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Silica and oesophageal cancer

CHARLES O'NEILL, PETER JORDAN, TARLOCHAN BHATT and RICHARD NEWMAN

Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London, WC2A 3PX, UK

Abstract. The growth of animal cells in culture can be stimulated very powerfully when they are allowed to extend upon a solid surface. In normal fibroblasts, the maximum is reached either on a plane surface with an area of 2500 μm^2 or on a narrow fibre with a length of 250 μm. This growth-stimulating effect of fibres could help to explain how asbestos causes cancer. All asbestiform minerals are complex mixtures of different lengths, but siliceous macrohairs with a uniform length are borne by several species of the grass genus *Phalaris*. Some of these species are common contaminants of the bread eaten in a part of Iran where oesophageal cancer has an unexplained high incidence. A pure preparation of 200 μm silica fibres from one of these species is a powerful promoter of cancer in the skin of mice. Similar fibres from millet (*Seteria italica*) are associated with the same disease in China, and plant silica has long been known to be associated with it in South Africa. In addition, a rare thoracic tumour, which normally only occurs after exposure to asbestos, has been detected among sugarcane farmers in the United States and in India; fine silica fibres are liberated into the air during the harvest.

1986 Silicon biochemistry. Wiley, Chichester (Ciba Foundation Symposium 121) p 214–230

Evidence that biogenic silica is involved in human cancer is beginning to accumulate. Various kinds of contamination with fibrous silica have been discovered in the diets of three populations in Asia and Africa who experience extremely high incidences of oesophageal cancer, and one of these contaminants has been purified and found to promote cancer in mice. Biogenic silica is therefore a new cause of cancer. It is still far from usual to look for it in the human diet or environment, and we have no idea yet how important it might eventually turn out to be.

We give here a brief description of our work on the proliferation of animal cells and how it has led us to suspect that fibres of a particular size (either mineral or biogenic) might act to cause cancer. We show how fibres of biogenic silica of this size contaminate the diets of populations at high risk for oesophageal cancer, describe the isolation of one of these fibres in essentially pure form, and show how it can produce cancer in mouse skin under standard

experimental conditions. Finally, we list those other biogenic silica contaminants of the environment which can be associated with unusual incidences of cancer in humans or in domestic animals, and describe the technical problems which face us.

Causes of cancer

In general, we do not know what causes cancer. Most human cancers are still unexplained, and tobacco contributes significantly only to the incidences seen in Europe or North America. Environmental contaminants produced by industry, such as asbestos, cannot account for more than 10% of the cancers seen even in those countries where they are common (Doll & Peto 1981). Some of the highest incidences are now known to occur in rural parts of Africa and Asia (Waterhouse et al 1975). However, it seems clear that the causes are environmental, in the sense that the incidences of different cancers differ by factors of 10 or 100 between different countries. Migrant populations eventually become free of the tumours characteristic of their parent countries (Cairns 1979, Cairns et al 1980). It therefore seems that it is preventable; our problem is to identify those elements of the environment, diet or lifestyle which give rise to it.

Cancer is not (except in rare instances) an inherited disease, but it is a genetic one. The controls of growth and differentiation which regulate the cells of healthy animals are complex and robust; a long process of somatic selection and evolution is necessary before one clone of cells eventually becomes autonomous and grows to destroy its host (Klein & Klein 1985). Cancer is caused by agents which can make this somatic selection happen. These agents are of two kinds, either mutagens or mitogens. That is, they are either agents which cause changes in the genome, or agents which stimulate proliferation so that selection among these changes can take place. In a typical experiment, an 'initiator' (almost all initiators can be shown to have mutagenic properties) is first applied to the skin of a mouse. A 'promoter' (which will generally have obvious mitogenic properties) is then painted on repeatedly. Well-known examples of initiators are polycyclic hydrocarbons such as benzo[*a*]pyrene which attack the DNA sequence chemically. The most powerful promoters known are the phorbol esters, a group of plant diterpenes which mimic the growth-promoting effects of the endogenous diacylglycerols on protein kinase C, one of the regulatory mechanisms referred to above. We still have no idea of the relative importance of these two types of aetiological agent to human cancer in real life.

