 1975, p 1243.
18 .Brown, R.C., Chamberlein, M.: The activity of two types of asbestos in tissue culture. In Wagner, JC. Ed. Biological Effects of Mineral Fibers, Lyon, International Agency Research on Cancer (IARC) Scientific Publications No:30, pp 401-407, 1980.
19. Casey, K.R., Shigeoka, J.W., Rom, W.N., Moatamed, F.: Zeolite exposure and associated pneumoconiosis. Chest 87:837-840, 1985.
20. Churg, A: Fiber counting and analysis in the diagnosis of asbestos-related disease. Hum. Pathol. 13:381-392, 1982
21. Deer, W. A., Howie, R.A., Zusman, J.: Rock-Forming Minerals, Vol 4 Framework Silicates London,. 1963, Longman pp 351-385.
22. Deffeys, K.S.: Erionite from cenozoic ruffaceous sediments, central Nevada. Am Mineralogist 44:501-509, 1959.
23. deShazo, R.D., Nordberg, J., Baser, Y,. Bozelka, B., Salvaggio, J.E.: Analysis of cell mediated immunity in asbestos workers. Clin Res. 30:512, 1982.
24. deShazo, R. D.: Current concepts about the pathogenesis of silicosis and asbestosis. J. Allergy Clin Immunol. 70:41-49, 1982.
25. Elmes, P.C.. Fibrous minerals and health. J. Geol. Soc. London, 137:525-535, 1980.
26. Fahr: Demonstration. Präparate und Mikrophotogramme von einem Falle von Pneumokoniose. Münchener Med. Wochenschr. 22:625, 1914.
27. Farley, M.L., Greenberg, S.D., Shuford, F.H., Hurst, G.A., Spivey, C.G., Christianson, C.S.: Ferruginous bodies in sputa of former asbestos workers. Acta. Cytol. 21:693-700, 1977.
28. Förster, H.: Ein mineralogisch-petrographische Untersuchung über mögliche Ursachen von Mesotheliomen in Kappodokien/Turkey. Zbl. Arbeitsmed. 32:18-27, 1982.

29. Gönen , Ö., Artvinli, M., Özesmi, M., Baysal, F., Baris, Y.I.: Asbestosis in Turkey. Paper presented at the 11th Turkish National Tuberculosis Congress, May 1973, Bursa, Turkey.
30. Harington, J.S., Allison, A.C., Badami, D.V.: Mineral fibers: chemical, physiochemical, and biological properties. Adv Pharmacol. Chemoter. 12:291-402, 1975.
31. Hillerdal, G., Baris, Y.I.: Radiological study of pleural changes in relation to mesothelioma in Turkey. Thorax 38:443-448, 1983.
32. Hillerdal G, Zitting A, Assendelft A.H.W, Kuusela T. Rarity of mineral fibre pleurisy among persons exposed to Finnish anthophyllite and with low risk of mesothelioma. Thorax 39:608-611, 1984.
33. Hillerdal, G: Malignant mesothelioma 1982: review of 4710 published cases. Brit. J. dis. Chest 77:321-344, 1983.
34. Hillerdal G. Asbestos-related pleuropulmonary lesions and the erythrocyte sedimentation rate. Thorax 39:752-758, 1984.
35. Holmberg, L.A., Ault, K.A.: Characterization of natural killer cells induced in the peritoneal exudates of mice injected with listeria monocytogenes: A study of their tumour target specificity and their expression of murine differention antigen and human NK associated antigens. Cellular Immunology 89:151-168, 1984.
36. Johnson, N.F., Edwards, R.E., Munday,. D.E., Rowe, N., Wagner, J.C.: Pluripotential nature of mesothelioma induced by inhalation of erionite in rats. Br. J. Exp. Path. 377-382, 1984.
37. Kagan, E., Jacobson, R.J.: Lymphoid and plasma cell malignancies: asbestosrelated disorders of long latency. Am. J. Clin. Pathol. 80:14-20, 1983.
38. Kelsey, K.T., Yano, E., Liber, H.L., Little, J.B.: The in vitro genetic effects of fibrous erionite and crocidolite asbestos. Brit. J. Cancer 54:107-114, 1986.
39. Kiviluoto, R.: Pleural calcification as a roentgenologic sign of non-occupational endemic anthophyllite asbestosis. Acta radiol, Suppl. 194, 1960.
40. Lilis, R.: Fibrous zeolites and endemic mesothelioma in Cappadocia, Turkey. J. Occup. Med 23:548-550, 1981.
41. Maltoni, C., Minardi, F., Morisi, L.: Pleural mesotheliomas in Sprague-Dawley rats by erionite: First experimental evidence. Environ Res. 29:238-244, 1982.
42. McDonald, J.C. and McDonald, A.C.: Epidemiology of mesothelioma from estimated incidence. Prev. Med. 6:426-446, 1977.

43. McDonald, J.C. and McDonald, A.D.: Malignant mesothelioma in North America. Cancer 46:1650-1650, 1980.
44. Mumpton, F.A.: Worldwide deposits and utilisation of natural zeolites. Industrial Minerals 73:2-11, 1973.
45. Mumpton, F.A.: Commercial utilization of natural zeolites. Industrial Minerals and Rocks. 4th edition. Lefon, S, J. (editor in chief) AIME publ. New York, 1975 pp 1262.
46. Mumpton, F.A.: Natural Zeolites: A new industrial mineral commodity. in Natural zeolites, Occurence, Properties, Use. Ed. Sand, L.B. and Mumpton, F.A.. Pergamon Press. Elmosford, New York 1978 p3-27.
47. Mumpton, F.A.: Report of reconnaissance study of the association of zeolites with mesothelioma cancer occurences in central Turkey. Brockprot, New York, Department of the Earth Sciences, State University Colllege, 1979.
48. Olson, H.R.: Introduction, Industrial Minerals and Rocks. 4th Edt. lefon, S.J. (editor in Chief) AIME Publ. New York, 1975, pp1235.
49. Özemi, M. Baris, Y.I., Artvinli, M., Göktepeli, A.: Environmental and paraoccupational asbestosis of Michaliccik Town, Prevalence study. Tüberkuloz ve Toraks (Turkey) 22:487-496, 1974.
50. Poole, A., Brown, R.C., Turner, C. J., Skidmore, J.E., Griffitihs, D.M.: *In vitro* genotoxic activities of fibrous erionite. Br. J. Cancer 47:697-705, 1983.
51. Preger, L.: Asbestos-related disease, Grune and Stratton 1978, New York, San Francisco, London. pp23-26.
52. Pylev, L.N., Krisvoskeeva, L.V., Bostashvili, R.G.: Possible carcinogenic hazard of zeolite-clinoptilolite. Gig. Tr. Prof Zabol 3:48-53, 1984.
53. Rohl, A.N., Langer, A.M., Moncure, G., Selikoff, I.J., Fischbein, A.: Endemic pleural disease associated with exposure to mixed fibrous dust in Turkey. Science 216:518-520, 1982.
54. Rom, W., Greaves, W., Parry, W., Moatamed, F.: Prevalence of pleural disease in a Nevada community hospital near a fibrous erionite deposit. Am. Rev. Resp. Dis. 123, suppl:s 143, 1981.
55. Rom, W.N., Casey, K.R., Parry, W.T., Corey. H.M., Moatamed, F.: Health implications of natural fibrous zeolites for the Intermountain West. Environ. Res. 30:1-13, 1983.
56. Saracci, R., Simonato, L., Baris, Y.I., Artvinli, M., Skidmore, J.: The age-mortality curve of endemic pleural mesothelioma in Karain, central Turkey. Br. J. Cancer 45:147-149, 1982.

57. Schniter, R.J.: Ad Cauces in Pharmacotopy and chemotherapy vol:12, 1975. Academia pures. New York, San Fransisco, London.
58. Sebastien, P., Gaudichet, A., Bignon, J., Baris, Y.I.: Zeolite bodies in human lungs from Turkey. Lab. Invest. 44:420-425, 1981.
59. Sébastien, P., Bignon, J., Baris, Y.I., Awad, L., Petit, G.: Ferruginous bodies in sputum as an indication of exposure to airborne mineral fibers in the mesotheiloma village of Cappadocia. Arch. Environ. Health 39:18-23, 1984.
60. Shepord, R.A.: Zeolites in sedimentary rocks. Industrial Minerals and Rocks. 4th edition. Lefon, S.J. editor in chief AIME. Publ. New York, 1975. pp 1257.
61. Stanton. M. F., Layard, M., Tegenis, A., Miller, E., May, M., Kent, E.: Carcinogenicity of fibrous glass. Pleural response in the rat in relation to fiber dimension. JNCI 58:587-603, 1977.
62. Stanton M.F., Layard M, Tegeris A, Miller E, May M, Morgan E, Smith A. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. JNCI 67:965-975, 1981.
63. Stumphius, J.: Epidemiology of mesothelioma on Walxheren Island. Brit. J. Ind. Med. 28:59-66, 1971.
64. Suzuki, Y.: Carcinogenic and fibrogenic effects of zeolites: Preliminary observations. Environ, Res. 27;433-445, 1982.
65. Suzuki, Y., Kohyama, N.: Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. Environ. Res. 35:277-292, 1984.
66. Timbrell V, Griffiths D.M., Pooley D.F. Possible biological importance of fibre diameters of South African amphiboles. Nature 232:55-56, 1971.
67. Timbrell, V.: Deposition and retention of fibres in the human lung. Ann. Occup, Hi. 26:347-369, 1982.
68. Wagner, J.C., Elmes, P.C.: The mineral fibre problem. Recent advances in Occupational Health. Ed McDonald, J.C. Churcill Livinstone Edinburg and New York, pp 1-13.
69. Wagner, J.C.: Experimental studies using erionite fibre. VIth International Pneumoconiosis Conference 1983, Vol 2. Bochum/West Germany. Deutsche Bergbaugenossenschaft, 1983:1047-1051.
70. Yazicioglu, S.: Pleural calcification associated with exposure to chrysotile asbestos in Southeast Turkey. Chest 70:43-47, 1976.
71. Yazicioglu, S., Öktem, K., Ilcayto, R., Balci, K., Sayli, B.S.: Association between malignant tumors of the lung and pleura and asbestosis. Chest 73:52-56, 1978.

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AN OUTBREAK OF PLEURAL MESOTHELIOMA AND CHRONIC FIBROSING PLEURISY IN THE VILLAGE OF KARAIN/ÜRGÜP IN ANATOLIA

BY

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An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Ürgüp in Anatolia

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Baris, Y. I., Sahin, A. A., Ozesmi, M., Kerse, I., Ozen, E., Kolacan, B., Altinörs, M., and Göktepeli, A. (1978). *Thorax*, **33**, 181–192. **An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Ürgüp in Anatolia.** The 575 inhabitants of the remote Anatolian village of Karain suffered 11 deaths from pleural mesothelioma in 1975/76 and .here were five cases of fibrosing pleurisy. In the previous five years there had been 25 cases of mesothelioma. Calcified pleural plaques were common on survey radiography. Asbestos does not occur in the local soil or rock, nor is it handled in the village, but a few fibres were found in the water. Fibres were also found in the pleural tissue of two of five cases examined. Inhabitants of the neighbouring villages are free of mesothelioma.

Karain is a village of 575 inhabitants lying in the district of Ürgüp, well known to tourists because of the picturesque rock dwellings called 'fairy chimneys'.

For a long time it has been known that the people of Karain die of cancer. The saying goes that 'The peasant of Karain falls ill with pain in the chest and belly, the shoulder drops, and he dies'.

This paper describes an investigation of the disease which has given the village this reputation. The investigation is divided into two parts: 1. An analysis of information already available up to 1975; and 2. A survey of conditions in 1975/76.

Methods

1. ANALYSIS OF INFORMATION AVAILABLE UP TO 1975

For the period up to 1970 elderly residents and the village headman (Muhtar) were the main sources of information as a formal health service had not been established in this area. More recent medical information was obtained from the Health Institute in Karain and from relatives. Information was obtained from hospitals about patients admitted from the village. Survey radiographs (70×70 mm) taken in 1971 and information on all deaths between 1970 and 1974 were reviewed.

2. SURVEY OF CONDITIONS IN 1975/76

A team of physicians, geologists, physicists, and mining and agricultural experts visited the area.

(a) They studied the physical and economic structure of the village, the working and living patterns of the inhabitants, the rock, the soil, and the water. Geological, biological, and chemical tests were done to detect toxic hazards in the environment. The tests were also applied to the neighbouring villages and the findings were compared.

(1) Airborne dust was collected on membrane filters. These and the samples of soil, rock, stucco, etc. were examined by optical and electron microscopy. Elemental analysis of soil and rock was carried out using optical spectrographic analysis, and a scintillometre model, Saphymostel, Spp 2-nf, was used to detect radioactivity.

(2) Samples of food and drink were examined for aflotoxin using the AOAC 26 Natural Poisons 20.0.14-CB (Published by the Association of Official Analytical Chemists, PO Box 540, Benjamin Franklin Station, Washington, DC 20044, USA). Soil and dust samples were extracted with cyclohexane and the extracts were examined spectrographically for benzpyrene.

(3) The water supplies were tested for nitrosamine using Preussman's reagents (Preussman and Eisenbrad, 1972).

(4) The water supplies were also analysed for fibrous minerals; 250 ml samples of water were filtered through a Sartorius membrane filter (Sartorius membrane filter Gmbh 3400 Göttingen Postfach 142) to collect particles of over 0·2 millimicrons. The filter was then ashed to 450°C for three hours and the residue was resuspended in 1 ml distilled water by ultrasonic vibration for 30 minutes. One drop was taken onto an EM grid for examination by transmission microscopy.

(b) The team studied the health of the villagers and that of the inhabitants of neighbouring villages.

(1) The mass chest radiographic survey previously carried out in 1971 using 70×70 mm films was repeated in 1975. Identification of the individuals surveyed in 1971 was incomplete so that this was not an individual follow-up study.

