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FINDINGS: Exposure to all forms of asbestos fiber types with the exception of anthophyllite and South African amosite leads to meso. Low and short exposure may also lead to meso. Cigarette smoking has nothing to do with the risk of developing meso. Cites Wagner for his finding of a proportional dose-response relationship in exposure to chrysotile and crocidolite. Wagner's animal experiments confirm that chrysotile can cause meso. Types of Canadian chrysotile were found to be as carcinogenic as crocidolite. The superfine chrysotile was considered the most carcinogenic of all the materials tested. Argues that Wagner experiments support the hypothesis that finer fibers are more aerodynamic and more cytoxic since they are able to get to the pleura. Wagner found mesos after exposure to chrysotile. Still argues that short fibers are not necessary harmless. They too are able to induce meso. Characterizes tremolite as long and fine, the type of fiber that habitually causes meso. Found that chrysotile fibers longer than 20 um were more prone to induce meso and lung cancer than fibes shorter than 20 um.

Guest Editorial

John S. Harington [1, 2]

Fiber Carcinogenesis: Epidemiologic Observations and the Stanton Hypothesis [3]

For nearly half a century, asbestos fiber has been associated in some way or another with human disease (*1*). Industrial exposure to several forms of fiber may cause severe asbestosis, which is often associated with lung cancer because both may be caused by the same agent. Lung cancer can occur without asbestosis (at least radiologically), and asbestosis can occur without lung cancer (Selikoff IJ: Personal communication). Type of fiber and nature of exposure lead to clear differences in risk of developing lung cancer. In addition, the synergistic effect of cigarette smoking for lung cancer by persons heavily exposed to asbestos is exceptional (*2*, *3*).

Exposure to all forms of asbestos used in industry, with the exception of anthophyllite and South African amosite, may also cause mesothelioma, although at much lower rates than lung cancer rates. **Exposure may be short, slight, and much earlier in time. The lapsed period for mesothelioma is usually very long, about 35-39 years for pleural mesothelioma and about 45 years for peritoneal mesothelioma (*4*). The development of mesothelioma and degree of asbestosis are not related.** Wagner et al. stated (*5*) that even with slight exposure the risk of developing this tumor in humans is dose-dependent, although at this stage it is probably wise to regard all dose-response data as provisional in nature. Unlike lung cancer associated with exposure to asbestos, **cigarette smoking does not increase the risk of developing mesothelioma.**

Concern is currently growing about possible human cancer risk posed by other natural or man-made fibers, such as sepiolite, attapulgite, fibrous glass, and fibrous zeolites (Wagner JC: Personal communication). Regarding exposure to fibrous glass, Pott et al. have suggested (*6*) that, although fibrous glass has been produced in Germany for 50 years, fibers less than 5 μm in diameter were not produced before 1961. Since then the tendency has been to produce fibrous glass of smaller diameters, less than 1 μm; consequently, such material has been inhaled only in the past 10 years, at least in Germany. It is thus too early to expect any development of mesothelioma, and no increase in frequency has been observed (*6*). Also, too few workers may be producing fibrous glass of diameters and lengths that may prove hazardous to humans. Glass is an extremely active skin irritant, so more effort may have been made to control the release of fibers into the air of factories and industries, thus providing effective worker protection.

At present, there are comparatively few man-made fibrous zeolites; some but not much of the total synthetic production is fibrous (*7*). Real concern arises when a natural, unavoidable exposure to fibrous zeolites exists, such as that occurring in Turkey (*see* "Turkey," p. 984).

The manner in which asbestos and other fibers cause lung cancer or mesothelioma is unknown. Most attention has been concentrated on asbestos. Here, mode of action has been ascribed to chemical effects, to the actual chemical constituents of the fiber, to the metals either forming part of its intrinsic structure or occurring as surface contaminants, or to polycyclic aromatic hydrocarbons occurring naturally with or on the fibers or in oils added during industrial processing [*see* (*1*)]. Most of these hypotheses have now been effectively challenged. Physical effects have been suggested too, e.g., "solid-state" (*1*) or "foreign-body" carcinogenesis. More recently, Hart et al. postulated that asbestos fibers interfere with DNA repair (*8*). For further clarification of these issues, many extensive animal experiments have been conducted; these are now described.

ANIMAL EXPERIMENTATION

The Wagner Experiments

Wagner (*9*) was the first to induce mesotheliomas (in rats) after the intrapleural inoculation of [illegible] fibers. Later, pure amosite, chrysotile, and [illegible] induced

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ABBREVIATION USED: UICC = International Union Against Cancer.

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[2] I acknowledge with gratitude formative discussions in the development of this paper with Dr. Neil McGlashan, Department of Geography, University of Tasmania, Hobart, Tasmania. Invaluable criticism was provided by numerous colleagues, in particular Dr. I. J. Selikoff and Dr. A. M. Langer, Mt. Sinai School of Medicine, New York, N.Y.; Dr. R. E. Shapiro, National Institute for Environmental Health Sciences, Research Triangle Park, N.C.; Dr. V. Timbrell and Dr. J. C. Wagner, Medical Research Council Pneumoconiosis Research Unit, Penarth, Wales; and Mr. R. E. G. Rendall, the National Centre for Occupational Health, Johannesburg, Republic of South Africa. I am especially grateful to Dr. Timbrell for allowing me access to his unpublished material and for permission to use text-figures 1 and 2, to Dr. Wagner for figure 1, and to Dr. Selikoff and Dr. Arthur N. Rohl, Environmental Sciences Laboratory, Mount Sinai School of Medicine of the City University of New York, New York, N.Y., for figure 2.

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Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.

appreciable proportions of pleural mesotheliomas in the same animal (*10*). Amosite produced fewer tumors than did chrysotile and crocidolite and needed a longer induction period. Risk of developing mesothelioma in the animals was proportional to dose (*11*). Mesotheliomas were also induced with anthophyllite asbestos and brucite [$Mg(OH)_2$] (the outer layer of the curved composite structure of chrysotile); a few mesotheliomas were induced with synthetic aluminum silicate (ceramic) fibers, and a single tumor was induced with each of barium sulfate, glass powder, and aluminum oxide. Of the UICC reference samples (*11*) used in the series (*12*) and not altered in any way by further milling, crocidolite was most carcinogenic and was followed very closely by six samples of Canadian crysotile B, then by amosite, three other samples of Canadian chrysotile, two of Rhodesian chrysotile, and anthophyllite. Carcinogenicity was apparently unrelated to the presence of iron, chromium, cobalt, nickel, scandium, manganese, or to organic matter on the fiber. The potency of several of the Canadian chrysotile samples, particularly the "superfine" grades, is noteworthy, especially in that it exceeds that of crocidolite; indeed, the superfine chrysotile was the most carcinogenic of all the materials used (*12*).