Surface area can regulate cell growth

One approach to the study of the regulatory mechanisms controlling growth is

to explant human or animal cells from tissues and grow them in culture dishes. Even under these simple conditions the cells show a very strict regulation. When they are seeded into a dish they rapidly attach, spread, start moving and grow out to colonize the surface, but all this growth ceases when a confluent sheet is formed. If the cells are then detached and seeded into a fresh dish, the same process occurs again. Nutrients and hormonal stimulators of growth are necessary, but not sufficient; even the medium from quiescent confluent cultures still retains the ability to support growth if it is applied to freshly seeded cells. The regulatory mechanism seems to depend on the amount of solid substratum available, since cells which are prevented from attaching to the surface of the dish will cease to grow almost immediately. In general, suspended cells can only be induced to grow if they have been derived from

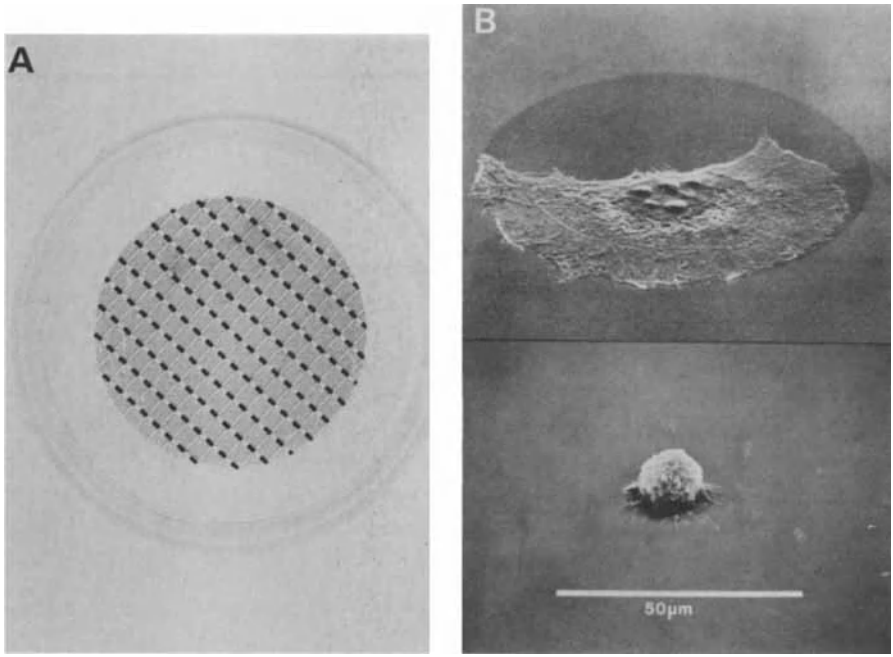

FIG. 1. (A) Dish bearing an array of adhesive islands, as described in the text. The islands range between 400 and 5000 μm^2 in area. In addition, larger areas (0.75 μm long) of unobstructed substratum are present; it is only these larger areas which can be seen clearly at this magnification. The islands are vacuum-evaporated in palladium onto a non-adhesive substratum of HEMA (polyhydroxyethylmethacrylate).
(B) Cells of the 3T3 mouse line attached to islands of 5000 μm^2 (upper picture) and 400 μm^2 (lower picture). Separation between the island centres is 150 μm, and hence only one island can be pictured in each case. The cells are from an active culture in random growth.

tumours, or their genomes have been altered by carcinogens or by the introduction of tumour cell DNA. Growth in suspension is a useful way of assessing the tumorigenicity of experimental cells and is often called anchorage independence.