(2) All patients developing symptoms of chest disease during 1975 and 1976 were investigated. When possible they were admitted to hospital and submitted to the usual radiological and other tests to find other primary sites of malignancy beside the pleura. Tests for delayed hypersensitivity were carried out with streptokinase-dornase, purified protein derivative, mumps, and Candida antigens. Lymphocyte counts and lymphocyte stimulation tests were also carried out on six of the patients. Histological material was obtained from patients suspected of pleural mesothelioma by punch biopsy, thoracoscopy, or thoracotomy. When underlying lung was obtained this was examined for asbestos and asbestos bodies by optical and electron microscopy. Pleural tissue from some cases was ashed and analysed by electron probe for asbestos and other minerals. Explant tissue culture from some of the biopsy material was attempted without success.

(3) The causes of all deaths in Karain and in the neighbouring villages in these two years were recorded and verified as far as possible.

The pedigrees of all new cases were investigated for evidence of inherited factors. Occupational and domestic histories were taken and investigated.

Results

1. ANALYSIS OF INFORMATION AVAILABLE UP TO 1975

The elderly residents admitted that the painful disease had been occurring in the village for many years. They said that because of it the village had always been known as Karain, which in Turkish means 'Abdominal pain'. However, records of the numbers of cases had been kept only since the establishment of a 'health institute' in the village. The villagers recalled that the disease affected the middle-aged of both sexes, and that the onset was usually with insidious breathlessness, less often with pain or cough. It was fatal.

More recent information from medical records showed that the disease was usually associated with a serofibrinous or serohaemorrhagic pleural effusion. Tests for tuberculosis were negative. Chest radiographs also showed an effusion often with confluent peripheral nodular shadows suggesting a mesothelioma of the pleura. In two cases pleural calcification was also present (Fig. 1).

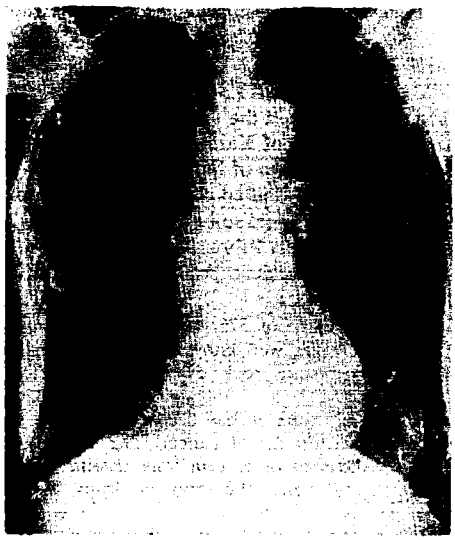

Fig. 1 *Radiograph of an old woman from Karain showing multiple bilateral calcified pleural plaques.*

Antituberculosis and other forms of treatment tried up to 1975 had been ineffective.

The incidence of the disease had been high (Table 1) and appeared to account for the very high overall death rate for a village of this size. The situation was particularly bad in 1974 when, in a population of only 604, there were 11 pleural mesotheliomas out of a total of 18 deaths.

The comparison with neighbouring villages for the year 1974 is shown in Table 2. The total number of deaths in Karain is high but not significantly higher than in the village of Akcaören. But these two villages have a significantly higher death rate than the rest (χ^2 30·45 8 degrees of freedom). However, the really significant difference lies in the causes of death. Whereas there were 11 pleural

Table 1 *(a) Deaths due to malignant disease in Karain 1970–74*

Year	Population	Deaths due to malignant disease			
		Male	Female	Total	Mean age
1970	808	3	5	8	55
1971	795	3	1	4	48
1972	656	4	1	5	49
1973	656	1	2	3	50
1974	604	8	6	14	50

(b) Causes of death

Year	Pleural mesothelioma	Ca. lung	GI cancer*	Non-malignant†	Total
1970	6	1	1	2	10
1971	2	1	1	5	9
1972	3	1	1	7	12
1973	2	0	1	3	6
1974	11	0	3	4	18

*May have included primary peritoneal mesotheliomas.
†Infantile deaths, cardiovascular diseases, and accidents.

mesotheliomas in Karain there was none in any of the other villages. A number of gastrointestinal deaths occurred without a precise diagnosis and these may have included peritoneal mesotheliomas.

The 564 photofluorograms taken in 1971 were reviewed and the findings are shown in Table 3. These indicate that there has been no significant change in the general pattern of chest disease in the village since 1971.

Table 3 *Radiological findings on mass survey 1971 and 1975 compared (70 mm films)*

Finding	Number of cases	
	1971	1975
Diffuse fibrosis	21	24
Pleural tumour	7	5
Pulmonary tubercle	7	3
Carcinoma of lung	4	0
Pleural calcification similar to that seen in asbestos exposure	2	2
Pleural and pulmonary calcification which resembles healed Tb, empyema, or haemothorax	2	2
Pleural thickening	0	3
Films taken	564	452

2. SURVEY OF CONDITIONS IN 1975/76

(a) The environment

The village of Karain lies in Cappadocia in central Anatolia (Fig. 2). Rock consists of volcanic tuffs. The soil is poor, and the climate very hot and dry in summer and cold with snow in winter. The soft volcanic rock is cut by adze for making walls of houses and buildings and is plastered with a greyish-white powdered rock from the neighbouring hills. Similar powdered material is used for clearing wine and making a sweetmeat called 'pekmez'. Most people work on the land growing potatoes and onions in the area between the village and the river. The work is very dusty and hot in summer. Apart from a few donkeys, mules, dogs, and poultry, no animals are kept and few fodder crops are grown.

Table 2 *Comparison of deaths in Karain and neighbouring villages, 1974*

Village	Distance from Karain (km)	Population	Total no. of deaths		Cause of death			
			Obs.	Exp.	Pleural mesothelioma	GI disease	Infantile	Other
Karain	0	604	18	6·60	11	3	1	1 accident 2 stroke
Akcaören	20	544	11	5·94	0	2	9	0
Akkoy	9	972	6	10·62	0	0	2	2 cardiac 1 psychosis 1 accident
Basdere	13	2764	28	30·19	0	4	13	2 stroke 1 ca. lung 3 Tb 1 suicide 1 unknown
Boyali*	3	530	3	5·79	0	1	1	1 renal disease
Demirtas	12	275	3	3·00	0	1	1	1 chronic obst. lung disease
Iltas	9	348	2	3·80	0	1	0	1 rh. fever
Karlik	4	645	5	7·04	0	1	2	1 cardiac 1 unknown
Yesilöz†	7	641	4	7·00	0	0	2	1 cardiac 1 genitourinary disease

*One patient from Boyali died of mesothelioma in 1976. She was a native of Karain and lived there until she was 25 years old (Table 4, case 11).
†A patient migrated to Yesilöz from Karain when she was 12 years old. She was found to have a mesothelioma in 1976, aged 52 (Table 4, case 13).

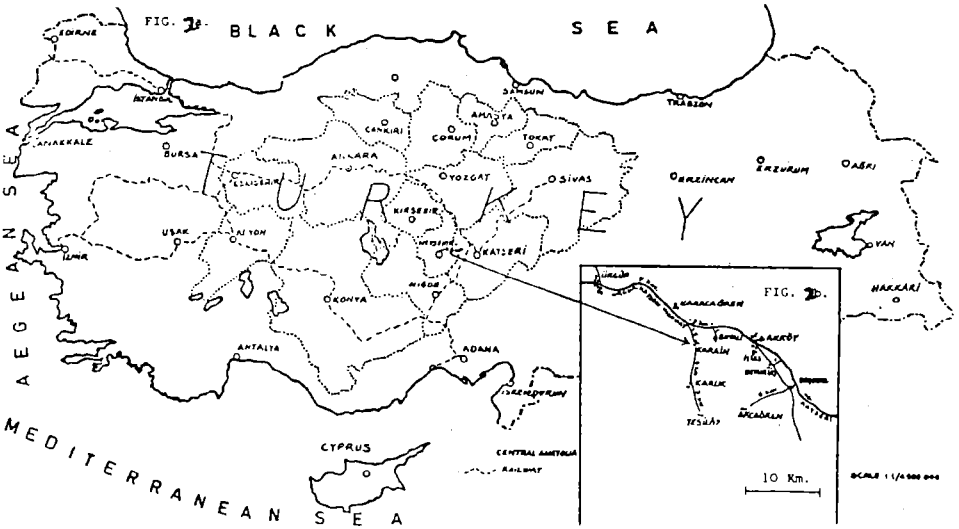

Fig. 2 *Map of Turkey showing Ürgüp district.*

In Karain some of the men are smokers; the women never smoke.

Water is obtained from: (a) the river; (b) the springs in the river bed; (c) wells in the fields; and (d) a spring in the hills behind the village which is piped to a communal tap in the village. The villagers are mainly dependent on the piped water, which has been available for over 100 years, for drinking, washing, and cooking. Recently, artesian wells have been dug close to the village and used to provide more piped water to it. None of the systems contains asbestos pipes. The other sources are used for irrigation but in hot weather the field workers drink from the wells and the springs in the river bed and all the villagers bathe in the river.

The village lies on the road up the valley (Fig. 2) to the villages of Karlik and Yesilöz. The road is not metalled and is very dusty. These villagers do not share Karain's piped water supply but are otherwise similar. Karain's disease makes it difficult for the peasants to sell their potatoes and onions so that they are even poorer than their neighbours. Young people moving from the village may develop the disease in later life; but young people moving to Karain do not develop the disease. The village population dwindles because of the economic difficulties, and the young people have to move far away to areas where the problem is not known.

There are no deposits of asbestos in the area nor has there been any processing of such material brought in from elsewhere. The soil, the rock, the stucco, the material used for making the sweetmeat, and the airborne dust contain volcanic debris. There are no asbestos fibres but there are a few fine barbed fibre fragments of volcanic glass.

Rock samples contained calcite, feldspar, quartz, volcanic glass, biotite, chlorite, muscovite, and augite. The main elements are silicon, aluminium, and magnesium with smaller quantities of calcium and iron. Optical spectrographic analysis did not indicate that arsenic, uranium, nickel or chromium were present in sufficient quantities to constitute a cancer risk.

Tests for aflotoxin on potatoes, bread, grape juice, wine, wheat, and fresh fruit used by the villagers were negative. Cyclohexane extracts of soil, dust, and stucco were negative for benzpyrene.

Nitrosamine tests on various water samples were negative. No significant radioactivity was found in the village or in the surrounding soil and rocks.

In special studies the various water supplies to Karain were compared with the water used in Karlik and Yesilöz (4 and 7 km further up the valley). Only in the original piped supply to Karain and in water from one of the old wells by the

river were any mineral fibres found (Fig. 3). There were small quantities of fibres ranging from 5 to 70 microns long.

(b) The health of the villagers

The mass radiographic survey showed no significant change in the incidence of chest disease between 1971 and 1975. But during 1975 and 1976, 16 new cases of pleural disease were found. Table 4 gives some of the details of these patients. None of the eight men and eight women had done anything but work in the fields or at home except case 7. They were between 27 and 65 years old and presented with chest pain and breathlessness.

Case 3 had been treated for pleurisy 20 years previously and the radiograph taken in 1971 had

Fig. 3 *Clumps of ? asbestos fibres in the water of Karain.* ×30 000.

Table 4 *Prospective study of new patients in 1975 and 1976*

Case	*Age/ sex*	*Clinical and radiological findings*	*Diagnostic methods*	*Histopathological diagnosis*	*Comments*
1	40 M	R massive haem. pl. effusion	Thoracoscopy	Malignant pl. mesothelioma	Died 5 mth later
2	65 M	L massive haem. pl. effusion	Thoracoscopy	Chronic fibr. pleurisy	Died 4 mth later
3	46 M	L pl. effusion	Thoracotomy	Chronic fibr. pleurisy	Pleurectomy. Well
4	50 F	R pl. effusion	Pl. punch biopsy	Chronic fibr. pleurisy	Died 2½ yr later
5	60 I	R massive haem. pl. effusion	Pl. punch biopsy	Malignant pl. mesothelioma	Died 4 mth later
6	50 I	R pl. thickening and peripheral nodular pulm. lesions	Radiology	—	Died 5 mth later
7	37 M	L pl. effusion	Thoracotomy	Chronic fibr. pleurisy Malignant pl. mesothelioma	Pleurectomy. L L lobectomy. Well
8	48 F	L pl. effusion	Thoracoscopy	Malignant pl. mesothelioma	Died 2 yr later
9	50 M	R pl. thickening with effusion	Pl. punch biopsy	Malignant pl. mesothelioma	Died 8 mth later
10	43 F	R pl. effusion	Pl. punch biopsy	Malignant pl. mesothelioma	Died 2½ yr later
11	63 F	L pl. thickening and peripheral nodular pulm. lesions	Thoracotomy	Malignant pl. mesothelioma	Died 4 mth later
12	45 M	L pl. thickening and peripheral pulm. nodule	Radiology	—	—
13	52 F	R pl. effusion with peripheral pulm. lesions	Thoracoscopy	Malignant pl. mesothelioma	Seriously ill after 2 yr
14	50 I	R pl. thickening with peripheral pulm. lesions	Radiology	—	Died 3 mth later
15	27 M	R hydropneumothorax	Pl. punch biopsy	Chronic fibr. pleurisy	Well
16	60 M	R pl. thickening	Radiology	—	—

shown a left pleural effusion. At thoracotomy dark yellow fluid filled the pleural cavity and the visceral and parietal pleura was leathery and 5 mm thick. There were partially calcified hyaline plaques in the parietal pleura. The histological diagnosis was chronic fibrosing pleurisy and the patient remains well after pleurectomy.

Case 7 was working as a road constructor in Germany when he was investigated there in 1972 and a diagnosis of left-sided pleurisy with effusion was made. A left lobectomy and pleurectomy was performed at the Ruhrlandklinik in Essen in September 1974 to remove what was thought to be a bronchiectatic lobe secondary to the pleurisy. A histological diagnosis of malignant pleural tumour (probably mesothelioma) was made. As the patient remains well we suspect that this may have been chronic fibrosing pleurisy.

Irrespective of the histological diagnosis the radiological findings showed pleural fluid, pleural thickening, and nodular peripheral lung shadows consistent with the diagnosis of mesothelioma. The usual tests for cancer of other primary sites, for other causes of pleural effusion, and for collagen disease were all negative.