A relationship between carcinogenicity and size distribution of fibers was found. For the UICC amphibole samples tested, fiber diameters were distributed in decreasing order of fineness from crocidolite to amosite to anthophyllite. Thus for a given weight of asbestos inoculated into the pleural cavity (and assuming all asbestos classes had the same proportion of fibers of appropriate length, e.g., >10-20 μm), anthophyllite provided fewer fibers than did amosite, which in turn provided fewer fibers than did crocidolite (*13*). Of these fibers, only a small proportion of anthophyllite would be below the threshold diameter, whereas the proportion of amosite would be somewhat larger, and that of crocidolite, larger still. This classification of the amphiboles is directly related to the efficiency with which the fibers produced mesotheliomas in the experiments.

Chrysotile fibers in animals proved difficult to assess or compare with the amphiboles, although a specific superfine chrysotile obtained from a Canadian mine (produced by water sedimentation separation from the most "fully milled" commercial product) was highly carcinogenic, much more so than the coarser UICC chrysotile B and crocidolite (*13*). The main conclusion derived from the work was that the fibrous nature of the materials used was the major factor in the production of mesotheliomas: The finer the fiber diameter, the more tumors were produced. Mesotheliomas were later also produced by glass fibers mostly of less than 0.5 μm in diameter, but coarser fibers with a median diameter of 1.8 μm did not cause the disease (*14*).

Results of the Wagner experiments support the hypothesis that "finer" fibers produce more pleural mesotheliomas in rats than do "coarser" fibers. And although confined to animal inoculation experiments, the results are consistent with the aerodynamic advantage finer fibers have in penetrating the periphery of the lung after fiber inhalation (*13, 15, 16*).

Later experiments by Wagner et al. (*17*) used more realistic but more expensive inhalation techniques by which laboratory animals inhale fibers rather than have large amounts implanted directly into the pleural cavity. Mesotheliomas were obtained with crocidolite, Canadian chrysotile, anthophyllite, and amosite. Two such tumors occurred after only 1-day's exposure to crocidolite and amosite. Finally, a positive association was found between asbestosis and malignant lung tumors (adenocarcinomas and squamous carcinomas) in the rats, though this is not borne out too strongly by comparison of time of exposure (equivalent to dose) and numbers of lung tumors, as shown by the data.

The Stanton Experiments

In their first experiments, Stanton and Wrench (*18*) applied crocidolite, chrysotile and amosite asbestos, six types of fibrous glass, two of fine, noncrystalline silica, and two of metal particles in gelatin glass vehicles (pledgets) to the pleurae of rats. In 2 years, four different specimens of crocidolite, chrysotile, and amosite yielded a high incidence (58-75%) of pleural mesotheliomas. Crocidolite excessively milled into submicroscopic fibrils (particles?) caused fewer tumors (20-32%) than did conventionally milled crocidolite, which gave rise to a high incidence of mesotheliomas. This is not surprising because milling (to reduce dimensions) is known to lead to surface changes on the fibers and to reduce their biological activity, as has been found in the case of chrysotile fibers (*19, 20*). As concerns glass fibers and in spite of their noncrystalline character, milling could conceivably alter a fibrous habit to a predominantly particulate one. Energy input in size reduction by milling severely changes the fiber. Pulverized fragments of nickel from the steel mill were inactive as were microspheres of noncrystalline silica and the intact fibrous glass vehicle to which the test fibers were attached (*18*).

Two types of fibrous glass with a mean diameter of 5-10 μm produced 4 mesotheliomas in 91 rats. Two other types of especially fine fibrous glass, 0.06-3 μm in diameter and specially milled to approach the length of the asbestos fibers, gave 12-18% of mesotheliomas. Pulverized chrysotile, crocidolite, and fibrous glass were much less carcinogenic than were the standard, original samples. Very short fibers were weakly carcinogenic, probably because they were trapped by macrophages and carried off.

In another study in 1977 by Stanton et al. (*21*), 17 samples of fibrous glass of diverse types and dimensions induced various incidences of pleural mesotheliomas. Carcinogenic response correlated well with dimensional distribution of the fibers: Fibers less than or equal to 1.5 μm in diameter and greater than 8 μm in length had the highest probability of induction of mesothelioma. Probability trends suggest that in these experiments the incidence of mesothelioma increased

with the number of fibers thinner than 1.5 μm and longer than 8 μm (especially for fibers thinner than 0.5

(...is primacy of length and diameter of fibers over physicochemical properties is borne out especially well by other Stanton work [Stanton (*22*) and Stanton and Layard (*23*)] based on the contention that if length and diameter of asbestos are important, durable fibers of similar dimensions but different composition should also induce malignant tumors. This theory was verified (*22*). The following fibers produced incidences of mesothelioma greater than 50% after implantation in the pleurae of rats: UICC standard reference samples of crocidolite and chrysotile A; two samples of fine, fibrous glass with diameters of 3 μm or less; and aluminum oxide whiskers. All samples were composed almost entirely of fibers predominantly below 5 μm in diameter. The aluminum oxide fibers are of interest because they are totally different from asbestos and glass, both in internal structure and chemical composition, yet their size distribution is remarkably like that of UICC crocidolite. Fibrous forms of aluminum oxide were carcinogenic; nonfibrous forms were not, another reminder of the importance of fiber size and shape to carcinogenic activity.

Stanton and Wrench (*18*) concluded that the essential feature for mesothelioma induction is "a durable fibrous shape, perhaps in a narrow range of size." Fibrous glass is as carcinogenic as asbestos: When ...ension is appropriate, fibers of attapulgite, dawsonite, aluminum oxide, silicon carbide, and potassium titanate can all induce mesotheliomas in animals (*21*). Two categories of fiber sizes gave good correlations with carcinogenicity: length greater than 64 μm and diameter between 0.25 and 1.5 μm on the one hand and length greater than 8 μm and diameter less than 0.25 μm on the other (*24*).

In summary and on the basis of results obtained after the intrapleural inoculation of a total of 37 man-made types of fibers of different size distributions [(*21, 23*); *see* (*25*)], the optimum correlation was obtained with fibers less than 1.5 μm in diameter and greater than 8 μm in length. Absence of correlation does not preclude a low level of tumor response outside these ranges.

Other Experiments

Studies suggesting a "physical" rather than a "chemical" mode of action for certain materials including glass have been reviewed [(*1*), pp. 366-367]. More recent information comes mainly from Davis (*26*) and Pott and his colleagues, as summarized recently by Pott (*25*). Their studies on fibrous glass of different fiber aspects (*6, 27, 28*) support the results obtained by Stanton and his group. In their testing of mineral fibers other than glass, Pott et al. (*6*) dealt carefully ...th the important relationships among *dose*, diameter, ... length. In his recent analysis, Leineweber (*29*) ...ows how the most recent data of Pott (*25*) are in good agreement with those of Stanton: Fiber diameter for maximum biological activity is about 0.25 μm, and length is greater than 10 μm [*see* fig. 1 of Pott (*25*)].