We have devised a way of measuring this dependence on the substrate by offering individual cells a graded series of minute islands of adhesive substratum. These islands are surrounded by a surface which does not allow the cells to attach (made from the same material as soft contact lenses) (Folkman & Moscona 1978). In this way, single cells can be kept in isolation and yet offered the whole range of substrate areas which they would encounter during their growth towards confluence (Fig. 1A). At its most extreme, the size of the island can be so small that the cells are forced to adopt a hemispherical shape (Fig. 1B). Only the size of the substratum can change; the possible effects of cell

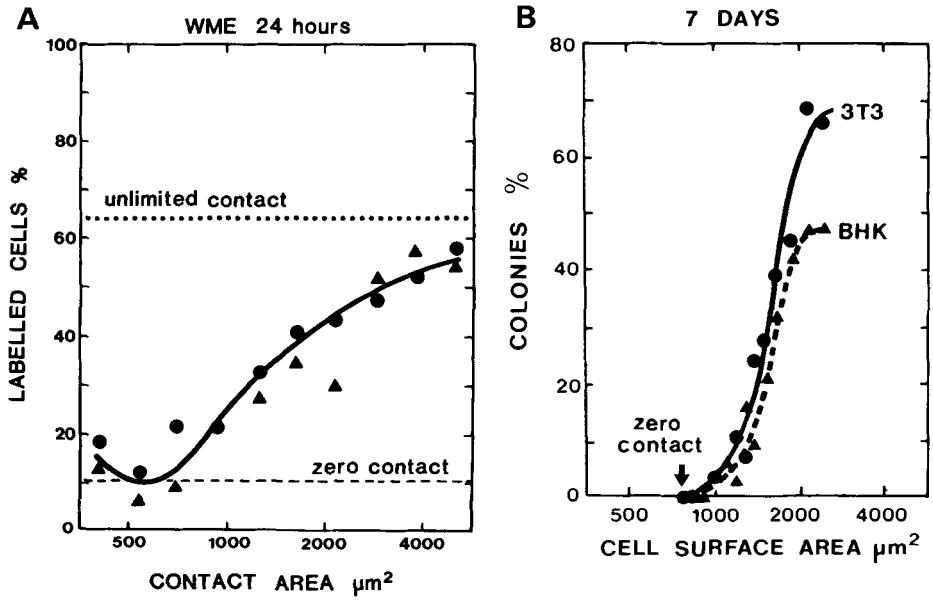

FIG. 2. (A) Proportion of whole mouse embryo (WME) fibroblasts undergoing DNA synthesis as a function of the size of island to which they are attached. Incubation is for 24 hours in growth medium containing [^{3}H]thymidine, and the percentage of cells incorporating thymidine is recorded for each island size. Different symbols refer to different experiments. The horizontal interrupted lines refer to cells allowed unlimited attachment on unobstructed substratum (. . . .), and cells denied any attachment at all, and maintained in suspension in soft gel above the island array (– – – –).
(B) Proportion of 3T3 and BHK fibroblasts stimulated to form colonies by fine glass fibrils. The surface area initially exposed by single cells is calculated from the fibre length on the assumption that the cells adopt an ellipsoidal shape whose volume is 2000 μm^3. 'Zero contact' indicates the surface area of unattached cells.

movement, encounters with other cells and general depletion of the medium can be discounted. These islands are imprinted over the whole surface of a culture dish so that the number of cells is the same as in an ordinary freshly seeded dish. Because the islands are so numerous, our measurements can be quite precise.

The results of a typical experiment are shown in Fig. 2A. This plots the proportion of cells which undergo DNA synthesis in preparation for division, as a function of island size. In addition, we include the proportions of cells kept in suspension above the surface of the dish which also undergo DNA synthesis, and similarly the proportion of cells which undergo DNA synthesis when they are allowed unlimited attachment. The experiment shows that the proportion of cells synthesizing DNA on some of the smallest islands can be the same as that seen in suspended cells. In each case, it is about 10% of the total. It is also interesting to note that the curve rises significantly at its lower end; this is a consistent feature of many different experiments.