Tests for delayed hypersensitivity were performed on cases 3, 7, 8, 10, 11, and 13 and all were negative.

The final diagnosis of mesothelioma was made in 11 of the 16 cases and was based on clinical and radiological grounds as well as on histology in cases 1, 5, 8, 9, 10, 11, and 13. In case 2 the diagnosis of mesothelioma was made in spite of the histological findings because of the thoracoscopy appearance, the fact that there was a massive haemorrhagic effusion, and the short fatal course of the disease. The clinical and radiological findings and fatal outcome also led to the diagnosis of mesothelioma in cases 4, 6, and 14.

The final diagnosis of chronic fibrosing pleurisy was made in the other five cases. The clinical course fitted the histological findings in cases 3 and 15. Case 7 has already been described. In cases 12 and 16 the diagnosis rests on clinical and radiological evidence.

Cases 4, 6, 12, 14, and 16 refused thoracotomy and some of the other investigative procedures.

The histological findings in case 8 are typical for mesothelioma. 'There are cuboidal cells with acidophilic cytoplasm and nuclei rich in chromatin, looking like mesothelioma cells forming sheet-like and adenoid structures. There is also dense mononuclear cell infiltration. Moreover there is calcification in hyalinised fibrous tissue' (Fig. 4). Histological sections from the fibrous pleurisies revealed fibrous tissue, rare mitosis, mononuclear cells, and hyalinised collagen tissue. Sometimes mononuclear cell infiltration formed follicles in the fibrous tissue and there was proliferation of mesothelial cells. Amorphous iron pigment in and outside the macrophages was also seen.

Asbestos bodies could not be demonstrated in these cases by optical microscopy.

Pleural biopsies from cases 2, 4, and 5 were examined by electron microscopy. In case 2 the cells and nuclei were large with prominent nucleoli. Mitochondria were moderate in number. Smooth and rough surface endoplasmic reticulum was abundant and enlarged in many cells. The cytoplasm contained dense inorganic material in

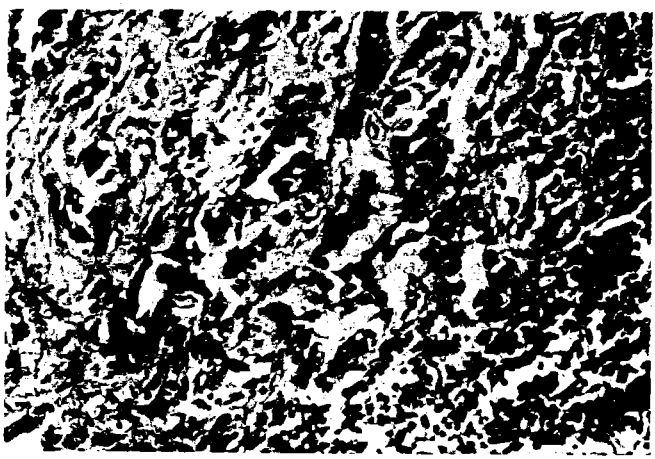

Fig. 4 *Case 8. Pleural biopsy shows malignant mesothelioma. Haematoxylin and eosin ×300.*

many cells (Fig. 5). In some cells ferritin could be demonstrated and tonofilaments were also visible. Most cells had the microvilli on the surface. Some other cells have shown granules varying in size and density (Fig. 6). The other two cases showed similar EM characteristics.

Electron microscopy of ashed pleural tissues from cases 1, 3, 8, 10, and 13 showed about 10 chrysotile fibres in case 1. In case 3 there were $1{\cdot}2\times10^5$ chrysotile and 3×10^5 amphibole asbestos fibres per gram of dry tissue. Traces of talc, calcium sulphate, feldspar, quartz, mica, and kaolinite were also found in the ashed tissue (Figs 7 and 8).

Genealogical surveys have proved that blood-relationship is not a necessary prerequisite for the incidence within families. The information suggests a common environmental aetiology. For instance, in the Sencan family (Table 4) case 2 is the mother of case 3. Case 13 is the sister-in-law of case 14 but the husband of case 14 had died of a mesothelioma in 1970. Neither parent in the Sencan family had died of mesothelioma.

Cases 10, 11, and 13 had been brought up in Karain and had moved to Ürgüp, Yesilöz, and Boyali when they married and before they developed mesothelioma. Conversely, no one brought up elsewhere who came to live in Karain developed mesothelioma.

Six other inhabitants of Karain became seriously ill or died during 1975/76. The results of the investigation of these patients are given in Table 5.

Table 5 *Deaths in Karain 1975–76*

Case	*Age/Sex*	*Comments*
17	46 M	Gastric biopsy proved gastric cancer. Died
18	65 M	Presently being followed with chylous ascites, chylothorax, and chronic renal disease
19	75 F	Died from stroke. Chest radiograph shows pleural calcifications (Fig. 1)
20	75 M	Died from chronic obstructive lung disease
21	61 F	Died from rheumatic heart disease
22	80 F	Died from stroke

Discussion

The investigation shows that diffuse pleural mesothelioma and chronic fibrous pleurisy are endemic in Karain. The disease affects the middle-aged and elderly in some families more than in others. There is no definite evidence of a genetic factor, and the main occupation of most of those affected is agriculture. Published epidemiological studies on mesothelioma (Wagner *et al.*, 1960; Newhouse and Thompson, 1965; Selikoff *et al.*, 1965; McDonald *et al.*, 1970; Rubino *et al.*, 1972) have emphasised that environmental or occupational exposure to asbestos is the important factor although tenuous evidence of a familial incidence has been reported by Webster (1965) and Milne (1976).

There is no obvious occupational or environmental source of asbestos exposure in Karain apart from the few fibres in the water. But asbestos fibres were found in the pleural tissue of two of the five cases investigated. Pleural calcification was seen quite frequently and was typical of that resulting from asbestos exposure. No other cause for the pleural lesions was found. Only three deaths due to primary lung cancer occurred between 1970 and 1976 (all men). Primary lung cancer is common in heavily exposed asbestos workers who smoke. The women of Karain did not smoke; the men smoked locally cured tobacco.

It is a feature of most carefully investigated series of mesothelioma that they include cases with no occupational or neighbourhood exposure to asbestos (McDonald *et al.*, 1970; Webster, 1972; Greenberg and Davies, 1974; Nurminen, 1975; Milne, 1976). It is difficult to ignore these cases although the possibility of avoiding contact with asbestos with its thousand or more occupational uses seems remote.

There are striking similarities between Rous and Studeny's survey (1970) and ours. They reported that plaques are endemic in the region of Pelhrimoy in Czechoslovakia, mainly in elderly farmers, with a familial occurrence and that pleural effusion was the common disease in their past history. Their geological survey showed that there is no asbestos in the vicinity of Pelhrimoy nor are any mines, mills or factories located there. But they failed to observe mesothelioma. However, we have found some villages in Turkey with endemic pleural calcification, and asbestos studies in these places gave negative results (Baris, 1975).

When there has been no occupational, neighbourhood, or domestic exposure to asbestos but asbestos fibres are detected in the pleural tissues of subjects with pleural plaques, with chronic fibrous pleuritis, or with diffuse mesothelioma, certain possibilities must be considered. The patient might have been exposed to asbestos from an as yet undetected source. These disorders may be caused by some unsuspected environmental agents, such as volcanic glass, talc, mica, kaolin, or feldspar. However, the same kinds of minerals have been found in other villages near Karain. The physical properties and concentration of volcanic glass and other fibres in the soil of this

Fig. 5 *Case 2. Electron microscopic view. N, nucleus; M, mitochondrium; DM, dense material; Fe, ferritin. Lead citrate-uranyl acetate. ×78 000.*

Fig. 6 *Case 2. Electron microscopic view shows three mesothelial cells. N, nucleus; DM, dense material; G, secretory granules, Mv, microvillus. Lead citrate-uranyl acetate.* ×11700.

region have not been studied analytically, and one could argue that there might be an actual difference in these particles from those in other villages. Another possibility is that the causative agent is asbestos, which reaches the body by means of water—that is, tap water, beverages, and foods are the sources of the asbestos fibres. Attention has recently been directed towards the widespread occurrence of asbestos fibres in certain natural sources and beverages (Biles and Emerson, 1968; Cunningham and Pontefract, 1971; Cook *et al.*, 1974; Masson *et al.*, 1974; Wells, 1975). But there is no agreement as to whether asbestos fibres in the water may be hazardous to health. Gross *et al.* (1974) have shown that the penetration of the ingested asbestos fibres through the walls of the gastrointestinal tract does not occur. Gross (1974) believes that the asbestos fibres in

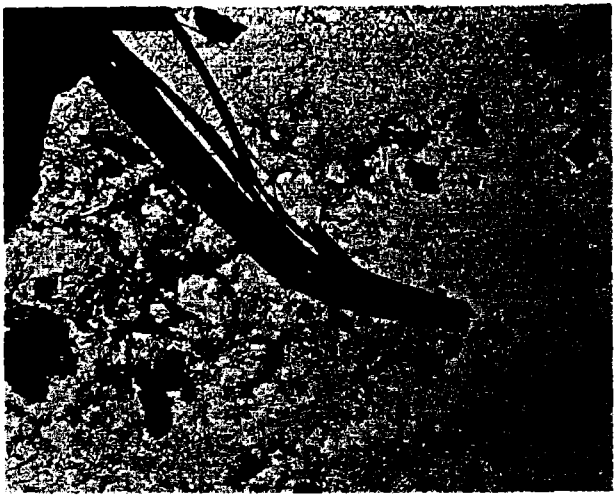

Fig. 7 *Case 3. Chrysotile asbestos fibres in the ashed pleural tissue. ×7500.*

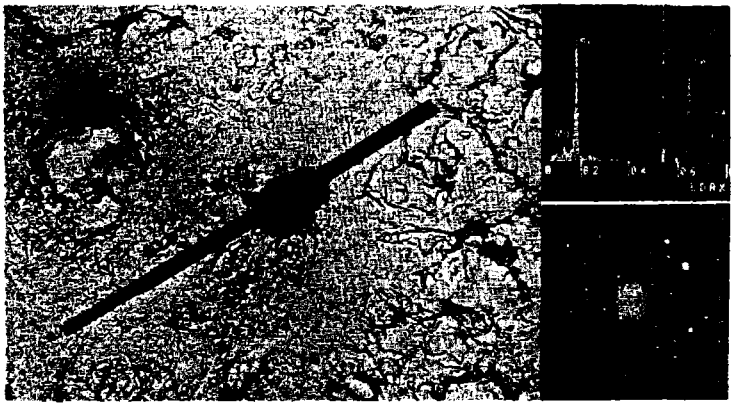

Fig. 8 *Case 3. Amphibole asbestos fibres in the ashed pleural tissue. ×16 750.*

the water and beverages are submicronic and that this kind of asbestos fibre is not carcinogenic or fibrogenic. However, some authors suggest that inoculation of asbestos fibres into the stomach results in penetration of the digestive tract by these fibres, spreading to various organs, and this condition may be important in asbestos carcinogenesis (Westlake *et al.*, 1965; Pontefract and Cunningham, 1973). The advisory committee of the Lyon Conference (Bogovsky *et al.*, 1973) judged there to be no evidence at present of 'an increased cancer risk resulting from asbestos fibres present in water, beverages or food or in the fluids used for the administration of drugs'. The outbreak of fibrous pleuritis and diffuse mesothelioma in Karain may well be an example of this route. We are unable to explain why these diseases occur in only one of the three villages, which are located quite close to each other and in the same valley, where the same kind of inhabitants live in the same living conditions. The only lifference between these villages is the source of the drinking water. Preliminary studies of the water of Karain, which showed asbestiform fibres, support our view. Perhaps the natural waters of Karain may be derived from the wearing down of asbestos-

containing rocks. The asbestos fibres in the water may be ingested or evaporation of the water may cause the asbestos fibres to become airborne. If our opinion is correct, asbestos in water may play a role in the pathogenesis of the endemic pleural calcification described earlier (Kiviluoto, 1960; Zolov, 1967; Burilkov and Michailova, 1970; Yazicioglu, 1976) and of certain cases of mesothelioma and chronic fibrous pleuritis whose exposure to asbestos could not be traced.

Mesothelioma has been induced by a strain MC29 *Avium leucosis* virus (Chabot *et al.*, 1970). It is very difficult to accept that this could be the cause of the localised disease in Karain, which is close to two other villages.

Further studies are required, such as exact measurements of the dimension of the fibres. The range of dimensions, the percentage of the different sizes, and their chemical composition should be reported and supported by experiments which show that the natural waters of Karain induce mesothelioma or chronic diffuse pleuritis.

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References

Baris, Y. I. (1975). Pleural mesotheliomas and asbestos pleurisies due to environmental asbestos exposure in Turkey. An analysis of 120 cases. *Hacettepe Bulletin of Medicine/Surgery*, **8**, 165–185.

Biles, B., and Emerson, T. R. (1968). Examination of fibres in beer. *Nature*, **219**, 93–94.

Bogovski, P., Gilson, J. C., Timbrell, V., and Wagner, J. C. (eds.) (1973). *Biological Effects of Asbestos. Proceedings of a Working Conference*. International Agency for Research on Cancer, Lyon.

Burilkov, T., and Michailova, L. (1970). Asbestos content of the soil and endemic pleural asbestosis. *Environmental Research*, **3**, 443–451.

Chabot, J. F., Beard, D., Langlois, A. J., and Beard, J. W. (1970). Mesotheliomas of peritoneum, epicardium, and pericardium induced by strain MC29 avian leukosis virus. *Cancer Research*, **30**, 1287–1308.

Cook, P. M., Glass, G. F., and Tucker, J. H. (1974). Asbestiform amphibole minerals: detection and measurement of high concentrations in municipal water supplies. *Science*, **185**, 853–855.

Cunningham, H. M., and Pontefract, R. (1971). Asbestos fibres in beverages and drinking water. *Nature*, **232**, 332–333.

Greenberg, M., and Davies, T. A. L. (1974). Mesothelioma register 1967–1968. *British Journal of Industrial Medicine*, **31**, 91–104.

Gross, P. (1974). Is short-fibered asbestos dust a biological hazard? *Archives of Environmental Health*, **29**, 115–117.