THE STANTON HYPOTHESIS

Evidence from Stanton's animal experiments and supported by differences in mesothelioma risk from fibers of different diameters in South Africa led him to formulate the following hypothesis (*30*): Durable fibers, of which asbestos is but one example, provided they subscribe to well-defined ranges of diameter and length, cause cancer irrespective of their physicochemical nature simply because they are fibers. Asbestos is but one example of this, a subset of a major generic group. Chemical nature is not the major carcinogenic determinant; morphologic quality is, i.e., the quality of being fibers of a certain dimension within an approximate range of less than or equal to 1.5 μm in diameter and greater than 8 μm in length.

The major experimental evidence, which was to establish this concept, was Stanton's inclusion of fibers other than asbestos in his animal experiments. The conclusions he and his colleagues drew from the distinctive results changed scientific thinking on the matter. Attention was now directed to the fiber's size and shape rather than to its innate chemical character. Asbestos became recognized for the first time as merely one of several fiber types capable of causing experimental mesothelioma in the rat.

Up to 14 fibrous materials are now known to produce "malignant fibrous neoplasms" (*29*) following implantation in the pleural or peritoneal cavities of animals: amosite, anthophyllite, chrysotile, crocidolite, and tremolite; borosilicate glass, aluminum silicate glass, and mineral wool; aluminum oxide, potassium titanate, silicon carbide, and sodium aluminum carbonate; and wollastonite and attapulgite. Leineweber (*29*) concludes that finding any other underlying cause for such biological activity other than fiber dimension is apparently impossible. If bulk composition, surface chemistry, or other factors do play a role, they must be secondary.

Difficulties in the Interpretation of the Animal Experiments

Stanton and Wrench (*18*) drew attention to deficiencies in the method of application, intrapleural inoculation, and the massive amounts of fibers used (standard dose, 40 mg). Such techniques are artificial and remote: "Direct application of the results to the problems in man would be unwise" (*18*). Wagner and his colleagues had mentioned this with regard to their animal work, hence their choice of the more "man-oriented" and realistic inhalation studies (*17*). Even here, complicating interactive factors occur in humans that are not found in animals; cigarette smoking is only one example. Obviously, both techniques have a place: In implantation, fibers are deposited directly into the pleural cavity, whereas after inhalation the

fibers must pass through a series of natural barriers and are much affected by aerodynamic considerations. The intrapleural deposition model is conserving of experimental material, and expensive equipment such as inhalation chambers is not needed. Essentially, the animal deposition model is the ultimate limiting condition for the inhalation test and saves resources when used as a possible prescreening technique for inhalation studies.

The value of the animal models used obviously depends on what is being predicted or sought, be it induction of mesothelioma, lung cancer, or asbestosis. With some reservations, the two techniques could supplement one another: As already noted, the intrapleural inoculation technique could possibly be used as a prescreen for inhalation studies.

Technical Problems in the Animal Experiments

A technical problem in Stanton's work lies in the use of the fibrous glass pledget as the vehicle for the fibers (*18, 21, 22*). While these pledgets alone did not essentially yield tumors [although incidences of mesothelioma as high as 3 of 30 rats in comparable "experiments could conceivably result from the vehicle fibrous glass alone" (*18*)], nor did their absence alter the incidence of crocidolite-induced tumors, it remains impossible to gauge a possibly synergistic effect by pledgets on the fibers applied to them. The pledgets weigh 45 mg, are flat, binder coated, and are made of coarse fibrous glass of the type commonly used as insulation material. Upon them the gelatin-suspended coatings of test fibers were allowed to harden. However, in view of Wagner's direct inoculation of fibers in saline suspension into the pleural cavity and of Stanton and Layard's later resorting to direct inoculation without vehicle (*23*), criticism of the use of the pledget is probably irrelevant.

Comprehensive and precise information on the technique of preparation of the Stanton fibers is not available; e.g., milling of fibers can greatly affect biological activity (*19, 20*). Furthermore, the Stanton hypothesis is restricted to induction of pleural mesotheliomas and their occurrence in the rat. It is no criticism, though essential, to stress that no judgment is made regarding fiber aspect in relation to lung cancer or to asbestosis in the rat; judgment is made neither in other animals nor in humans. Stanton and Wrench (*18*) warned against the direct application of their results to what might occur in human exposure; Timbrell's important work is relevant here (*see* "Aerodynamic Considerations of Fiber Deposition in the Respiratory System," p. 981).

The possible role of submicroscopic fibers in the experimental and nonexperimental induction of malignant tumors cannot be ignored. Rendall and Skikne (*31*) pointed out that since such fibers are too fine to be resolved under a light microscope, they are not included in routine evaluations of airborne fibrous dust clouds encountered in industrial atmospheres (and are often ignored in hypothetical reasoning). Fibers are optically visible down to 0.4 μm in diameter. Below this diameter, fibers 0.25 or 0.1 μm in diameter, although long, will not be seen. Thus an "invisible" proportion of submicroscopic fibers is certainly present in all fiber samples, including, of course, those used by Stanton and his associates; a sample may occasionally have up to three times more submicroscopic fibers than it has optically visible fibers (Rendall RE: Personal communication). **Even if we disregard such submicroscopic fibers, the carcinogenic potency of *short* fibers may be weak, but many short fibers may induce a tumor as easily as a few long fibers, though this is not necessarily incompatible with the Stanton hypothesis** (*25*).

Other shortcomings to the hypothesis that morphology plays a major role in inducing mesothelioma can be summarized from questions raised by A. M. Langer (personal communication): Is a critical number of fibers required to induce a specific tumor in a specific animal model? Does fiber diameter merely reflect fiber number? Pott et al. (*6*) emphasized the importance of dosage in conjunction with critical diameter and length; increasing particle numbers is accompanied by an increase in surface areas and unsatisfied surface valencies. What could be the role of these observations? Acid leaching and milling of chrysotile (and of amphibole) asbestos significantly alter biological activity. Does the hypothesis therefore require that crystalline surfaces remain *unaltered*? Fiber populations present in lung parenchyma and lung pleura of the same human individual show short and very fine (chrysotile) fibers (<5 μm long) at the pleura compared to mixed fiber populations in the lung parenchyma (*32*). Fibers larger than those in the pleura (including many amphiboles) were found simultaneously in the lung. These findings argue against the Stanton concept, although they refer to fibers that remain and not fibers that might originally have been there.

The hypothesis as a whole has been criticized by Gross (*33*) in his negative assessment of glass being carcinogenic to humans. Some interesting questions have also been raised by Gibbs and Hwang (*34*).