Both these features are what would be predicted if the surface area of the cell exposed to the medium were the controlling factor. Knowing the volume of the cells, we can calculate their surface area when they adopt a spherical shape in suspension, and similarly the area of the upper surface exposed to the medium on the various sizes of island. These two figures reach equality on islands of about 700 μm^2. Ordinary considerations of geometry show that when cells adopt a hemispherical shape they expose about 20% less surface area than spheres of equal volume. Further reduction in island size should cause the exposed surface area to begin to increase slightly in just the way that is observed. Hence, both the effect of progressively decreasing island size and the relative numbers of suspended and attached cells which undergo DNA synthesis can be predicted from the surface area of the cells. This experiment therefore shows that it is the surface area of the cell which determines its rate of growth in culture.

This brief description cannot, of course, be a complete account of the complex field of studies of growth regulation. It must also be remembered that only a few of the various kinds of tissue cell can yet be cultivated outside the body. Nevertheless, the general principle that an increase in surface area can give a powerful stimulus to growth seems quite secure.

One consequence of this principle is readily open to test. If surface area is the critical parameter, then spreading in one dimension should be as effective as in two. If cells extended along a line or a rod, their surface area could be equally increased, and they should be equally stimulated. This can be tested by allowing the cells to attach to glass fibres less than 1.0 μm thick, suspended in soft gel culture medium (Maroudas 1973). In this case it is more difficult to detect DNA synthesis, but it is simple to determine the proportion of the fibres which subsequently give rise to large colonies of cells. Two experiments of this sort are shown in Fig. 2B, which shows the response of 3T3 mouse and BHK

hamster fibroblast cells to different lengths of fibre. In this figure, we have plotted colonies against the initial surface areas of the cells when they first attach. The area of the fibre in contact with the cells is minute in comparison with the islands we used before. It can be seen that the proportion of the cells stimulated to grow by fibres in each case reaches a maximum when the surface area is about 2500 μm^2. This area is little different from the area exposed by cells on the islands described above when maximum growth was stimulated. It is strong confirmation of the idea that surface area determines growth in these cells (O'Neill et al 1986).

Asbestos and cancer

One immediate consequence of this idea is that it might explain the way in which asbestos causes cancer. Perhaps it stimulates the proliferation of whatever cells it touches, by allowing them to spread and so increase their surface