Gross, P., Harley, R. A., Swinburne, L. M., Davis, J. M. G., and Green, W. B. (1974). Ingested mineral fibers: Do they penetrate tissues or cause cancer? *Archives of Environmental Health*, **29**, 341–347.

Kiviluoto, R. (1960). Pleural calcification as a roentgenologic sign of non-occupational endemic anthophyllite-asbestosis. *Acta Radiologica*, Supplement **194**.

Masson, T. J., McKay, F. W., and Miller, R. W. (1974). Asbestos-like fibres in Duluth water supply. Relation to cancer mortality. *Journal of the American Medical Association*, **228**, 1019–1020.

Milne, J. E. H. (1976). Thirty-two cases of mesothelioma in Victoria, Australia: a retrospective survey related to occupational asbestos exposure. *British Journal of Industrial Medicine*, **33**, 115–122.

McDonald, A. D., Harper, A., El Attar, O. A., and McDonald, J. C. (1970). Epidemiology of primary malignant mesothelio tumors in Canada. *Cancer*, **26**, 914–919.

Newhouse, M. L., and Thompson, H. (1965). Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. *British Journal of Industrial Medicine*, **22**, 261–269.

Nurminen, M. (1975). The epidemiologic relationship between pleural mesothelioma and asbestos exposure. *Scandinavian Journal of Work, Environment and Health*, **1**, 128–137.

Pontefract, R. D., and Cunningham, H. M. (1973). Penetration of asbestos through the digestive tract of rats. *Nature*, **243**, 352–353.

Preussman, R., and Eisenbrad, G. (1972). *Topics in Chemical Carcinogenesis*, p. 323. University of Tokyo Press.

Rous, V., and Studeny, J. (1970). Aetiology of pleural plaques. *Thorax*, **25**, 270–284.

Rubino, G. F., Scansett, G., Donna, A., and Palestro, G. (1972). Epidemiology of pleural mesothelioma in north-western Italy (Piedmont). *British Journal of Industrial Medicine*, **29**, 436–442.

Selikoff, I. J., Churg, J., and Hammond, E. C. (1965). Relation between exposure to asbestos and mesothelioma. *New England Journal of Medicine*, **272**, 560–565.

Wagner, J. C., Sleggs, C. A., and Marchand, P. (1960). Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *British Journal of Industrial Medicine*, **17**, 260–271.

Webster, I. (1965). Mesotheliomatous tumors in South

Africa: Pathology and experimental pathology. *Annals of the New York Academy of Sciences*, **132**, 623–646.

Webster, I. (1972). The pathology of asbestosis. In: *Medicine in the Mining Industries*, edited by J. M. Rogan, pp. 39–55. Heinemann Medical Books, London.

Wells, A. H. (1975). Asbestos in Duluth water. *Minnesota Medicine*, **58**, 458–459.

Westlake, G. E., Spjut, H. J., and Smith, M. N. (1965). Penetration of colonic mucosa by asbestos particles. An electron microscopic study in rats fed asbestos dust. *Laboratory Investigation*, **14**, 2029–2033.

Yazicioglu, S. (1976). Pleural calcification associated with exposure to chrysotile asbestos in Southeast Turkey. *Chest*, **70**, 43–47.

Zolov, C., Bourilkov, T., and Babadjov, L. (1967). Pleural asbestosis in agricultural workers. *Environmental Research*, **1**, 287–292.

Requests for reprints to: Professor Y. I. Baris, Hacettepe University School of Medicine, Ankara, Turkey.

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Peritoneal mesothelioma and malignant lymphoma in mice caused by fibrous zeolite

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Peritoneal mesothelioma and malignant lymphoma in mice caused by fibrous zeolite

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ABSTRACT Dust from the village of Karain containing the fibrous zeolite erionite, talc, and physiological saline were tested by intraperitoneal injection in 486 Swiss albino mice. Malignant tumours were found in 84 (41 mesotheliomas, 31 lymphomas, 1 peripheral epidermoid carcinoma, and 11 lymphomas and mesotheliomas together) of the 321 animals which died spontaneously within nine to 32 months after injection of Karain dust (26·1%). Three mesotheliomas and no lymphomas were found among 24 animals injected in the same way with talc during the same time (12·5%). In 46 control animals injected with physiological saline three mesotheliomas and one lymphoma were seen (8·7%). Thus Karain dust appears to be a potent carcinogen, causing both mesotheliomas and malignant lymphomas.

Since 1974 the Baris group from Hacettepe University of Ankara, Turkey, has worked on the problem of mesotheliomas in middle Anatolia, where in certain villages there is an endemic occurrence of this disease. The highest incidence is in the village of Karain.[1] Volcanic tuffs form the bedrock of the area. In the villages with a high incidence of mesothelioma the tuffs have converted into zeolites, both non-fibrous (clinoptilolite and chabasite) and fibrous (erionite and mordenite.[2 3] Erionite fibres can also be found in biopsies of the lung[2 4] and pleura[4] in inhabitants of the affected villages. Since some doubt as to the causation of the mesotheliomas still remains,[5 6] it was decided to carry out some experiments beginning in 1980.

Material and methods

A rock from Karain was ground to dust at the Geological Institute of Hacettepe University. In this Karain dust (KD) zeolite was shown, both in fibrous and non-fibrous form. There were no signs of any asbestos in the dust. The dust was divided into packages of 5, 10, 15, 20, 30, and 40 g and suspended in 1 ml of physiological saline and sterilised with ultraviolet light. Commercial talc, 20 mg, was treated in the same way. The suspensions were injected into 6 week old Swiss albino mice. As a control, 1 ml of physiological saline was used in several animals.

The animals were checked every day until spontaneous death occurred or until there were obvious signs of tumour, such as a greatly enlarged abdomen. Animals which died before nine months had elapsed after injection were excluded.

Postmortem examination was performed on all dead animals. Macroscopical findings were noted and biopsy specimens from the injection site, parietal and visceral peritoneal mesothelium, peritoneal lymph nodes, spleen, liver, kidneys, adrenals, mediastinal lymph nodes, and tumours were taken for microscopical investigation. The pathologist was unaware of the type of dust to which the animals had been exposed.

Results

As seen in the table, KD caused tumours in 26·1%, talc in 12·5%, and physiological saline in 8·7% of the animals. There was no evident relation between the amount of dust and the incidence of tumours. No tumours occurred before nine months had elapsed after injection.

Of the 321 animals given KD, mesothelioma developed in 41, lymphoma in 31, peripheral epidermoid carcinoma in one, and both lymphoma and mesothelioma in 11. Thus in all there were 52 meso-

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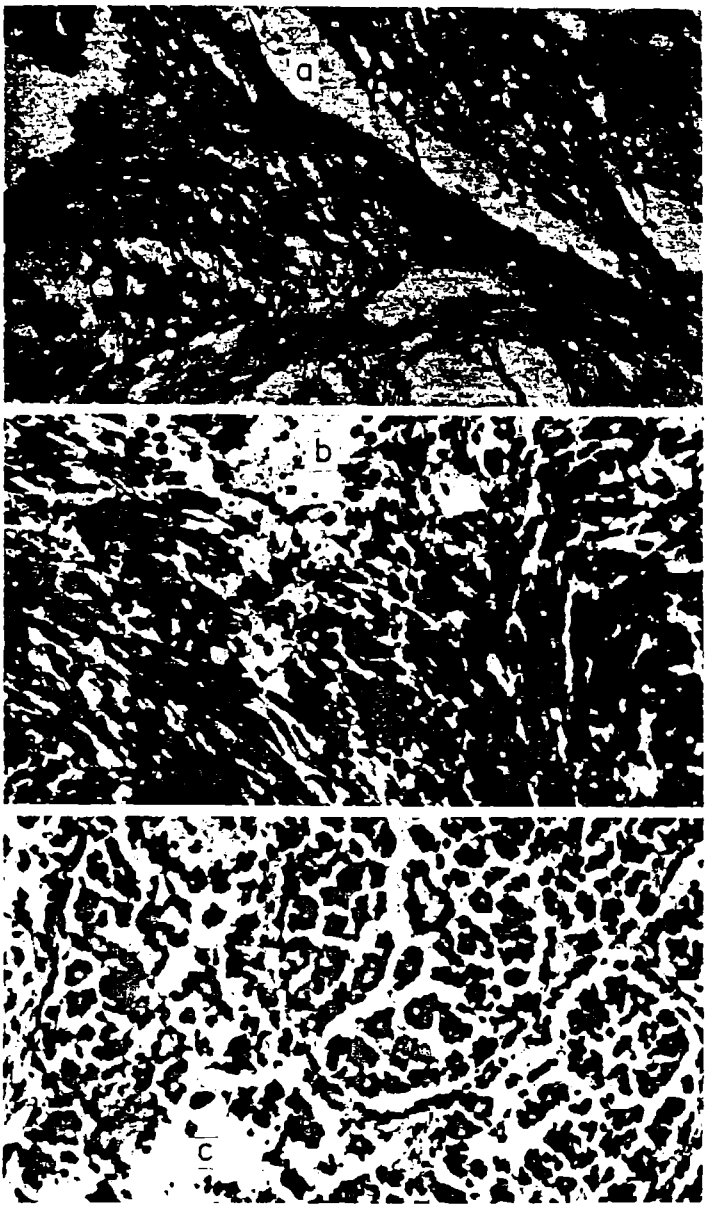

Peritoneal mesotheliomas in mice: (A) fibrous mesothelioma with few mitoses, (B) sarcomatous type, (C) epithelial mesothelioma. (HE stain, original magnification × 400.)

Results of intraperitoneal injection of Karain dust (KD) and of talc into mice

Injected material	*No of mice*	*Dead before six months*	*Remaining*	*Mesothelioma*	*Lymphomas*	*Both lymphoma & mesothelioma*	*Total tumours*	*%*
Saline	55	9	46	3	1	0	4	8·7
5 mg KD	69	14	55	6	—	4	10	18·1
10 mg KD	97	16	81	12	11	4	27	33
15 mg KD	98	25	73	10	7	—	17	23·3
20 mg KD	45	2	43	6	5	0	12*	28
30 mg KD	45	6	39	3	4	1	8	20·5
40 mg KD	37	7	30	4	4	2	10	33
All KD	391	70	321	41	31	11	84*	26·1
Talc (20 mg)	40	16	24	3	—	—	3	12·5

*One animal had an epidermoid tumour of the lung.

theliomas (16·5%), and 42 lymphomas (13·3%). All types of mesotheliomas (fibrous sarcomatous, and mixed) were seen (figure). The lymphomas were of the malignant lymphomatous type.

Depending on the stage of disease at death, the liver or spleen, or both, were often invaded in both types of tumours, and in some cases there were also distant metastases.

Discussion

The first international report from the village of Karain was published by Baris *et al* in 1978.[1] Extremely high incidences of malignant mesothelioma and benign pleural changes were reported, but apart from some asbestos fibres in some of the water used in the village (a finding which has not since been confirmed[7]), no mineral fibres had been found in the environment to that date. The extensive subsequent investigations showed that fibrous zeolites, the most important of which is erionite, were present in the village of Karain and in two other villages—Tuzköy and Sarihidir—where mesotheliomas also occur.[2,4,8]

There is still some doubt as to whether fibrous zeolite is the cause of the malignant mesotheliomas in this part of Turkey, and it has been suggested that it is mixed fibrous dust, containing asbestos fibres in addition to erionite, that is the culprit.[5,6] It has been claimed that there is some asbestos in the surroundings of Karain,[5] and lung biopsy specimens from patients from Karain contained other fibres in addition to erionite.[6] Measurements of the fibre levels in environmental air samples from Karain have shown such low figures[7] that doubt has been cast on their importance. Furthermore, it has been claimed that erionite fibres may be found in nearby villages where there are no known cases of mesotheliomas.[5] In an investigation by Mumpton, 15 of 28 probes from the three villages showed erionite fibres, whereas only four of 48 from control villages showed such fibres.[9] Förster has since reported that zeolites occur only in the northern part of Karain,[3] whereas the probes in Mumpton's study were taken in the southern part.

Asbestos fibres may be seen in the lungs of many people even without occupational exposure and their occurrence in people from Karain could be a chance finding. The vast majority of the fibres in the lungs of the Turkish villagers were erionite, and only a small minority were asbestos.[5,6] In patients from Tuzköy fibres of erionite in high concentrations and ferruginous bodies on erionite have been found.[10] Mesotheliomas in experimental animals after intrapleural or intraperitoneal injection or even inhalation of erionite have also been shown.[11-13]

The results of the present study confirm that dust from Karain is carcinogenic, and since it contained no asbestos it seems most probable that it is the erionite that caused the mesotheliomas. Maltoni *et al* state that pure erionite injected intrapleurally in rats causes mesothelioma in the pleura in 90% of the cases,[11] and Wagner reported 100% of mesotheliomas with both intrapleural injection and with inhalation.[13] In the present material the frequency of tumours was not as high, since dust from Karain was used rather than pure erionite, and perhaps also because the intraperitoneal rather than the intrapleural route was chosen. According to Maltoni *et al*, erionite causes more mesotheliomas than crocidolite, which is generally considered to be the most dangerous type of asbestos in this regard.[11] Wagner states that erionite is the only dust so far known to cause mesotheliomas in 100% of experimental animals on inhalation.[13]

An unexpected observations was the relatively common occurrence of malignant lymphomas in the experimental animals. There are no epidemiological findings to confirm this. Artvinli and Baris, however, state that tumours other than mesotheliomas may not be uncommon in Tuzcöy.[4] So far, however, with the exception of lung cancer,[7] no tumour other than malignant mesothelioma has been shown to have an increased incidence in the zeolite area.

Suzuki used two types of American erionite (from

Nevada and Colorado), which on intraperitoneal injection caused malignant peritoneal tumours in 50% of the animals,[12] a frequency higher than that among animals injected with asbestos (33% with amosite and 17% with crysotile). Of the 39 animals with erionite induced malignant peritoneal tumours, five had plasmocytomas, and in two other animals mesothelioma was combined with plasmocytoma. There were no plasmocytomas or lymphoid tumours in the animals exposed to asbestos. Thus erionite seems to be able to cause tumours of the lymphoid system, which fits with the present results.