A certain amount of controversy still exists regarding the histologic nature of the malignant tumors induced by the intrapleural inoculation of fibers into animals: Are they malignant mesenchymal neoplasms, malignant fibrous neoplasms, fibrosarcomas, sarcomas, mixed tumors, or mesotheliomas? This contentious issue cannot be dealt with here, though reference to the views of Kannerstein and Churg (*35*) are appropriate.

ANALYSIS OF THE STANTON HYPOTHESIS IN RELATION TO MESOTHELIOMA IN HUMANS

Stanton's work shows conclusively that a wide range of conventionally milled, durable, fibrous materials with one feature in common, a carefully defined narrow range of size (i.e., <1.5 μm in diameter and >8

μm in length), induces high and very similar rates of pleural mesotheliomas in rats. The hypothesis draws attention to the *possible* danger from a specific-dimension set of carcinogenic fibers, a "subset" within the total exposure of humans to fibrous materials. R. E. Rendall (personal communication) has pointed out that, even at low concentrations, millions of fibers are inhaled, making the probability of subsets being present strong indeed. The very low concentration of 0.1 fiber cm^3 is equivalent to an inhalation of 10^6 fibers/day (≈10 m^3 air is inhaled/day). The internationally recommended threshold limit value is 2 fibers, optically visible only, per cm^3, i.e., about 20×10^6 fibers inhaled/day. The threshold limit value is of obvious importance to understand the mechanisms of fiber action and to legislate regulatory practice if only because the nonoptically visible fibers increase the numbers by a minimum of three or four.

Support for the hypothesis comes from other experimental animal studies designed explicitly for different purposes, notably those of Wagner et al. (*5*), Pott (*25*), and Pott et al. (*6*). Although the size ranges used in the Wagner series cannot be compared precisely with those used by Stanton, there is general agreement that finer fibers are more carcinogenic than are coarser fibers when applied to the pleurae of rats.

Aerodynamic Considerations of Fiber Deposition in the Respiratory System

When extrapolating the Stanton hypothesis to the induction of mesothelioma in humans, we must realize that two sets of separate criteria are demanded: one for the induction of mesotheliomas in rats by the direct inoculation of fibers into the pleurae and another for the respirability of fibers by humans in occupational and environmental circumstances. These two sets are entirely different and must not be confused. Bearing this in mind and before embarking on descriptions of occupational and environmental induction of mesothelioma in human populations, let us now give some attention to the respirability characteristics of fibers as they affect the human lung.

Armed with the criteria made available by Stanton and his associates from their work on the induction of mesothelioma in the rat, we can speculate whether these criteria can be integrated with the important work of Timbrell and his colleagues (*13*, *15*, *16*), part of which has been briefly reviewed recently by Leineweber (*29*). Both theoretical and experimental studies by Timbrell and his group [*see* (*29*)] show that, while aerodynamic diameter is a major determinant of how deeply fibers can penetrate the distal portion of the lung and the alveoli, considerable significance should also be placed on fiber length. Fibers as long as 200 μm could penetrate in this fashion, while the limiting width for fiber respirability could be a diameter of approximately 3 μm. From these dimensions, a fiber 3 μm or less in diameter can be regarded as respirable, even if its length might exceed 200 μm [*see* (*29*)]. Thus the distinguishing mechanisms that affect deposition in or penetration of the lung are controlled aerodynamically by the fiber's mean aerodynamic diameter and to a limited but finite extent by its length (*36*). If its diameter is less than about 3 μm, the fiber stands a good chance of escaping deposition and of penetrating the lung. The more symmetric a fiber is, the greater is its chance of being deposited.

An overall limitation on the lengths of the fibers that reach the pulmonary air spaces is imposed by the nasal hairs and by the small diameters of the respiratory bronchioles. Furthermore, each fiber type appears to possess a unique diameter spectrum, i.e., not one single diameter, but perhaps a distinctive, narrow band or range. Anthophyllite fibers are significantly thicker than are amosite fibers, which in turn are significantly thicker than are crocidolite fibers. **Tremolite fibers may be long and fine, generally similar in dimension to the fibers mentioned above. The** curliness of chrysotile poses an aerodynamic problem of its own, causing the fibers to adopt a random orientation in the lung airways, thus making interception much more efficient than that for amphibole fibers.

Relating these aerodynamic considerations to the intrapleural inoculation investigations and bearing in mind the important introductory precautions stated in the first paragraph of this section, we can see that the results of the Wagner animal experiments both anticipate and support the Stanton hypothesis that finer fibers are more carcinogenic than are coarser ones. **Although intrapleural inoculation of fibers was used, the results are consistent with the aerodynamic advantage finer fibers have in penetrating the periphery of the lung (*13*, *15*, *16*); coarser fibers are "screened out," although coarser fibers that do get into the lung would not be accounted for (hypothetically, at least) in direct deposition studies in which groups of carefully selected fibers are chosen for use.** Again, the curly nature of chrysotile places a special facet on all this.

From size ranges drawn directly from the growing pool of experimental evidence, fibers considered "significant" in inducing mesotheliomas in the rat (*12*, *16*) are those less than 0.5 μm in diameter and greater than 10 μm in length (*12*). Decreasing carcinogenicity occurs as the number of such significant fibers decreases in the inoculum. By suitable comparison of the results with those of Stanton and Wrench (*18*), good agreement with number of significant fibers and mesothelioma induction was obtained. Moreover, physical properties of the fibers (in terms of dimension only) appear distinctly relevant. Since compared to coarser fibers, the finer fibers are more carcinogenic when applied to the pleura and since they are also able to penetrate the pleura more easily after inhalation, these two factors together would give the finer fibers a much greater importance than even aerodynamic differences would suggest.

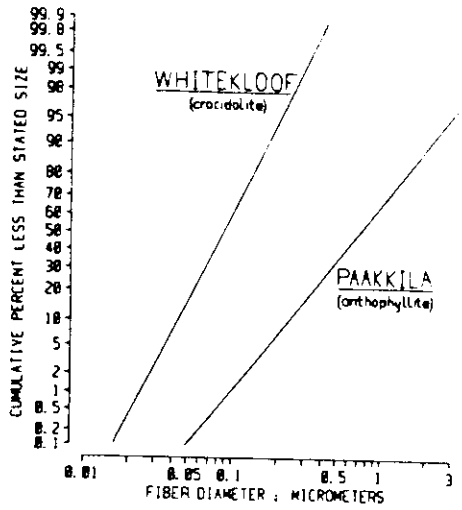
A notable advance in the study of human exposure to various types of asbestos was made by Timbrell et al. (*15*) when they suggested that fiber diameter could

be useful in distinguishing exposure to the same or different types of fiber in different geographic localities (*37*). Cape crocidolite, which is responsible for most mesotheliomas found in South Africa and which is mined and milled in the northwestern Cape, is distinctly shorter than are crocidolite and amosite produced in the northeastern Transvaal where up to 1979 as few as 5 mesotheliomas had been reported (Webster I: Personal communication) after almost 60 years of mining and milling (*37*). Such diameter differences could explain the clear connection between exposure to northwestern Cape crocidolite with the development of mesothelioma there and the rarity of this tumor in the Transvaal (*37*). Stanton (*30*) was aware of these distinct differences and drew on them for support for his hypothesis (*30*).