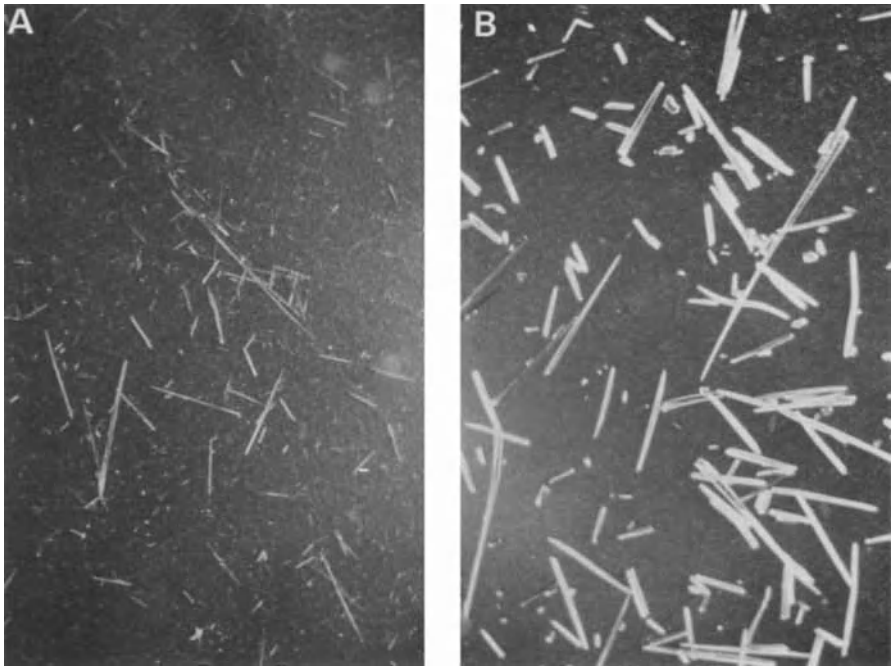

FIG. 3. Comparison of the appearance of carcinogenic mineral and biogenic silica fibres. (A) Crocidolite, standard sample from the Union International Contre le Cancer. (B) *Phalaris canariensis* from Queensland, isolated as described by Bhatt et al (1984). The fibres are suspended in water and visualized by differential interference contrast. Both pictures represent fields measuring 1.0 mm × 1.5 mm.

area. In this view, asbestos fibres act as promoters rather than initiators, and it is their mitogenic properties which allow them to cause cancer. One way of testing this hypothesis is to determine the lengths of fibre which are effective. The fibres used in the experiment described above first began to have an effect at 50 μm, and they reached their maximum stimulating power at 250 μm. It should be these lengths which cause cancer, if we suppose (not unreasonably) that the cells which they attack have similar volumes to the cells we have studied here. Unfortunately, all the fibrous carcinogenic minerals, both native and manufactured, are extremely heterogeneous in size. A standard preparation of crocidolite is shown as part of Fig. 3A. A substantial proportion of fibres

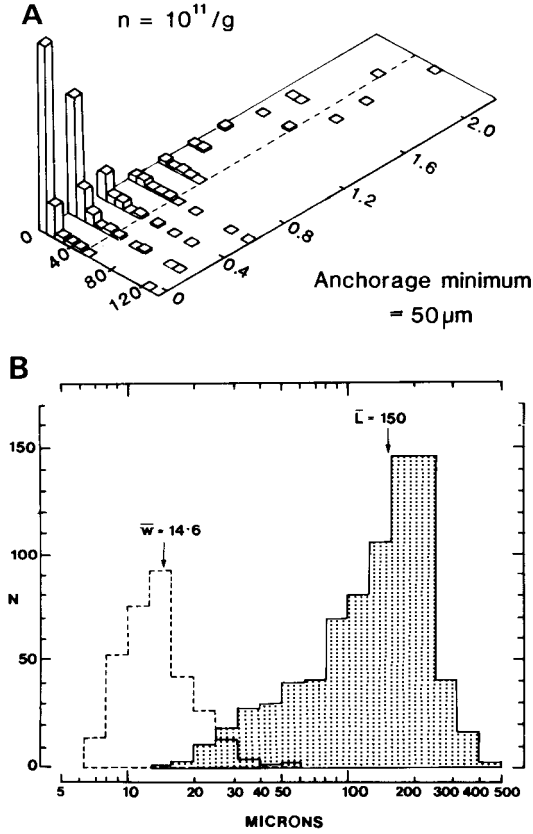

FIG. 4. Size distributions of carcinogenic mineral and biogenic silica fibres. (A) Tremolite, adapted from Wagner et al (1982). The dotted line indicates a length of 50 μm, the minimum found to cause anchorage stimulation of growth as described in the text. (B) *Phalaris canariensis* from Queensland; in this case diameter is independent of length and no attempt has been made to associate them. The hatched area shows length, and the clear area shows diameter. Mean length = 150 μm; mean width = 14.6 μm.

are clearly over 250 μm in length, but a typical size distribution of a carcinogenic mineral fibre (in this case tremolite) shows that most fibres are less than 10 μm (Fig. 4A) (Wagner et al 1982). There is clear evidence that the material of fibrous carcinogens is not important so long as they are durable and sharp, but the idea that they might be most effective at lengths greater than 250 μm is quite novel. It occurred to us that contaminations of the environment with fibres of this size might hitherto have gone unnoticed.