From a study with intraperitoneal inoculation and inhalation of asbestos, Wagner reported some lymphosarcomas.[14] All animals that had received asbestos showed pronounced stimulation of the reticular endothelial system. Tumours of the haematopoietic and lymphatic systems have also been described in people exposed to asbestos.[15] Since the effects of erionite are thought to be similar to those of asbestos, stimulation of the lymphatic system with the ultimate formation of lymphomas seems a possibility. On the other hand, Wagner *et al* have also shown that crystalline silica when injected intrapleurally in rats can cause lymphoproliferation and lymphomas.[16 17] Thus it is possible that the other ingredients of the Karain dust—those that are not fibrous—caused the lymphomas in our study. There seem to be great differences in the susceptibility of various strains of animals to the development of lymphomas, Wistar rats being most affected.[16] Swiss albino mice might be such a susceptible strain of animals. These questions necessitate further studies, which are now in progress.

We thank Professors Y Baris and G Ataman for their help and support in the present study

References

[1] Baris YI, Sahin AA, Özesmi M, *et al*. An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Urgüp in Anatolia. *Thorax* 1978;**33**:181–92.

[2] Ataman G. The zeolitic tuffs of Cappadocia and their probable association with certain types of lung cancer and pleural mesothelioma. *Comptes Rendus d'Academie de Sciences de Paris, Série D*, 1978;**287**:207–10.

[3] Förster H. Eine mineralogisch-petrographische Untersuchung über mögliche Ursachen von Mesotheliomen in Kappadokien Türkei. *Zentralblatt Arbeitsmedizin* 1982;**32**:18–27.

[4] Artvinli M, Baris YI. Malignant mesotheliomas in a small village in the Anatolian region of Turkey: an epidemiological study. *JNCI* 1979;**63**:17–22.

[5] Rohl AN, Langer AM, Moncure G, Selikoff IJ, Fischbein A. Endemic pleural disease associated with exposure to mixed fibrous dust in Turkey. *Science* 1982;**216**:518–20.

[6] Boman G, Schubert V, Svane B, *et al*. Malignant mesothelioma in Turkish immigrants residing in Sweden. *Scand J Work Environ Health* 1982;**8**:108–12.

[7] Baris YI, Saracci R, Simonato L, Skidmore JW, Artvinli M. Malignant mesothelioma and radiological chest abnormalities in two villages in central Turkey. *Lancet* 1981;**i**:984–7.

[8] Artvinli M, Baris YI. Environmental fiber-induced pleuropulmonary diseases in an Anatolian village: an epidemiological study. *Arch Environ Health* 1982;**37**:177–81.

[9] Mumpton FA. *Report on reconnaissance study of the association of zeolites with mesothelioma cancer occurrences in central Turkey*. Brockport, New York: Department of the Earth Sciences, State University College, 1979

[10] Sebastien P, Eng A, Bignon J, Baris YI. Zeolite bodies in human lungs from Turkey. *Lab Invest* 1981;**44**:420–5.

[11] Maltoni C, Mikardi F, Movisi L. Pleural mesotheliomas in Sprague-Dawley rats by erionite: first experimental evidence. *Environ Res* 1982;**29**:238–44.

[12] Suzuki Y. Malignant mesothelioma following intraperitoneal administration of asbestos and zeolite. In: *VIth International Pneumoconiosis Conference 1983*. Vol 2. Bochum, West Germany: Bergbaugenossenschaft, 1983:1047–51.

[13] Wagner JC. Experimental studies using erionite fibre. In: *VIth International Pneumoconiosis Conference 1983*. Vol 2. Bochum, West Germany: Bergbaugenossenschaft, 1983:1009.

[14] Wagner JC. The induction of tumours by the intrapleural injections of various types of asbestos dust. In: Severil L, ed. *Lung tumours in animals. Proceedings of the 3rd Quadrennial Conference on Cancer, Perugia, 1965*. Perugia: Division of Cancer Research, 1966:589–608.

[15] Kagan E, Jacobson RJ. Lympoid and plasma cell malignancies: asbestos-related disorders of long latency. *Am J Clin Pathol* 1983;**80**:14–20.

[16] Wagner MMF, Wagner JC. Lymphomas in the Wistar rat after intrapleural inoculation of silica. *JNCI* 1972;**49**:81–9.

[17] Wagner MMF, Wagner JC, Davies R, Griffith DM. Silica-induced malignant lymphocytic lymphoma: incidence linked with strain of rat and type of silica. *Br J Cancer* 1980;**41**:908–17

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Phenotypic characterisation of peripheral blood lymphoid cells in people exposed to fibrous zeolite

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Phenotypic characterisation of peripheral blood lymphoid cells in people exposed to fibrous zeolite

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ABSTRACT Among inhabitants of the village of Karain in Turkey there is an extremely high incidence of malignant mesothelioma, most probably due to exposure to erionite, which is a fibrous zeolite and similar in appearance and properties to asbestos. This mineral may be found in the dust in the village. To characterise possible disturbances in the immune system of people exposed to fibrous zeolite, a phenotypic characterisation of lymphoid cells in the peripheral blood of 74 immigrants to Sweden from Karain was performed. Compared with normal controls, the mean percentages of Leu 4^+ cells (Pan-T) and Leu $3a^+$ cells ("helper/inducer" T cells) were significantly decreased, whereas the mean percentage of Leu $2a^+$ cells ("suppressor/cytotoxic" T cells) was normal, leading to a significant reduction of the Leu 3a/Leu 2a subset ratio. The percentage of B cells (Leu 12^+ cells) was significantly increased, whereas the percentages of both HLA-DR^+ and HLA-DQ^+ cells were normal. The percentage of natural killer cells (NK) and killer (K) cells as defined by the monoclonal anti-Leu 7 and anti-Leu 11b were also normal. These findings indicate that exposure to fibrous zeolite causes a numerical imbalance between the two phenotypically different T cell subsets similar to that seen in asbestos exposed individuals.

Inhabitants of the village of Karain in Anatolia have an extremely high incidence of malignant mesothelioma,[1] probably caused by the inhalation of fibrous zeolite (erionite) which is present in the environment.[1 2] The mineralogical characteristics[3 4] and the deleterious effects on human health are similar to those of asbestos.[5] In asbestosis several immunological stigmata such as hypergammaglobulinaemia (IgG), in vitro cellular immune dysfunction, and abnormal peripheral blood levels of different immunoregulatory T cell subsets have been reported.[6 7]

Since an intact immunoregulatory network is thought to be of great importance for effective surveillance against tumours, such immunological derangements are of interest in the search for pathogenetic mechanisms underlying the development of mesothelioma. An experimental study on rats suggested that dust from Karain may affect the lymphoid system.[8] To our knowledge, however, no study of the immunological status of people exposed to erionite has been reported previously.

In Stockholm there are about 150 individuals who were born in Karain or lived there for many years but have now immigrated to Sweden. Among the immigrants from Karain there have been several deaths due to malignant mesothelioma, and more are to be expected.[9] The aim of the present study was to investigate whether there are any aberrations in the number of lymphoid cells in the peripheral blood of these people and if so to correlate them with radiographic findings, a family history of mesothelioma, and to future development of disease, particularly mesothelioma.

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Material and methods

The names and addresses of individuals from the village of Karain now living in Stockholm were obtained from a list of an earlier study[9] and completed with personal information from some of the people in the group who were invited to participate in the present study. The subjects underwent clinical examination and chest radiography and their history (personal and familial) was taken. Blood samples for investigation of different lymphoid cells and for determination of the concentrations of albumin, immunoglobulins, and orosomucoid were taken.

CONTROLS

Healthy blood donors were used as controls.

PERIPHERAL BLOOD MONONUCLEAR CELLS

Mononuclear cells in heparinised peripheral blood were isolated by standard methods on a Ficoll-Hypaque density gradient. The cells were then washed three times in phosphate buffered saline (PBS) containing 0·1% bovine serum albumin (BSA) at 10^6 cells/ml. About 10 μl of the cell suspension was dropped into each well of a multiple well microscope slide. The slides were air dried at +37°C for one hour and then stored at −70°C. After storage the cell preparations were immediately fixed in acetone diluted 1:1 with water (+4°C) for one minute and finally fixed in 100% acetone (+4°C) for five minutes before staining.[10]

ANTISERA AND OTHER REAGENTS

The murine monoclonals denoted anti-Leu 4, -Leu 2a, -Leu 3a, -Leu 7, -Leu 11b, -Leu 10, -Leu 12, and anti-HLA-DR were all obtained from Becton Dickinson (Sunnyvale, Calif). Anti-Leu 4 defines all peripheral T cells,[11] whereas anti-Leu 2a defines the "suppressor/cytotoxic" T cell subset.[12] Anti-Leu 3a reacts with the "helper/inducer" T cell subset,[12] but also reacts with some cells of the monocyte/macrophage lineage[13] in prefixed cell samples. Anti-Leu 12 defines all peripheral B cells (T C Meeker *et al*, in preparation). Anti-Leu 10 is specific for the HLA-DQ antigen, a human D region associated antigen,[14] that differs from the HLA-DR antigen recognised by the anti-HLA-DR monoclonal.[15] The human D region associated antigens are expressed in B lymphocytes, monocytes/macrophages, and activated T cells. Anti-Leu 7 and anti-Leu 11b monoclonals are thought to define the major NK and K cell subsets.[16 17]

The FITC conjugated goat antimouse antibody was obtained from the National Bacteriological Laboratory (SBL), Sweden. Biotinylated horse antimouse IgG, avidin DH, and biotinylated horseradish peroxidase H ("ABC" kit) were obtained from Vector laboratories (Burlingame, Calif). 3-Amino-9-ethylcarbazole was obtained from Sigma (St Louis, Mo).

IMMUNOENZYME STAINING

After the fixation procedure the slides were washed in PBS (pH 7·4) for five minutes. The subsequent incubations were carried out sequentially at room temperature and the slides were washed for five minutes in the PBS buffer between each step. Endogenous peroxidase was blocked by incubation in 0·3 H_2O_2 for 15 minutes. The cell preparations were then incubated for 30 minutes in humid atmosphere with 25 μl portions of monoclonal antibodies (dilution 1/32 for anti-Leu 4, -Leu 2a, -Leu 3a, -Leu 10, and -Leu 12, and 1/128 for anti-HLA-DR in PBS containing 4% BSA). Biotinylated horse-antimouse IgG (dilution 1/400) was allowed to react with the cell preparation for 30 minutes. A complex of 5 μl of avidin DH (10 mg/ml) and of 5 μl of biotinylated horseradish peroxidase H (5 mg/ml) in 400 μl of PBS was then layered on the cell preparation for 30 minutes. The peroxidase reaction was developed with use of a carbazole containing buffer for 15 minutes. The cell preparations were exposed to haematoxylin for nuclear staining and mounted in Kaiser's glycerin-gelatin (Merck, Darmstadt). The percentage of positive cells with a marginal membrane staining pattern[11] was determined by counting 200–500 mononuclear cells in a routine light microscope.

IMMUNOFLUORESCENCE STAINING

When using first step monoclonals of IgM class (anti-Leu 7 and anti-Leu 11b antibodies) FITC conjugated goat antimouse Ig was used as second step reagent. The cell preparations were incubated for 30 minutes with 25 μl portions of monoclonal antibodies (dilution 1/80 for Leu 7 and 1/20 for Leu 11b in PBS containing 4% BSA), washed for five minutes in PBS and then incubated with FITC antimouse immunoglobulin (dilution 1/320 in PBS containing 4% BSA) for 30 minutes. After being washed in PBS for 10 minutes, the cell preparations were mounted in PBS-glycerine. The cells were examined under a Leitz epifluorescence microscope. The percentage of positive cells was determined by counting 200 cells. The primary antibody was omitted for the negative controls.

STATISTICAL METHODS

Student's *t* test was used for tests of significance.

Results

Ninety four individuals responded to the invitation and participated in the study. In 74 (38 women and 36 men) blood samples were taken. Ten of these 74 had lived in Karain for less than ten years, 38 for ten to 20 years, and 26 for over 20 years. Seven showed radiological pulmonary abnormalities (pleural plaques).

The serum concentrations of albumin, IgG, IgA, and IgM were within the normal range (table). Neither sex, radiological abnormalities, nor the duration of residence in Karain showed any correlation with the serum immunoglobulin concentrations. The plasma orosomucoid concentration was slightly raised in those aged over 45 (n = 7) (mean value 1·2, 2 SD 0·19).

As seen in fig 1, the mean percentage of Leu 4^+ cells

Serum concentrations of albumin, immunoglobulins, and plasma orosomucoid in the Karain group. Normal range is given for comparison

	Karain group		
	No of samples	*Mean value and SEM (g/l)*	*Normal range (g/l)*
Serum albumin	71	45·8 (0·45)	37–52
Serum IgG	71	11·7 (0·25)	7–15
Serum IgA	58	2·05 (0·11)	0·8–3·8
Serum IgM	58	1·39 (0·07)	0·4–2
Plasmaorosomucoid	71	1·0 (0·03)	0·5–1

was lower ($p < 0·01$) and that of B cells (Leu 12^+) higher ($p < 0·05$) in the Karain group than in the normal controls. The mean percentages of HLA-DR and HLA-DQ (Leu 10) expressing cells did not differ from those in the control group. NK/K cell numbers as defined by the anti-Leu 7 and anti-Leu 11b monoclonals were also normal.

The percentage of Leu $3a^+$ cells (helper/inducer T cells) was significantly lower ($p < 0·01$) in the Karain group than in the controls, whereas that of Leu $2a^+$ cells (suppressor/cytotoxic T cells) was normal. Thus there was a significant decrease ($p < 0·05$) in the Leu 3a/Leu 2a ratio in the Karain group (fig 2).

No correlation was found between the percentages of different lymphoid cells and age, sex, family history of mesothelioma or duration of residence in the village of Karain. People with chest *x* ray abnormalities, however, showed a more profound reduction of Leu $3a^+$ cells, leading to a lower Leu 3a/Leu 2a ratio. These differences from the rest of the Karain group were not statistically significant.