Bivariate Analyses: Fiber Diameter and Length Ranges

Recent unpublished work (Timbrell V: Personal communication) has brought three-way differences among northwestern Cape, northeastern Transvaal, and Finland into sharper focus. Bivariate (diameter and length) analysis of airborne fibers at the Paakkila anthophyllite mine in Finland, which closed in 1975 and from which fibers have reportedly caused no human mesotheliomas since the mine first went into industrial use in 1918 (*38*), shows distinct differences in diameter and length compared to those of the mesothelioma-inducing airborne fibers in the northwestern Cape of South Africa. Substantial quantities of anthophyllite fibers were detected at Paakkila in the lungs of employees and local inhabitants. Airborne dust levels were such that even if only a small percentage were in the carcinogenic range, some mesotheliomas would be expected. The absence of these tumors at Paakkila suggests that the range of the fibers there does not include any from within the carcinogenic range, at least for development of mesothelioma, though not necessarily for lung cancer.

The valuable feature of these examples is that the diameter range (text-fig. 1) is extremely wide; for Paakkila only a narrow band which could be carcinogenic is left at the lower end of the range. Text-figure 1 shows that 1% of the Paakkila fibers is less than 0.1 μm in diameter, and if we suppose that under these dusty conditions, 1% provides enough fibers to produce tumors, then absence of mesothelioma indicates that the upper limit of fiber diameter for the induction of mesotheliomas must be below 0.1 μm. If we take the figure of 0.1% of fibers as necessary for some mesotheliomas to occur, the upper diameter limit drops to 0.05 μm; by extrapolation, if we take the figure of 0.01%, the limit falls to 0.035 μm, and if 0.001%, it becomes 0.023 μm. The value 0.05 μm seems a reasonable one, although it could be even lower. This is well below previous estimates including an earlier one of 0.5 μm. If 0.5 μm were correct, then virtually one-half the Paakkila fibers would be carcinogenic. This limit is clearly much too high. Stanton's limit of 1.5 μm would embrace virtually all the fibers.

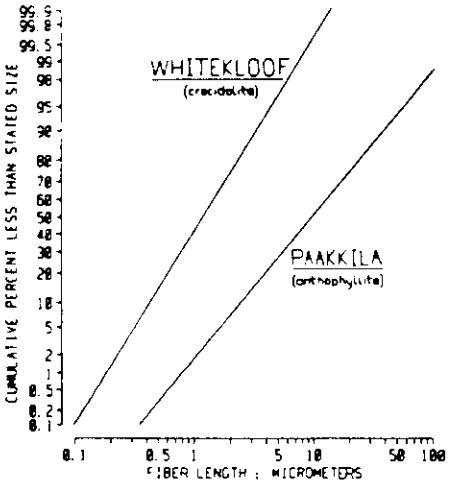


TEXT-FIGURE 1.—Comparison of diameters of airborne fibers from Paakkila anthophyllite mine, Finland (negative for mesothelioma induction), with diameters of airborne fibers from Whitekloof crocidolite mine, northwestern Cape (positive for mesothelioma induction); published by courtesy of Dr. V. Timbrell.

The data for the northwestern Cape (positive for mesothelioma) and the northeastern Transvaal (virtually negative for mesothelioma) fit very well with this concept of a very low upper limit. For the northwestern Cape mine (Whitekloof underground) where crocidolite is mined, it can be estimated that 60% of the fibers are less than 0.1 μm in diameter (60 times the Paakkila figure), 17% are less than 0.05 μm (170 times Paakkila), 6% are less than 0.035 μm (600 times Paakkila), and 1% are less than 0.023 μm (1,000 times). The Paakkila versus northwestern Cape discrimination rises sharply with decrease in the limit. Again, probably 0.05 μm would be enough to explain the very different mesothelioma incidences.

Of the Transvaal crocidolites (virtually negative for mesothelioma) so far examined by Timbrell, about 1% of the fibers are below 0.1 μm in diameter, closely approximating the data for Paakkila anthophyllite. The relative distributions are not much different when fiber length is considered (text-fig. 2). Sixty percent of the Paakkila fibers are longer than 8 μm, whereas 1% of the Whitekloof fibers are (Whitekloof 60 times < Paakkila). When retention data are applied by a comparison of the diameters of fibers extracted from the lungs of the Paakkila subjects with the diameters of airborne fibers, not much change is noted. If anything, an even lower diameter limit than 0.05 μm is suggested. Interestingly, the limit seems even more closely related to the fiber-tissue reaction than to respirability and other considerations.

Timbrell (personal communication) concludes that if the concentration of Paakkila fibers is increased 100 times compared with the data provided in text-figure 1, fibers below 0.05 μm in diameter begin to appear in small, absolute numbers. The normal inoculation dose



TEXT FIGURE 2—Comparison of lengths of airborne fibers from Paakkila anthophyllite mine, Finland (negative for mesothelioma induction), with lengths of airborne fibers from Whitekloof crocidolite mine, northwestern Cape (positive for mesothelioma induction); published by courtesy of Dr. V. Timbrell.

to the pleura in the animal experiments is probably 10,000 times or more higher than that which occurred at Paakkila; therefore, that this anthophyllite caused mesotheliomas in animal experiments is not surprising. The dose was so excessive that saturation was approached with a loss of discrimination against, for ample, northwestern Cape crocidolite. This same ection against the inoculation experiments can be led against in vitro screening tests, although it must be emphasized that both techniques bear little relevance to the "real world."

Unfortunately, comparatively few data concerning fiber length in relation to mesothelioma development in humans are available. **Regarding induction of mesothelioma and lung cancer, especially in rats, chrysotile fibers longer than 20 μm were found to be more dangerous than were fibers shorter than 20 μm (*39*);** this observation supports Stanton's criterion that sets fiber length at greater than 8 μm. Definition of the range of fiber length crucial for mesothelioma development is important in any attempt to relate the results of Stanton's work to the human situation. Comparatively little is known about fiber lengths involved in mesothelioma induction in humans, but Timbrell's recent data (summarized in "Aerodynamic Considerations of Fiber Deposition in the Respiratory System" and "Bivariate Analyses: Fiber Diameter and Length Ranges," pp. 981 and 982, respectively) go far in dealing with this deficiency.