Oesophageal cancer

Oesophageal cancer reaches extremely high, unexplained, incidences in several parts of Africa and Asia, including the Turkoman region of North-East Iran (Cook-Mozaffari 1979). A cursory examination of the grain which is the staple diet here showed us that it was contaminated with the seeds of several species of the grass genus *Phalaris* (O'Neill et al 1980). All these seeds bear pointed siliceous macrohairs about 15 μm in diameter and over 250 μm in length. The tip radius is about 0.25 μm. Fortunately, one of these seeds ('canary grass'; *Phalaris canariensis*) is commercially available and is used as a fodder crop in arid areas. The grain merchants take care to remove as many of the macrohairs as possible because the hairs are extremely irritant to human skin (Wrigley et al 1980). It is simple to make pure preparations of these hairs from the contents of dust-extraction bags. When we treated these hairs in hot nitric acid, the product was a very pure preparation of biogenic silica fibre (Mann et al 1983).

The appearance of our preparation is shown in Fig. 3B, and its size distribution in Fig. 4B. It is a fine pure white flocculent powder which can be rubbed on the skin of mice with no obvious effect. The mice live out their lifespans in apparently robust health. However, if the groups of 20 mice are first treated with a subminimal dose of an initiating carcinogen, most of them develop tumours, as shown in Fig. 5 (Bhatt et al 1984). These silica fibres are therefore an unusual form of tumour promoter. In quantitative terms, they are fully as effective as phorbol esters, and they promote the appearance of more than twice as many tumours; although the tumours themselves are rather slower in appearing and smaller in dimension, the proportion which go on to form invasive carcinomas seems to be the same. Both the pure acid-treated fibre (free from any organic material) and the native grain are equally effective; the presence of the carbohydrate cell wall which must have originally enveloped the native fibre seems irrelevant to its tumorigenicity.

This is the first demonstration that fibres can act to cause cancer by promotion. Mineral fibres can only be shown to cause cancer in soft tissues, perhaps because their relatively narrow diameter renders them too fragile to penetrate the tough keratin layer of the skin, and soft tissue tumours are slow to detect and difficult to analyse.

The same disease, oesophageal cancer, reaches similarly high incidences in a

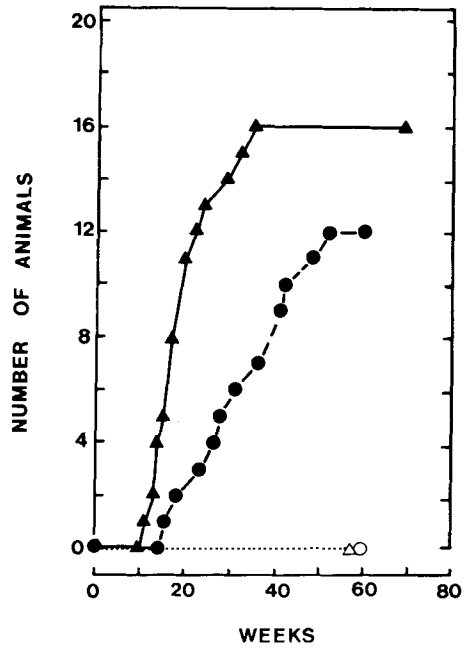

FIG. 5. Incidence of tumours initiated in mice when the skin was painted with 0.4 μg of 11–methyl–15–cyclopenta [*a*] phenanthrene seven days before the animals were treated with croton oil or *Phalaris canariensis* fibre. Triangular symbols: promotion with 0.1 mg of croton oil (1% in toluene) painted on the skin twice weekly. Circles: promotion with acid-purified fibre, rubbed on twice weekly. Open symbols: no initiator. Only the number of animals bearing tumours is shown (16 with croton oil, 12 with *P. canariensis* fibre); number of tumours per animal is twice as high with fibre as with croton oil.