Discussion

Earlier epidemiological studies have shown that environmental exposure to fibrous zeolite can cause pleural plaques, pleural calcification, pleural and peritoneal mesothelioma, and pulmonary fibrosis,[1 15 18] diseases similar to those caused by exposure to asbestos. Experimental exposure of animals to erionite has resulted in mesothelioma,[8 19] which supports these epidemiological data.

In asbestosis several immunological derangements have been shown, including a numerical imbalance between different immunoregulatory T cell subsets.[20] In people exposed to erionite, however, no such immunological studies have been reported. Our finding of a reduced number of Leu $3a^+$ cells (helper/inducer T lymphocytes) leading to a decrease in the Leu 3a/Leu 2a ratio is, to our knowledge, the

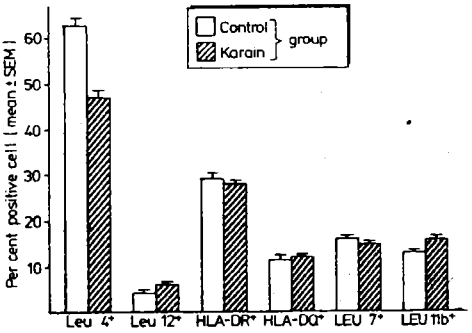

Fig 1 *Per cent positive cells (mean SEM) of different phenotypes in peripheral blood of Karain group (n = 74 for Leu 1, Leu 12, HLA-DR, and HLA-DQ, n = 15 for Leu 7 and Leu 11b) and control group (n = 10 for all monoclonals examined).*

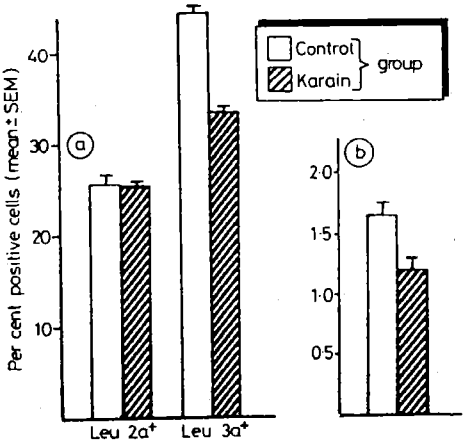

Fig 2 *(a) Per cent positive cells (mean SEM) of Leu 3a and Leu 2a phenotype in peripheral blood in Karain group (n = 74) and control group (n = 34). (b) Leu 3a/Leu 2a ratio in Karain group (n = 74) and control group (n = 34).*

first evidence of a T cell subset derangement in people exposed to erionite. This finding is similar to the derangement in T cell subsets found in individuals exposed to asbestos.

Since an intact immunoregulatory network is thought to be of great importance for effective surveillance against tumours, such observed imbalance between different immunoregulatory T cell subsets are of interest. It is still unclear, however, whether this imbalance is of aetiological importance for the development of mesothelioma, or if it represents a secondary epiphenomenon. Further clinical and immunological follow up of this group might shed some light on this particular question.

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References

1 Baris YI, Sahin AA, Özesmi M, *et al.* An outbreak of pleural mesothelioma and chronic fibrosing pleurisy in the village of Karain/Urgup in Anatolia. *Thorax* 1978;33:184–92.
2 Elmes PC. Fibrous minerals and health. *Journal of the Geological Society of London* 1980;137:525–35.
3 Förster H. Eine mineralogisch-petrograpohische Untersuchung uber mögliche Ursachen von Mesotheliomen in Kappadokien/Turkei. *Zentralblatt für Arbeitsmedizin* 1982;32: 18–27.
4 Helfferrich F. A simple identification for zeolites (molecular sieves). *American Mineralogist* 1964;49:1752–4.
5 Artvinli M, Baris YI, Environmental fiber-induced pleuropulmonary disease in an Anatolian village: and epidemiological study. *Arch Environ Health* 1982;37:177–81.
6 Preger L. *Asbestos-related disease*. London: Grune and Stratton, 1978:23–6.
7 de Shazo RD, Nordberg BA, Baser Y, Bozelka B, Weill H, Salvaggio J. Analysis of depressed cell-mediated immunity in asbestos workers. *J Allergy Clin Immunol* 1983;75:418–24.
8 Özesmi M, Patiroghe TE, Hillerdal G, Özesmi C. Peritoneal mesothelioma and malignant lymphoma in mice caused by fibrous zeolite. *Br J Ind Med* 1985;42:746–9.
9 Boman G, Schubert V, Svane B, *et al.* Malignant mesothelioma in Turkish immigrants residing in Sweden. *Scand J Work Environ Health* 1982;8:108–12.
10 Karlsson-Parra A, Forsum U, Klareskog L, Sjöberg O. A simple immunoenzyme batch staining method for the enumeration of peripheral human T lymphocyte subsets. *J Immunol Methods* 1983;64:85–90.
11 Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and T cytotoxic/suppressor subpopulations in mouse and man. *J Exp Med* 1981;153:310–23.
12 Engleman EG, Benike CJ, Glickman E, Evans RL. Antibodies to membrane structures that distinguish suppressor-cytotoxic and helper T lymphocyte subpopulations block the mixed leucocyte reaction in man. *J Exp Med* 1981;153:193–8.
13 Wood GS, Warner NL, Wranke RA. Anti Leu 3/Tu antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J Immunol* 1983;131:212–6.
14 Brodsky FM. A matrix approach to human class II histocompatibility antigens: reactions of four monoclonal antibodies with the products of nine haplotypes. *Immunogenetics* 1984;19:179–94.
15 Lampson LA, Levy R. Two populations of Ia-like molecules on a human B-cell line. *J Immunol* 1980;125:293–9.
16 Abo T, Balch CM. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J Immunol* 1981;127:1024–9.
17 Phillips JH, Le AM, Lanier LL. Natural killer cells activated in a human mixed lymphocyte response culture identified by expression of Leu-12 and class II histocompatibility antigen. *J Exp Med* 1984;159:993–1008.
18 Casey KR, Shigeoka JW, Rom WN, Moatamed F. Zeolite exposure and associated pneumoconiosis. *Chest* 1985;87:837–40.
19 Maltoni C, Mikardo F, Moviso L. Pleural mesothelioma in Sprague-Dawley rats by erionite: first experimental evidence. *Environ Res* 1982;29:238–44.
20 de Shazo RB, Hendrich AJ, Diem JE, *et al.* Immunologic aberrations in asbestos cement workers: dissociation from asbestosis. *J Allergy Clin Immunol* 1983;72:454–61.

PROSPECTIVE CLINICAL AND RADIOLOGIC STUDY OF ZEOLITE-EXPOSED TURKISH IMMIGRANTS TO SWEDEN

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ABSTRACT

Ninety-four persons from the village of Karain, now residing in Stockholm, were investigated. In this village environmental exposure to erionite, a fibrous zeolite, occurs and there is a high risk of mesothelioma among the villagers. Since an earlier investigation of the cohort in 1980, another four young persons have fallen ill with malignant mesothelioma in this group, where the incidence thus approaches 1 per 100 per year and is the most common cause of death. Pleural plaques were noted in 7.4 per cent, and there were radiological signs of affection of the visceral pleura (increased visibility and enlargement of the minor fissure), but otherwise no abnormal findings at radiography. This is probably due to the low mean age (36 years). Further follow-up of this cohort could give clues to the pathogenesis of malignant mesothelioma.

INTRODUCTION

Diffuse pleural mesothelioma is the most frequent cause of death in the village of Karain in Anatolia, Turkey (1). The emigration from this small village of about 500 persons has been relatively large, and about 150 immigrants from there now live in Stockholm, Sweden. Up to 1980 when a study was performed (2) three cases of mesothelioma had been reported in this group. From 1981 to the end of 1985, another four cases of malignant mesothelioma have occurred in this small cohort. The present study is a follow-up of the 1980 study of clinical and radiological findings and a short report on the four new cases of mesothelioma.

SUBJECTS AND METHODS

An invitation to participate in a control survey was sent to all known Turkish immigrant families from Karain, Turkey, who were living in the city of Stockholm or in its suburbs. The survey was conducted between February and June 1985. The list comprised those who attended the first survey (2) and in

addition a number of other persons from Karain, the names of whom were obtained from friends and relatives, and who were initially willing to participate. Ninety-four persons, 47 men and 47 women, were investigatied. The following examinations were carried out:

a/ Personal questioning comprising family history, particularly regarding the occurrence of respiratory diseases and tumours; occupational history; smoking habits; length of time spent in the village; respiratory and other diseases and symptoms. This history was taken in Turkish by a Turkish doctor with knowledge of the area and considerable experience from field studies in the village (MÖ).

b/ Clinical examination.

c/ Vital capacity and forced expiratory volume were measured in a Vitalograph.

d/ Chest roentgenograms with posterio-anterior (PA), lateral and two oblique views were taken.

e/ Blood tests were analyzed. The results from this investigation has been reported elsewhere (3).

RESULTS

Participation. The full list comprised 117 persons. From the original 1980 list, three had died, all of mesothelioma (see case reports), and four had moved back to Turkey. Another approximately 25 persons either refused to attend or could not be identified with certainty. In the final count, twenty-three persons who had originally been willing to attend never showed up. Thus, there remained 94 persons, 47 men and 47 women.

Age. The age distribution of the participants can be seen in figure 1. The mean age was 36 years with the youngest being 17 and the oldest 64 years old.

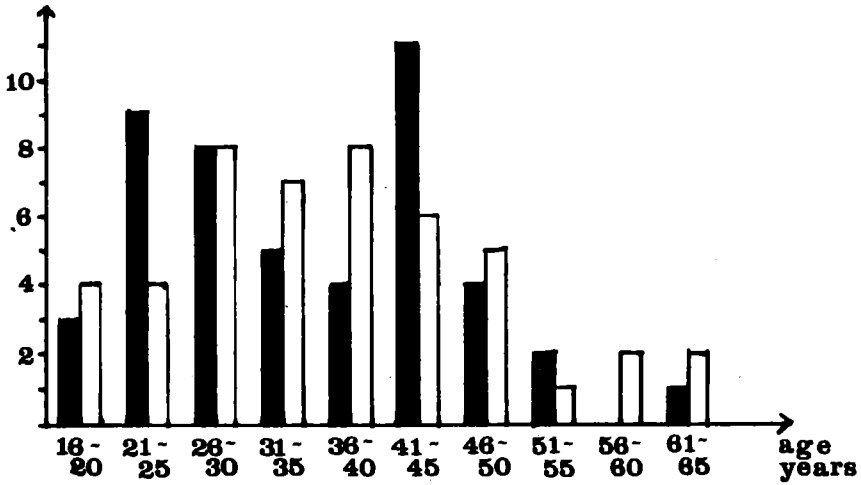

Figure 1. Age distribution. Filled bars, men; unfilled, women.

Time in Karain. Eighty-nine patients were born in Karain and five were married with persons from the village and had lived a great part of their lives in it. The time spent in the village can be seen in figure 2. Although the majority had immigrated directly to Sweden from their home village, a few had resided in other places in Turkey as well. Nine individuals had lived in Sweden less than five years, 16 between 5 and 10 years, 44 10-15 years, and 23 between 15 and 20 years.

Occupation and smoking habits. In Karain most individuals had been agricultural workers. In Sweden, 63 were currently employed as janitors, 13 were cooks or restaurant workers, five technicians, six teachers or clerks, two housewives and five were studying. Only two were occupationally exposed to asbestos. Twenty-nine of the men (60 per cent) were smokers. None of the women smoked.

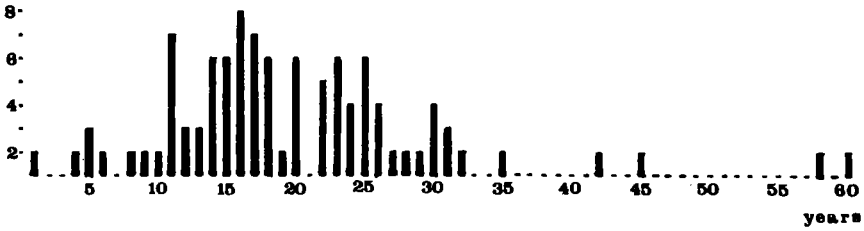

Figure 2. Length of time resided in the village of Karain. (Five people who were married to villagers and moved there in adult life excluded.)

Mesothelioma in the family. Sixty-four individuals (65 per cent) gave a history of malignant mesothelioma among their close relatives. Many of those 64 reported more than one case, the mean being 1.9 per person. Sixteen persons had had both their parents dying in the disease, and one each of them in addition a husband or a brother. Most of these mesotheliomas had not been pathologically verified, but the signs and symptoms of the disease are well known by the inhabitants of the village.

Respiratory symptoms and some other findings. Two patients complained of dyspnea and three of chest pain. At examination, rhonchi were heard in five persons. Four suffered from shoulder pains, 15 from low-back pains, 9 joint pains, and 15 abdominal pains, none of them severe.

Pulmonary function. There were no gross abnormalities in lung function.

Chest roentgenograms. Pleural plaques, most often unilateral, were seen in 7 persons (7.4 per cent). Only one person showed abnormalities consistent with pulmonary fibrosis. The transverse fissure was visible in 71 cases (75.8 per cent) and was thickened in 8 (8.5 per cent) at the PA view. In the lateral view, the oblique fissures were seen in 79 cases (84 per cent). There were no tumours or pleural exudates.

Sixty-three of the participants had also participated in the 1980 study. No evident progression was seen on the chest roentgenograms, except in a few cases where the width of the transverse fissure had progressed.

CASE REPORTS

Case 1. This man was 18 years old when he left Karain in 1960. Both grandfathers, his father, and a cousin had died in mesothelioma. At age 37, he started having right-sided chest pain and chest roentgenogram revealed a pleural effusion. Repeated thoracocenteses and a thoracoscopy did not lead to diagnosis, and a thoracotomy with decortication was performed. Biopsies showed only mesothelial cell proliferations. The symptoms continued, and 15 months after the operation a lump appeared on the back, the biopsy of which showed metastasis from papillary mesothelioma. Treatment with radiotherapy and cytostatics gave some improvement, but subsequently the tumour progressed, giving exudate on the other side as well and ascites, and the patient died in 1984, 4 years and 8 months after the first symptoms.