These results suggest that a length of 8 μm is too high a threshold for fiber carcinogenicity. In the northwestern Cape only about 1% of the fibers are longer than 8 μm. A much smaller percentage, approximately 0.1%, is both longer than 8 μm and narrower than 0.05 μm in diameter. These percentages are probably insufficient to explain the northwestern Cape mesotheliomas. If the threshold diameter is about 0.05 μm, then the threshold length must be reduced to 3 μm for inclusion of a few percent of the fibers in the carcinogenic range. The probability that very short fibers are carcinogenic is very low, but the probability increases with increasing fiber length, being significant even at 5 μm. It is essential that both critical dimensions, i.e., diameter and length, of airborne fibers associated with human exposure be known and that both dimensions are included in the appropriate ranges. One dimension alone is insufficient, though the suggestion is that the Stanton and Timbrell concepts could be reconciled, perhaps quite readily if, in light of our increasing knowledge, we were to make some adjustment in the dimension limits that we consider critical.

Yet another provocative question is posed by the observations that amosite and anthophyllite induce mesotheliomas in animals (*12*), though not to any great extent in humans (*37, 38*); **however, the evidence is clear that in the United States both mesothelioma and lung cancer have been found in [illegible] workers exposed to amosite (*40*), and the development of lung cancer is strongly synergized by the smoking of cigarettes (*41*).** The UICC fibers, predominantly amosite and anthophyllite, used in the animal experiments contain Stanton subsets of critical diameter and length. But are such subsets absent in the human's total exposure to amosite in South Africa and to anthophyllite in Finland? Are the subsets present in amosite from the United States, where old amosite stocks from World War II have been shown to be carcinogenic to humans (*40*)? Or are Timbrell's fiber diameter-length criteria more convincing determinants of mesothelioma induction in humans (*see* "Aerodynamic Considerations of Fiber Deposition in the Respiratory System, p. 981)? The implications of these matters have not yet been fully worked out, though they may be resolved with further animal experimentation and measurement of airborne fibers in the human environment and, possibly, in human tissues.

In animal experiments in progress, Suzuki (in preparation) found that a single dose of fibrous erionite, a natural zeolite mineral 0.5–30 μm in fiber length (average, 1 μm) and 0.05–0.2 μm in fiber diameter (average, 0.1 μm), has so far produced 6 malignant peritoneal tumors (4 consistent with mesothelioma) in 10 mice 8–22 months after ip administration of the fiber. Of 4 chrysotile-treated controls, 2 developed malignant mesotheliomas. Another zeolite, mordenite (a mixture of fibrous and granular forms), has thus far produced no malignant tumors. In the fibrous form this zeolite has lengths ranging from 0.5 to 30 μm (average, 1.5 μm) and diameters averaging 0.5 μm. The granular form is 5–30 μm in the long axis and 3.64–10 μm in the short axis.

The dimensions for both these zeolites are roughly similar, though conclusions are premature concerning the Stanton hypothesis inasmuch as one form of zeolite (erionite) produces malignant peritoneal tumors and the other form (mordenite) does not. The fibrous forms may be disproportionate in the two zeolites (fewer

fibers in the mordenite), and the preliminary study in question must be completed, extended to include more animals and experimental fibers, and eventually confirmed.

EPIDEMIOLOGIC EVIDENCE OF MESOTHELIOMA IN HUMAN POPULATIONS

Two situations concerning mesotheliomas in people exposed to mineral fibers will now be described. Each could possibly be used to integrate the Stanton hypothesis, which was confined to animals, with the Timbrell aerodynamic criteria that relate human exposure and fibrous materials. Wagner (*42*) points out that a possible hazard to humans may exist when the ultimate parameter of fibers in both environmental and occupational exposure is in the suspected critical range of a diameter less than 0.5 μm and a length of 5–30 μm for development of mesotheliomas and a diameter of 2 μm or less and a length of 10–50 μm for development of pulmonary fibrosis, which, particularly in cigarette smokers, may lead to excess cancer of the lung.

The first situation is the well-known outbreak of mesothelioma in South Africa (*1*, *5*, *43–46*), and the second is the fairly recent outbreak of the disease in small, well-defined areas in Turkey (*47–52*). A description of industry-associated cases of pleural mesotheliomas in Western Australia (26 cases among 6,200 male employees) appears elsewhere (*53*), and Wagner (*42*) provides an interesting contemporaneous account of areas other than Turkey in which mesotheliomas occur in association with fine, naturally occurring mineral fibers.

South Africa

In February 1956, a case thought to be tuberculous pleurisy was found post mortem to be a large gelatinous tumor in the right pleural cavity (fig. 1). The tumor was later demonstrated as a mesothelioma, and a few asbestos bodies were found in the lungs. Subsequently, it was established that the patient had been herding goats in the vicinity of the Cape asbestos mines. Meanwhile, Dr. C. A. Sleggs, superintendent of a tuberculosis hospital in Kimberley, Republic of South Africa, found that after 1952, when antituberculosis drugs became available, a number of his patients, mainly from the northwestern Cape, failed to respond to therapy (*46*). He was puzzled by this, and later inoperable cancer was established in these cases. Biopsy material from 12 of Sleggs' patients showed histologic features of pleural mesothelioma (*43–46*). In 3 cases, small fragments of lung tissues were present, and occasionally asbestos bodies were seen. On this evidence Wagner (*46*) examined the detailed history of the 12 patients and eventually established an association with exposure to a specific form of asbestos, "Cape crocidolite," in the northwestern Cape, where even today this fiber is being extensively mined and milled.

At this stage lungs were being received from all asbestos workers who had died on other asbestos mines. No evidence of cases of mesothelioma from the amosite mines in the northeastern Transvaal or from the chrysotile mines in the eastern Transvaal was found, nor was there any knowledge of these tumors occurring at Havelock in Swaziland or at Shabani in Zimbabwe. Cases continued to come from the northwestern Cape and occasionally from the South African railways where crocidolite had been used for lagging. Most cases were among people who had not actively worked with actual production of fiber but who had been domestically or environmentally exposed. The average period between first exposure to fiber and subsequent recognition of the tumor was 44 years (*46*).

By 1959, domestic or occupational exposure to asbestos had been found in 32 of 33 patients with histologically confirmed pleural mesotheliomas. Little or no asbestosis was seen, and in a number of the patients, exposure seemed extremely slight. Wagner et al. (*43*) concluded (and as it has transpired, correctly) that the mesotheliomas were probably related more to exposure to northwestern Cape crocidolite asbestos than to any consequence of asbestosis. Subsequent extension of the investigation revealed that a related and serious problem also existed in the United Kingdom, although at that time in an occupational context. Later, similarly high incidences were reported in the United States by Selikoff and Lee (*47*).