part of North China. Here it is closely localized in the uplands around the Tai Han Shan mountain range. In this region there is (or was until recently) a traditional dish made from the bran of millet (*Setaria italica*) which consists chiefly of the outer coat of the grain but also includes the macrohairs which cover the inflorescence pedicels. These pedicel hairs are siliceous and seem as fine and sharp as those of *P. canariensis*; in addition, the bracts coating the grain readily shed thin siliceous sheets, and the total weight of silica in the bran is as much as 20% (O'Neill et al 1982, Hodson & Parry 1982, Parry et al 1984). Another localized area of high incidence occurs in the Transkei region of South Africa; in the diet of this region there are several broad-leaved plants, including *Bidens pilosa*, with high silica content (Rose & Guillarmod 1974). The anatomy of these plants has been summarized (Parry et al 1984), and *Bidens* has been found to have a small promoting effect on carcinogenesis in the oesophagus of rats initiated by methyl-*n*-amylnitrosamine when it is included in the diet (Mirvish et al 1985).

Other forms of cancer

A very different and theoretically interesting association between silica and cancer in humans has been reported from India (Miraj) and Louisiana. In both places, a few cases of mesothelioma have been diagnosed among sugarcane farmers (Das et al 1976, Rothschild & Mulvey 1982). This disease is interesting in spite of its general rarity because of its apparent confinement to people who have been exposed to mineral fibres (Cochrane & Webster 1978). It has now been found that sugarcane is heavily silicified, and that fine silica fibrils are liberated in the ash produced by burning the leaves (Newman 1983). The leaves are customarily burnt *in situ* to make harvesting easier, and the cane itself is used as fuel when the sap is boiled to extract the sugar. The fibres are often below 1 μm in diameter, and so are small enough to be breathed into the lungs; a substantial proportion of them are greater than 250 μm in length.

There are several other possible associations which deserve examination. Epidemiologists in the south of Brazil (Rio Grande do Sul) are investigating a moderate incidence of oesophageal carcinoma which might be associated with a reputedly common enrichment of wheat flour with *P. canariensis*. Neither the adulteration nor even the commerce in *Phalaris* grain is legally sanctioned, which makes investigation difficult, and the few samples of bread we have examined from this region are apparently free of silica fibres. Silica fibres can be detected in stomach tumours from Japan, where this disease is very common (Henderson et al 1975).

In many parts of the Scottish Highlands (Jarrett et al 1978) cattle suffer from a notably high (25%) incidence of stomach cancer, which has a histological resemblance to oesophageal cancer. This is clearly associated with consumption of bracken. Bracken (*Pteridium acquilinum*) is highly toxic and unpalatable, but when it decays the residue of insoluble detritus among the grass is rich in silica fibres (Parry et al 1985). We have been told that cancer of the eye is common in Queensland among cattle allowed to graze on *Phalaris* when it comes into seed but we have not yet investigated this interesting report.

Opaline silica in human tissue

We have found all these various associations between silica and cancer by examining the environments of people or animals known to exhibit exceptionally high or unusual incidences of cancer. This crude strategy is all too common but is not the way to arrive at any sort of final judgement on the importance of silica in this disease. In an attempt at a more intelligent approach, we have made some studies of silica in human and animal tissue using acid extraction and energy-dispersive X-ray analysis. This has shown that total silica in patients in China is about 10 times higher than in London, but we have not detected any fibres (O'Neill et al 1982). We are far from convinced of

the ability of acid extraction to isolate fibres without breaking them up, or of the relevance of crude estimates of total silica to cancer incidence. Dissolution in acid also prevents us from determining the local effects of silica on the growth of cells *in situ*, which is the only sure way of establishing how it affects the cancer process. The fibres of *Phalaris* are at least 100 times more massive than typical asbestos fibres and hence must be far easier to detect. Nevertheless, even this great advantage has not yet allowed us to find (far less, count) them *in situ* with any confidence. Some sort of detection method is an absolute necessity if we are to undertake the geographical epidemiology and experimental studies which could define the importance of biogenic silica in cancer.