Case 2. This woman was born in Karain where she lived and worked until she moved to Sweden at age 22. At age 35, she suffered from coughing and pain in the right shoulder; thoracoscopy revealed sanguinolent fluid in the pleura and multiple scattered mushroom-like tumours. Pleurodesis was performed with Mepacrine with good result, but the tumour relentlessly progressed despite apparent palliation with radiotherapy and cytostatics, and the patient died 14 months later.

Case 3. A woman who emigrated from Karain at age 18 and after 10 years in Sweden developed the first symptoms of the tumour at age 28, also on the right side. Thoracotomy confirmed the diagnosis of mesothelioma, treatment with cytostatics and radiotherapy was of no avail, and she died 14 months after the first symptoms.

Case 4. Also a woman, who came to Stockholm at age 36. After two years there she developed right-sided pleurisy, and further investigations including thoracoscopy revealed malignant mesothelioma. Cytostatics were given due to increasing pain but to no avail and the patient died 18 months after the first symptoms.

DISCUSSION

In the village of Karain malignant mesothelioma affects both sexes equally, usually during middle age (35-50 years), or somewhat later in life. Smoking habits do not appear to affect the risk of developing the disease, as the men smoke and the women do not. This is in accordance with the literature (4). The disease appears to be familial but is not due to a genetic defect, since some patients are not born in Karain but have gone there from other places in Turkey as brides. In these instances, the tumours develop about 35 years later.

Extensive investigations as to the cause of the disease in Karin village has revealed the occurrence of a nonasbestos mineral fibre of the zeolite family in the ground and in the air of the village (5,6). This fibre is called erionite and has also been shown to coccur in lung specimens from people living in the village (7,8). Experimental studies with Karain dust (9) and pure erionite (10,11) has shown a high incidence of mesothelioma. Thus, there seems to be no doubt that the large number of cases of mesothelioma in Karain villagers is due to erionite.

The effects of erionite are very similar to those of asbestos, and therefore not surprisingly a high incidence of pleural changes, including plaques, has also been reported (1). In the present study, only 7.4 per cent had visible pleural plaques. This comparatively low figure is probably due to the fact that pleural plaques need many decades to develop (12) and the mean age of the investigated persons in this study is relatively low.

A more exhaustive list of Karain villagers living in the Stockholm area comprised 117 persons. This list was obtained thanks to their own cooperation and with the aid of various organisations, but it is unfortunately not complete. Approximately 50more persons from the village are known to live in the area but showed no interest in participating in the survey. This should, however, not affect the results of the study, since their reasons for not participating are not medical.

The visceral pleura in asbestos exposure has been the subject of few investigations, mainly due to the reason that it is not very easily studied. It can be seen only in the fissures and especially in the minor fissure of the PA view. If carefully looked for, this fissure is seen in a high proportion of normal individuals as a hair-fine line. Felson claims that it can be seen in 56 per cent (13). Even a very slight thickening of it will increase the chances of it being seen. Thickening of the minor fissure is included in the ILO system for pneumoconiosis as an obligatory comment (14), but unfortunately it is rarely commented upon.

Studies from Finland (15) and Turkey including the erionite villages (16) are, however, available. In the erionite villages, in persons without pleural calcifications, 34 per cent did have an enlarged minor fissure; in Finland, in an area with environmental asbestos pollution, this figure was 7.9 per cent. In controls from both countries no such thicke-

ning of the minor fissure occurred. In a recent paper, it was claimed that the first sign of asbestos exposure is a diffuse thickening of the visceral pleura (17). Thus, it is probable that the increased visibility and the 8.5 per cent of clearly thickened minor fissures in this study reflects early pleural changes caused by by erionite. This theory is further strengthened by the progression seen in a few cases.

The "normal" or "background" incidence of malignant mesothelioma in a population not exposed to asbestos has been estimated to be 1 per million per year (3). In the present small group of 150 persons, there is about one case every year. There might be other patients who go back to Turkey when they fall ill and thus have been missed by us. Thus, the incidence approaches 1 per 100 per year and is at least half that - a risk increase of 5 to 10 thousand times. Further follow-up of this small group is therefore of great importance and might give information as to various risk factors other than the exposure for erionite per se.

REFERENCES

1. Baris YI, Sahin AA, Özesmi M, Kerzse I, Ozen E, Kolacan B, Altinörs M, Göktepeli A. An outbreak of pleural mesothelioma and chronic fibrosin pleurisy in the village of Karain/Urgüp in Anatolia. Thorax 1978; 33: 181-192.

2. Boman G, Schubert V, Svane B, Westerholm P, Bolinder E, Rohl AN, Fischbein A. Malignant mesothelioma in Turkish immigrants residing in Sweden. Scand J Work Envir Health 8: 108-112, 1982.

3. Özesmi M, Karlsson-Parra A, Hillerdal G, Forsum U. Brit J Ind Med 46: 630-833, 1986.

4. Hillerdal G. Malignant mesothelioma 1982: review of 4710 published cases. Brit J Dis Chest 77: 321-344, 1983.

5. Baris YI, Saracci R, Simonato L, Skidmore JW, Artvinli M. Malignant mesothelioma and radiological chest abnormalities in two villages in central Turkey. Lancet 1: 984-987, 1981.

6. Baris YI, Simonato L, Artvinli M, Pooley F, Sacci R, Skidmore J, Wagner J.C. Epidemiological and environmental evidence of the health effects of exposure to erionite fibers: A four-year study in the Cappadocian region of Turkey. Int J Cancer 39: 10-17, 1987.

7. Elmes PC. Fibrous minerals and health. J Geol Soc London 137: 525-535, 1980.

8. Rohl AN, Langer AM, Moncure G, Selikoff IJ, Fischbein A. Endemic pleural disease associated with exposure to mixed fibrous dfust in Turkey. Science 216: 518-520, 1982.

9. Özesmi M, Patiroglu TE, Hillerdal G, Özesmi C. Peritoneal mesothelioma and malignant lymphoma in mice caused by fibrous zeolite. Brit J Ind med 1985; 42: 746-749.

10. Suzuki Y, Koyama N. Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. Environ Res 35: 277-292, 1984.

11. Wagner JC. Experimental studies using erionite fibre. In: VIth International Pneumoconiosis Conference 1983, Bochum/ West Germany. Deutsche Bergbaugenossenscahft, vol. 2: 1047-1051.

12. Hillerdal G. Pleural plaques: occurrence, exposure to asbestos, and clinical importance. Acta Universitatis Upsaliensis 363, 1980.

13. Felson B, Chest roentgenology. W:B: Saunders Company, USA 1973. ISBN 0-7216-3591-1.

14. Guidelines for the use of ILO international classification of radiographs of pneumoconioses. ILO Occupational Safety and Health Series 22, rev 1980.

15. Hillerdal G, Zitting A, van Assendelft AHW, Kuusela T. Rarity of mineral fibre pleurisy among persons exposed to Finnish anthophyllite and with low risk of mesothelioma. Thorax 39: 608-611, 1984.

16. Hillerdal G, Baris YI. Radiological study of pleural changes in relation to mesothelioma in Turkey. Thorax 38: 443-448, 1983.

17. Bohlig H, Calavrezos A. development, radiological zone patterns, and importance of diffuse pleural thickening in relation to occupational exposure to asbestos. Brit J Ind Med 1987; 44: 673-681.

PHENOTYPIC CHARACTERISATION OF PERIPHERAL BLOOD LYMPHOID CELLS IN PATIENTS WITH ASBESTOS-RELATED PLEURAL LESIONS

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ABSTRACT

Asbestos-related parietal pleural plaques develop slowly and are of little clinical significance. Other asbestos-related pleural reactions, as for example acute exudative pleurisy and progressive pleural fibrosis, often occur suddenly and are of clinical importance. The pathogenesis of these reactions is unknown, but immunological disturbances may be involved. To investigate this a phenotypic characterization of lymphoid cells was performed in the blood of 45 patients with asbestos-related pleural lesions; 20 with pleural plaques (PP), 15 with diffuse pleural fibrosis (DPF), and 10 with acute benign asbestos pleurisy (BAPE). Twenty-four healthy blood donors were used as controls. The percentage number of "helper/inducer" T cells was significantly lower in the BAPE and DPF groups than in the control and PP groups. All asbestos groups together had a significantly higher percentage of B cells than the controls. Thus, significant differences in peripheral blood lymphoid cells were found in various groups of asbestos-related pleural diseases, and in particular patients with DPF and BAPE differed from patients with PP only who were similar to normals.

INTRODUCTION

Exposure to asbestos can cause both benign reactions and malignant tumours in man (1) and can also affect the immune system (2). Pleural changes are the most common of the asbestos-related findings, and most often seen are parietal pleural plaques. These consist of dense collagen tissue and typically show a gradual development without any sudden progression (3). The lung itself is not affected by the plaques, although a parenchymal fibrosis may be present concomitantly.

Other asbestos-related reactions of the pleura also occur. An exudative pleurisy can appear suddenly (4). Extensive pleural fibrosis with adhesions and involvement of the lung parenchyma affecting the pulmonary function can also be seen. This type of change can result from an exudative pleurisy, but it can also occur without any apparent such episode. These changes can be progressive (5). Navratil and Dobias gave the first extensive description of these pleural changes and named them "hyalinosis complicata", while the plaques were called "hyalinosis simplex" (6). The erythrocyte sedimentation rate (ESR) is significantly more often elevated in patients with the diffuse pleural fibrotic changes than in those with pleural plaques alone, indicating some type of inflammatory activity (7).

The present study was undertaken with the aim of determining the nature of some possible disturbances in the immune system of persons with different asbestos-related pleural changes.

MATERIAL AND METHODS

Patients: At the Department of Lung Medicine in Uppsala, Sweden, a large number of persons with asbestos-related pleural and parenchymal changes are seen (3). They were divided into three groups according to the findings on their chest roentgenograms:

1/ Pleural plaques only (PP), the criteria being bilateral plaques at least 5 mm thick (3);
2/ benign asbestos pleural effusion (BAPE), with a proven exudate and tuberculosis, malignancy and other possible causes of the effusion excluded;
3/ bilateral diffuse pleural thickening of various degrees (DPF), with rounded costo-phrenic angles but without pleural effusion.

Consecutive patients, all male, were collected. The number of patients with PP was limited to 20. There were 15 patients with DPF and 10 with BAPE. They were all occupationally exposed to asbestos, mainly as building workers, and most had had only intermittent exposure. They had been exposed for in the mean20 years with extremes of 3 to 35 years. There was no difference in the intensity or length of exposure between the various groups (PP,BAPE och DPF).

Controls: Twenty-four healthy blood donors were used as controls.

Peripheral blood mononuclear cells:

Mononuclear cells in heparinized venous blood were isolated by standard methods on a Ficoll-Hypaque density gradient. The cells were then washed three times in phosphate-buffered saline (PBS) containing 0.1 % bovine serum albumin (BSA) at 10^6 cells/ml. Approximately 10 ul of the cell suspension was dropped into each well of a multiple well microscope slide. The slides were air-dried at +37°C for one hour and then stored at -70°C. After storage the cell preparations were immediately fixed in acetone diluted 1:1 with water (+4°C) for 1 min and finally fixed in 100 % acetone (+4°C) for 5 min before staining (8).

Antisera and other reagents:

The murine monoclonals denoted anti-Leu 4, -Leu 2a, -Leu 3a, -Leu 7, -Leu 11b, -Leu 10, -Leu 12 and anti-HLA-DR, were all obtained from Becton Dickinson (Sunnyvale, Calif., USA). Anti-Leu 4 defines all peripheral T cells (9), whereas anti-Leu 2a defines the "suppressor/cytotoxic" T-cell subset (10). Anti-Leu 3a reacts with the "helper/inducer" T-cell subset (10), but also reacts with some cells of the monocyte/ macrophage lineage (11) in prefixed cell samples. Anti-Leu 12 defines all peripheral B cells (12). Anti-Leu 10 is specific for the HLA-DQ antigen, a human D region associated antigen (13), which differs from the HLA-DR antigen recognized by the anti-HLA-DR monoclonal (14). The human D

region associated antigens are expressed in B lymphocytes, monocytes/macrophages and activated T cells. The anti-Leu 7 and anti-Leu 11b monoclonals are thought to define the major NK and K cell subsets (15,16).

FITC-conjugated goat anti-mouse antibody was obtained from the National Bacteriological Laboratory (SBL), Sweden. Biotinylated horse anti-mouse IgG, avidin DH and biotinylated horseradish peroxidase H ("ABC" kit) were obtained from Vector laboratories (Burlingame, Calif., USA). 3-amino-9-ethylcarbazole was obtained from Sigma (St Louis, Mo, USA).

Immunoenzyme staining:

After the fixation procedure the slides were washed in PBS (pH 7.4) for 5 min. The subsequent incubations were carried out sequentially at room temperature and the slides were washed for 5 min in the PBS buffer between each step. Endogenous peroxidase was blocked by incubation in 0.3 percent H_2O_2 for 15 min. The cell preparations were then incubated for 30 min in a humid atmosphere with 25-ulportions of monoclonal antibodies (dilution 1/32 for anti-Leu 4, -Leu-2a, -Leu 3a, -Leu 10 and -Leu 12, and 1/128 for anti-HLA-DR in PBS containing 4 per cent BSA). Biotinylated horse-antimouse IgG (dilution 1/400) was allowed to react with the cell preparation for 30 min. A complex of 5 ul of avidin DH (10 mg/ml) and of 5 ul of biotinylated horseradish peroxidase H (5 mg/ml) in 400 ul of PBS was then layered on the cell preparation for 30 min. The peroxidase reaction was developed with use of a carbazole-containing buffer for 15 min. The cell preparations were exposed to haematoxylin for nuclear staining and mounted in Kaiser´s glycerin-gelatin (Merck, Darmstadt). The percentage of positive cells with a marginal membrane staining pattern (9) was determined by counting 200-500 mononuclear cells in a routine light microscope.