Turkey

After the extensive research on occupationally and environmentally occurring mesotheliomas found in the Republic of South Africa, the significance of the story soon broadened to a matter of world public health importance. Some 20 years after Wagner's findings, another unusual story began to unfold. The Anatolian village of Karain in the district of Ürgüp in Cappadocia is well known to tourists because of the picturesque volcanic rock dwellings called "fairy chimneys" or "fairy castles" found there (fig. 2). The rock consists of volcanic tuff: The soil is poor and the climate is very hot and dry in summer and cold with snow in winter. The soft volcanic rock is cut by adzes for making walls of houses and is plastered with a grayish-white rock from the neighboring hills. Most people work the land, growing potatoes and onions 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 (*48*).

Karain has a population of about 700 people. In 1978 Baris et al. (*48*) reported that 35 cases of pleural mesothelioma, over 40% of all deaths, had occurred in the village between 1970 and 1976. The 35 cases included 2 women from nearby villages, Boyali, 2.2 miles (3 km) from Karain, and Yesilöz, 4 miles (7 km) from Karain. Both women had moved to those villages from Karain where they had spent their early years. The 35 deaths, all attributable therefore to Karain, were

the only ones to have been found in a group of eight villages only 2-12.5 miles (3-20 km) from Karain. M eliomas have since been found also in Tuzköy, w , like Karain and about 25 miles (40 km) from it, is situated in an area of abundant volcanic tuff.

By 1979 (*49*) the circumstances had extended to include not only the circumscribed area of Karain and environs but also rural parts of central Anatolia, where asbestos is common. The 1979 data show two fairly distinct groups of occurrence of mesothelioma in Turkey (*49*). Group 1 consisted of 63 cases: Most were from Karain (51 cases); the remainder were from Tuzköy (10) and Ürgüp (2). All three villages are in Cappadocia, in rural central Anatolia. The cases were comprised of 37 male and 26 female patients. Asbestos neither occurs in the local soil or rock nor is handled in Karain or Tuzköy. Recent preliminary mineralogical studies show that many fibers are of respirable size in the stones (tuff) from which some of the patients' homes were constructed. These fibers were shown to be erionite-type zeolite fibers found in the tuff of Karain and Tuzköy but not in the tuff from the other regions (zeolites are a large family of natural and synthetic minerals that resemble, particularly in adsorptive and ion-exchange properties, clay minerals). Half the patients (all males) were smokers; all the female patients were nonsmokers.

Group 2 consisted of 122 subjects, mostly hospital case from other rural parts of central Anatolia, of w 5 had had possible occupational exposure to as s. **Mineralogical studies of white stucco, soil samples, and airborne dust samples revealed that "domestic" asbestos (mostly tremolite and chrysotile) was present in the patients' villages and not in other villages.** No smoking information seems available for the 82 male patients; **all 40 female patients were nonsmokers (*49*).**

Apart from the "endemic" pleural mesotheliomas, both groups showed clinical evidence of pleural effusions, calcified pleural plaques, and chronic fibrosing pleurisy (*48-52*). The two villages of Karain and Tuzköy have no asbestos but have a much higher incidence of mesothelioma "probably due to a . . . zeolite" (*49*) found in the ubiquitous tuff in the areas concerned but not in others. Risk is omnipresent: Life in fairy castles consists of collecting fertilizer from pigeons' nests, using tuff powder and calcite and gypsum as residential whitewash, and playing or working in dry fields, gray with tuff dust. Epidemiologic studies of these villages point to the zeolite (apparently erionite) as being more potent than the tremolite or chrysotile in causing mesothelioma (*49*). Exposure to mineral fibers of various kinds begins virtually from birth. Exposure during childhood may lead to development of mesothelioma in middle age, wh as exposure at a later age causes its development af bout 60 years of age. Cigarette smoking does not see o have a major contribution in the development of the mesotheliomas.

Data on lung cancer and other cancers are limited, mainly because of the general quality of health statistics. The very low rate of postmortem examinations, the fact that many deaths occur at home and not at the hospital, and unreliable death certificate data all contribute to underestimation of cases (Lilis R: Personal communication).

Occupational Studies

The period between the observation of mesothelioma in the Republic of South Africa in 1956 and that in Turkey in 1975 is notable for a remarkable expansion of research into the biological effects of mineral fibers, especially of asbestos, an effort quite unlike anything else in the history of occupational medicine. From a welter of findings, most of which have been accumulated since the first germinal international meeting convened by the New York Academy of Sciences in 1964, a number of general principles concerning the biological effects of asbestos on humans, animals, and living cells has been ascertained. Most of these principles can now be considered as established beyond reasonable doubt [*see* (*1*, *47*)]. Currently, three major asbestiform fibers are used commercially: chrysotile, crocidolite, and amosite. Of some 40 naturally occurring minerals, some in *rare* instances produce or contain asbestos fibers (Langer AM: Personal communication). Asbestos has over 1,000 commercial uses, and world production is increasing.

Contact with the various minerals and possible exposure to asbestos dust by the general population are widespread. Real concern about the possible health effects of exposure to asbestos both on workers and the general population has been firmly expressed in recent publications (*5*, *47*). Attention is being directed to replacement of natural materials with artificial fibers. This further underlines the importance of defining the dangerous parameters already discussed and avoiding their man-made replication. In looking for substitutes for asbestos, one must avoid anything as hazardous or more so. Our increased awareness of size ranges of fiber diameters and lengths that may be dangerous to humans is thus of some value.

SIGNIFICANCE OF THE HYPOTHESIS

The Stanton hypothesis offers a conceptual framework for etiologic investigations and helps toward perceiving the causative mechanisms, although it is not without certain shortcomings (*see* "Difficulties in the Interpretation of the Animal Experiments," p. 979, and "Technical Problems in the Animal Experiments," p. 980). As a general criticism extending well beyond the Stanton hypothesis, the use of such terms as "structural" versus "physicochemical," "foreign-body" and "solid-state" carcinogenesis, "the Oppenheimer phenomenon," and "physical" as opposed to "chemical" needs to be given real meaning or alternatively abandoned. Boyland (*54*) draws attention to a report of the World Health Organization in 1969 that sarcomas

(occurring at the site of injection) ". . . may or may not result from the operation of physical factors—i.e., physical properties of the test material that are unrelated to any chemical carcinogenic potential it may possess." Boyland trenchantly points out that this statement, though made by a group of experts, does not conform to fact because physical properties are indeed necessarily dependent on chemical structure and properties.

In the context of fiber activity, even glass is hemolytic and cytotoxic. Acid leaching of chrysotile fibers reduces hemolytic and cytotoxic activities and the ability of the fibers to induce mesotheliomas in rats (*1*, *55*, *56*). It is hard to believe that this is a solely "physical" action, though it should be borne in mind that leaching implies that the integrity of the basic crystalline structure of the fiber is not impaired and that the fragility of the fibers after leaching may not result in their breakdown into smaller fibers or fragments. Heating may also reduce biological activity of certain fibers [*see* (*25*)]. Partially pulverized crocidolite is much less carcinogenic than is intact fiber (*18*). Glass powder by itself produced 3 mesotheliomas in rats after intrapleural inoculation (*14*). Does pulverization alter "physical" character by changing dimension? Or is reduction of carcinogenicity a "chemical" effect because the orderly, linear arrangement of cations (mainly metals) on the fiber has been destroyed or disturbed? Basically, in biological activity both "morphology" and "surface chemistry" conceivably complement each other (*57*).

Comprehensive in vitro testing of the wide range of fibers used in the Stanton and other animal experiments is a still compelling need, although an effective start (supportive of the hypothesis) has already been made by Lipkin (*58*) and Brown et al. (*59*). Techniques for testing the toxicity of fibers on macrophages and other cells and for analyzing the effects of fibers on target cells in carcinogenesis are already available. Stanton or Timbrell subset preparations could be used to attempt in vitro malignant transformation of mesothelial cells or tissues or appropriate organ cultures (*60*).

The Stanton hypothesis allied with the important criteria established by Timbrell and his colleagues may in time be applicable to regulatory practices, although more refined experimental techniques may be needed to detect further dimensional subtleties and help to extend the animal findings more into the human experience. The different and important feature of this hypothesis is the demonstration of a definite carcinogenic potential of nonasbestos fibers in animals and therefore probably in humans. Other investigators, e.g., Pott et al. (*6*) and Wagner (personal communication) established experimentally that fibers other than asbestos (glass and nemalite) can induce mesotheliomas in animals. However, to try to equate the number and size distribution of fibers with the total quantity of all fibers in human tissues (*30*, *61*) is a gargantuan task. Even if the results were of any biological significance, the statistical appraisal alone defies the imagination. Nevertheless, the specific tissue burden of asbestos minerals and other minerals under certain exposure conditions may be useful in establishing quantification of dose-response curves for humans after exposure. In general, it seems clear that by far the most inviting challenge to future work in this field, insofar as human experience is concerned, is to integrate the Stanton hypothesis based upon animal studies with the obviously more relevant aerodynamic data defined by Timbrell and his associates.

Both practically and philosophically, the work inspired by Stanton is a provocative stimulus to further analyses of fiber carcinogenesis. In spite of some remaining deficiencies regarding actual and specific dimensions and some other features, the basic idea remains promising, and the hypothesis is plausible in relation to the induction of mesotheliomas and related malignant pleural tumors in rats. A convincing challenge to earlier (and future) hypotheses is allowed, and the way is opened to more rational in vitro experimentation to provide the "clues to the intimate events between cell and fiber that lead to cancer" (*30*). Above all, allied with aerodynamic considerations, no reason is apparent why the hypothesis should not be extended into human experience.

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FIGURE 1.—Wagner's first case of human mesothelioma of the pleura (43), published by courtesy of Dr. J. C. Wagner. *Bar* = 5 cm. × 0.5

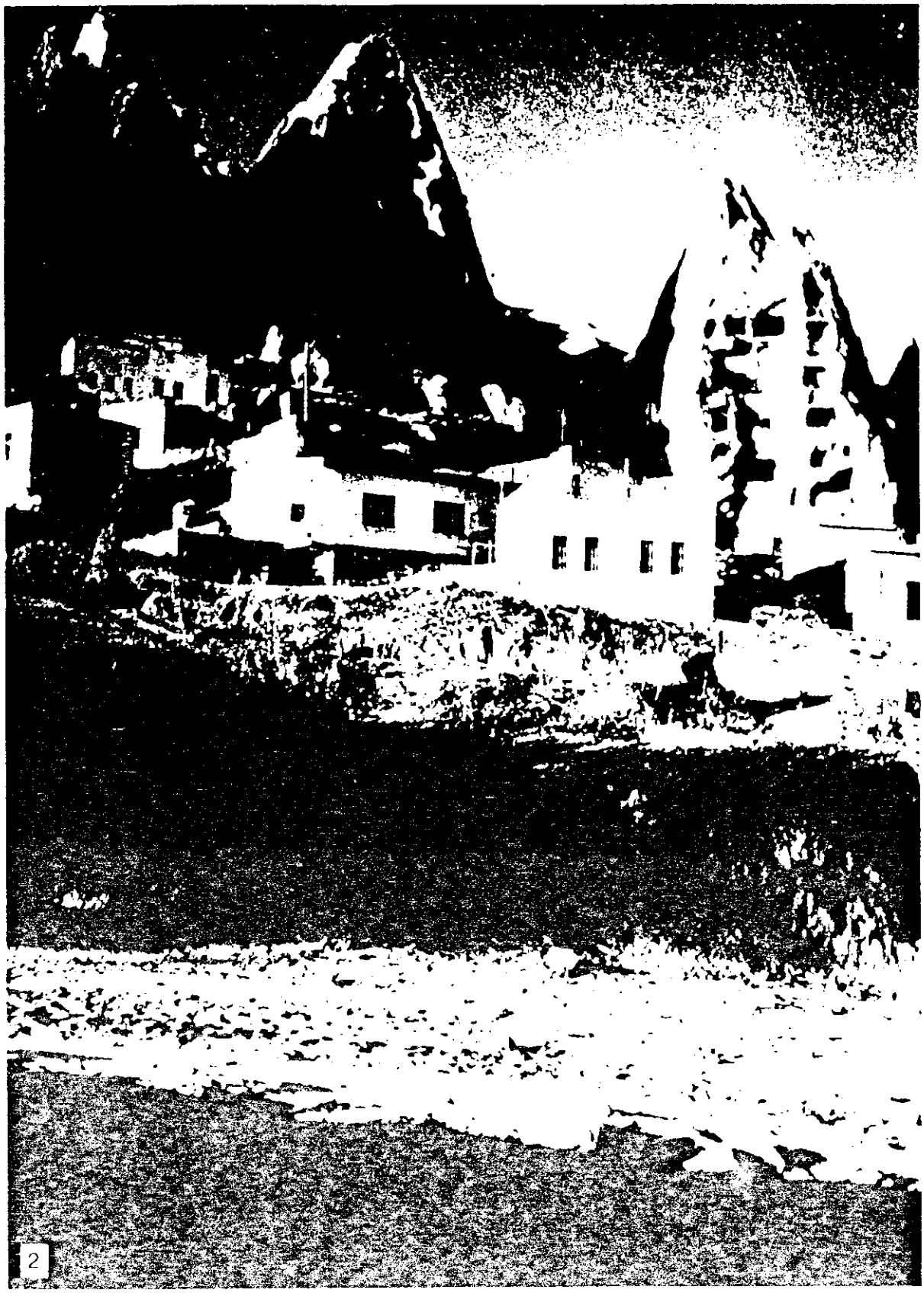

FIGURE 2.—Lava castles in Karain, Turkey, published by courtesy of Dr. I. J. Schkoff and Dr. A. N. Rohl