Immunoflourescence staining:

When using first-step monoclonals of IgM class (anti-Leu 7 and anti-Leu 11b antibodies), FITC-conjugated goat anti-mouse

Ig was used as the second-step reagent. The cell preparations were incubated for 30 min with 25-ul portions of monoclonal antibodies (dilution 1/80 for Leu 7 and 1/20 for Leu 11b in PBS containing 4 per cent BSA), washed for 5 min in PBS and then incubated with FITC anti-mouse immunoglobulin (dilution 1/320 in PBS containing 4 per cent BSA) for 30 min. After being washed in PBS for 10 min, the cell preparations were mounted in PBS-glycerine. The cells were examined under a Leitz epifluorescence microscope. The percentage of positive cells was determined by counting 200 cells. The primary antibody was omitted for the negative controls.

Statistical methods. Student's t test (two tails) was used.

RESULTS

There were no significant differences between the various asbestos groups regarding age, but the normal controls were a little younger. The mean values for ESR and for various subsets of lymphocytes are given in Table 1.

Lymphoid cell phenotypes. The mean percentage of T cells (Leu 4) was lower in the PP and, particularly, in the DPF group than in the normal controls ($p<0.05$ and <0.005, respectively), but there was no significant difference between the BAPE group and normals. The mean value for the PP group was lower than that for the BAPE group but not significantly so, and lower than the DPF group ($p<0.05$); the mean value for the DPF group was significantly lower than that for the BAPE group ($p<0.005$).

For the "helper/inducer" (Leu3a) T cells, groups DPF and BAPE had a significantly lower value than the controls ($p<0.005$). The PP group, however, was not significantly different from the normals, but there was a significant difference between groups PP and DPF ($p<0.05$) and PP and BAPE ($p<0.05$) and between PP and the combined DPF and BAPE groups ($p<0.01$), PP

TABLE 1

AGE, ERYTHROCYTE SEDIMENTATION RATE (ESR) AND VARIOUS LYMPHOCYTE SUBSETS
(WITHIN BRACKETS TWO STANDARD DEVIATIONS)

	NORMALS	PLEURAL PLAQUES (PP)	DIFFUSE PLEURAL FIBROSIS (DPF)	ASBESTOS EFFUSION (BAPE)
n	24	20	15	10
AGE (YEARS)	48.6	65.9	61.7	62.4
	(15.0)	(16.7)	(19.6)	(24.2)
ESR	-	14.1	20.2	31.6
		(19.4)	26.0)	(34.9)
HLA-DR	29.1	28.3	28.9	32.3
	(9.8)	(12.4)	(14.8)	(11.2)
Leu 10	11.5	11.4	13.67	13.33
(HLA-DQ)	(7.6)	(10.2)	(17.2)	(12.3)
Leu 4	63.0	56.8	50.1	59.4
(pan-T)	(11.4)	(16.2)	(19.3)	(13.1)
Leu 2a	24.1	27.5	23.5	26.4
(suppr./cytotox.)	(9.0)	(17.8)	(18.7)	(13.1)
Leu 3a	42.9	41.7	32.8	33.3
(helper/inducer)	(12.2)	(18.5)	(22.0)	(17.2)
Leu 12	4.2	5.2	4.8	6.3
(B cells)	(4.2)	(8.2)	(4.1)	(5.6)

having a higher value. No significant difference was found for the "suppressor/cytotoxic" (Leu2a) cells.

There were no significant differences between the groups regarding the Leu 10^+ (HLA-DQ), HLA-DR^+ or Leu 12^+ cells (the B cells). However, all asbestos groups (PP,DPF and BAPE) combined had a significantly higher percentage of B cells than the normal controls ($p<0.01$).

The changes in Leu 3a and the non-significant changes in Leu 2a subsets followed each other to some extent, and hence there were no differences between the various groups for the Leu 3a/Leu 2a ratio. In normal controls, this ratio was 1.89; in the PP group 1.66; in the BAPE group 1.52; and in the DPF group, 1.66. For all three asbestos groups combined, the figure was 1.63, which was not statistically different from that of the normal controls.

ESR. The sedimentation rate differed significantly between the PP and the BAPE groups ($p<0.005$) and between the PP group and the combined group of BAPE and DPF ($p<0.005$).

Correlations. ESR correlated with changes in the percentage numbers of B cell (Leu 12^+) in group BAPE ($r=0.51$) and in groups DPF and BAPE combined ($r=0.49$), but not in group DPF alone, nor in group PP ($r=-0.03$). There were no correlations between any other lymphocyte subsets and ESR in any group.

DISCUSSION

In persons exposed to asbestos several immunological derangements have been demonstrated (2). The most consistent findings are an increase in the B-cell lymphocyte activity, with increased levels of various immunoglobulins and autoantibodies, and defective T-cell activity, manifested by weak or absent delayed cutaneous reactions to common antigens such as tuberculin protein (17). A decrease in T lymphocytes in the blood has been reported (18,19,20) and both subsets ("helper/inducer" (18) and "suppressor/cytotoxic" (18,21,22)) seem to be affected.

The number of B lymphocytes has been reported to be both decreased (23) and increased (21) in the blood of persons exposed to asbestos, but the consistent reports of elevated levels of immunoglobulins (2,19,24) and autoantibodies such as rheumatoid and anti-nuclear factors (2,25,26,27) indicate that these cells must be hyperactive. This hyperactivity

might be due to a direct effect of asbestos on B cells and/or defective suppressor T-cell function. In the present study the B cells were increased in number in the asbestos group.

More interestering than the changes in the combined group of asbestos-exposed persons is the fact that significant differences in the lymphocyte subsets were observed between groups with clinically and radiologically different asbestos-related changes. In particular, the PP group differed in many aspects to a greater extent from the BAPE/DPF group than from the normals, as seen in table 1. This was especially true for the "helper/inducer" T cells. These findings are in accordance with the clinical findings of Navratil and Dobias, who divided the pleural changes into "hyalinosis simplex" and "complicata" (6) and also fit with the clinical impression that the diffuse pleural fibrosis with rounded cocto-phrenic angle is secondary to a benign asbestos pleural effusion (4,5).

A hypothesis which fits with these data is that in some individuals exposed to asbestos only minor immunological changes will develop; these persons will show pleural plaques only. Other persons, however, are prone to a more severe immunological derangement; these are the ones who develop BAPE and/or DPF.

Since an intact immunoregulatory network is thought to be of importance for protection against tumours, the observed numerical imbalance between different lymphocyte subsets might have further consequences. DPF and BAPE seem to be fairly common in populations with a high risk of malignant mesothelioma but rare in other asbestos-exposed populations where this risk is low (28,29). The question of whether the immunological imbalance observed here is of any aetiological importance for the development of malignant tumours or represent only a secondary epiphenomena necessitates further studies.

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REFERENCES

1. Becklake M. State of the art: Asbestos-related diseases of the lung and other organs: their epidemiology and implications for clinical practice. Am Rev Resp Dis 1976: 114: 187-227.

2. Morris DL, Greenberg SD, Lawrence EC. Immune responses in asbestos-exposed individuals. Editorial. Chest 1985: 87: 278-280.

3. Hillerdal G. Pleural plaques in a health survey material. Frequency, development and exposure to asbestos. Scand J Resp Dis 1978: 59: 257-263.

4. Hillerdal G, Özesmi M. Bnign asbestos pleural effusion: 73 exudates in 60 patients. Eur J Respir Dis 1987: 71: 113-121.

5. Hillerdal G. Non-malignant asbestos pleural disease. Thorax 1981: 36: 745-751.

6. Navratil M, Dobias J. Development of pleural hyalinosis in long term studies of persons exposed to asbestos dust. Environ Res 1978: 15: 108-118.

7. Hillerdal G. Asbestos-related pleuropulmonary lesions and the erythrocyte sedimentation rate. Thorax 1984: 39: 752-758.

8. Karlsson-Parra A, Forsum U, Klareskog L, Sjöberg O. A simple immunoenzyme batch staining method for the enumeration of peripheral human T lymphocyte subsets. J Immunol Methods 1983: 64: 85-90.

9. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and T cytotoxic/suppressor subpopulations in mouse and man. J Exp Med 1981: 153: 310-323.

10. Engleman EG, Benike CJ, Glickman E, Evans RL. Antibodies to membrane structures that distinguish suppressor/cytotoxic and helper T lymphocyte subpopulations block the mixed leucocyte reaction in man. J Exp Med 1981: 153: 193-198.

11. Wood GS, Warner NL, Wranke RA. Anti Leu 3/Tu antibodies react with cells of monocyte/macrophage and Langerhans lineage. J Immunol 1983: 131: 212-216.

12. Meeker TC, Miller R, Link M, Bindl J, Warnke R, Levy R. A novel human B lymphocyte antigen defined by a monoclonal antibody. Manuscript in preparation, Berton Dickinson Source Book section 4.32.

13. Brodsky FM. A matrix approach to human class II histocompatibility antigens: reactions of four monoclonal antibodies with the products of nine haplotypes. Immunogenetics 1984: 19: 179-194.

14. Lampson LA, Levy R. Two populations of Ia-like molecules on a human B-cell line. J Immunol 1980: 125: 293-299.

15. Abo T, Balch CM. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 1981: 127: 1024.

16. Phillips JH, Le AM, Lanier LL. Natural killer cells activated in a human mixed lymphocyte response culture identified by expression of Leu-12 and class II histocompatibility antigen. J Exp Med 1984: 159: 993.

17. Bozelka BE, Jones RN, de Shazo RD. is the immune system a contributing factor to the pathogenesis of asbestosis? An evaluation. Sem Resp Med 1984: 5: 289-299.

18. de Shazo RD, Nordberg BA, Baser Y, Bozelka B, Weill H, Salvaggio J. Analysis of depressed cell-mediated immunity in asbestos workers. J All Clin Immunol 1983: 75: 418-424.

19. Kagan E, Webster I, Cochrane JC, Miller K. The immunology of asbestosis. In: Inhaled particles 1977: IV: 429-433. Ed. by WH Walton. Pergamon Press.

20. Kang KY, Sera Y, Okochi T, Yamamura Y. T lymphocytes in asbestosis. New Engl J Med 1974: 291: 735-736.

21. Gaumer HR, Doll NJ, Scuyler M, Salvaggio JE. Diminished suppressor cell function in patients with asbestosis. Clin Exp Immunol 1981: 44: 108-116.

22. Miller LG, Sparrow D, Ginns LC. Asbestos exposure correlates with alterations in the circulating T cell subsets. Clin Exp Immunol 1983: 51: 110-116.

23. de Shazo RB, Hendrich AJ, Diem JE, Nordberg JA, Baser Y, Bevier D, Jones RN, Barkman HW, Salvaggio JE, Weill H. Immunologic aberrations in asbestos cement workers: dissociation from asbestosis. J All Clin Immunol 1983: 72: 454-461.

24. Lange A. An epidemiological survey of immunological abnormalities in asbestos workers. II. Serum immunoglobulin levels. Envir Res 1980: 22: 176-183.

25. Doll J, Diem JE, Jones RN, Rodriguez M, Bozelka BE, Stankus RP, Weill H, Salvaggio JE. Humoral immunological abnormalities in workers exposed to asbestos cement dust. J Allergy Clin Immunol 1980: 39: 176-182.

26. Huuskonen MS, Räsänen JA, Härkönen H, Asp S. Asbestos exposure as a cause of immunological stimulation. Scand J Resp Dis 1978: 59: 326-332.

27. Turner Warwick M, Parkes WR. Circulating rheumatoid and antinuclear factors in asbestos workers. Brit Med J 1970: 3: 492-495.

28. Hillerdal G, Baris YI. Radiological study of pleural changes in relation to mesothelioma in Turkey. Thorax 1983: 38: 443-448.

29. Hillerdal G, Zitting A, van Assendelft AHW, Kuusela T. Rarity of mineral fibre pleurisy among persons exposed to Finnish anthophyllite and with low risk of mesothelioma. Thorax 1984: 39: 608-611.

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THE FIBROUS ZEOLITE "ERIONITE":

MEDICAL IMPORTANCE AND EXPERIMENTAL FINDINGS AND SOME STUDIES ON IMMUNOLOGICAL EFFECTS OF MINERAL FIBRES.

by

MUSTAFA ÖZESMI

Akademisk avhandling som för avläggande av medicine doktorsexamen kommer att offentligt försvaras i Lungklinikens Föreläsningsal, ingång 40, Akademiska Sjukhuset, Uppsala, torsdagen den 17 mars kl 13.00 (exakt).

ABSTRACT

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Among the 575 inhabitants of village of Karain, Turkey, there were 18 deaths during 1975/76, therof 11 due to malignant pleural mesothelioma. In neighbouring village no mesothellomas were found. No asbestos occurs in the village. The bedrock is volcanic zeolite and contains a fibrous component called erionite.

Rocks from Karain containing erionite were grinded into dust. This dust was injected into the peritoneum of mice. In 321 mice which died spontaneously between 8 and 32 months after injection, malignant mesothelioma was seen in 41 and malignant lymphoma in 31. In addition, 11 animals had both malignant mesothelioma and malignant lymphoma. In control animals, few mesotheliomas and lymphomas were seen.

Immigrants from Karain living in Stockholm, Sweden, were invited to a health survey including clinical and radiological investigation and blood tests. Phenotypic characterisation of peripheral blood lymphoid cells was performed in 74 of them. There was a significant decrease in the percentage of T helper/inducer cells, while suppressor/cytotoxic cells were normal. Pleural reactions (visible fissures, pleural thickening and pleural plaques) were observed on chest roentgenograms. Lung function and clinical investigation were unremarkable.

Forty-five persons occupationally exposed to asbestos and with pleural plaques, diffuse pleural fibrosis or acute benign asbestos pleurisy were next investigated. Helper/inducer T cells were significantly decreased among those with diffuse pleural fibrosis and acute pleurisy, but not among those with pleural plaques only.

Thus, erionite is highly carcinogenic as seen from epidemiological and experimental studies. The mineral also affects the immune system and causes abnormal blood levels of various immunregulatory T-cell subsets. Whether these immunological changes are only secondary epiphenomena or if they have any pathogenetic importance should be further investigated.

Key words: Erionite, mineral fibre, asbestos, mesothelioma, Immune system, lymphoid cells, lymphoma.

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PRINTING ERRORS

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16	4	Tuz Gölü	"Tuz Gölü"
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