Acknowledgements

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DISCUSSION

Volcani: What is known about the effects of synthetic fibres of the same sizes as your siliceous fibres?

O'Neill: If you can get the fibres into the pleura, tumours can be induced with alumina or glass fibres. The reason these synthetic fibres do not cause cancer in human may be aerodynamics. Asbestos fibres are much thinner and thus can be inhaled (Selikoff & Lee 1978).

Volcani: I am wondering whether the stimulus is purely a physical irritation, and silicon as such has nothing to do with the effect. I recall that Dr Timbrell at the Medical Research Council Pneumoconiosis Unit in Penarth, Wales, was studying this question. Sincock & Seabright (1975) observed chromosome changes in Chinese hamster cells induced by chrysotile or crocidolite asbestos; fibreglass and glass powder were inert. Hesterberg et al (1982) recently observed that chrysotile, crocidolite and certain fibreglass and quartz dusts induced neoplastic transformation of cultured Syrian hamster cells.

O'Neill: Yes, the stimulus could well be purely physical. It is accepted that non-biogenic mineral fibres can cause karyotypic abnormalities—that is, they can act as mutagens. But we have not yet seen anything of this sort with the biogenic fibres that we study. Nor do they affect the viability of Vero cells, or secretion of growth factors by macrophages (O'Neill et al 1980). Synthetic fibres are very different from these biogenic fibres. Both synthetic fibres and asbestos are extremely heterogeneous in size distribution. The presence of large numbers of smaller particles might account for some of the differences we see.

Last: Animal and epidemiological studies suggest that crystalline silica can cause cancer in the lung; the tumours are epithelial rather than pleural in origin (adenocarcinomas, for example). Silica, as distinct from silicates such as asbestos, seems also to have some sort of specific tumour-causing property, perhaps as a direct carcinogen (that is highly controversial), and as a promoter in the presence of a substance such as benzo[*a*]pyrene. There seems to be a distinction between the response of the rat and hamster in such studies; there is a peculiar effect of crystalline silica on rat macrophages, which seems not to occur in hamsters. The same process by which crystalline silica does harmful things to macrophages and causes fibrosis, as we discussed earlier (p 190), may have a counterpart in long-term experiments, where silica seems able to cause tumours in rats. And there is very weak epidemiological evidence in humans for an increased incidence of cancer in silicosis patients in Scandinavia, but unfortunately smoking has not been controlled to the point where we can confidently attribute the effect to silica. This is independent of the effects of silicates and asbestos, except where the two might go together; but generally they do not.

O'Neill: There do seem to be two separate mechanisms. Crystalline silica has toxic properties in cell cultures that our biogenic silica fibres have not. I like to think that asbestos works in both ways—the way you have just suggested, and the way that I put forward. That is why it is so effective as a carcinogen. The attractive point about the work on plant silica is that we may be able to isolate a single mechanism for study.

Williams: Is a mineral surface of a fibre more effective in enhancing the spreading of a cell than most organic surfaces, or would this be a feature of any fibre? I can understand why, say, your polyhydroxyethyl methacrylate areas

around the adhesive islands (Fig. 1) do not allow cells to attach, since there is no hydrogen bonding capacity, but what about heavily cross-linked collagen, or cellulose? Are they effective?

Last: Collagen is a superb surface for the spreading of cells, probably the best there is.

Williams: So what direct relevance does silica have to the problem of disease, other than that you happen to find silica fibres?

O'Neill: I have no evidence at all that the chemical nature of silica is relevant. We should mention that you can also produce tumours in rats by implanting sheets of plastic or metals subcutaneously (Brand 1975). The reason why fibres cause cancer is that you are not liable to breathe in a Petri dish, but you could breathe in fibres! Implanted plates of plastics, metals or glass are all equally effective in producing cancer.

Wilson: Tumours can be induced in rats by subcutaneous implantation of discs of the right size of almost any material (Oppenheimer et al 1958). You are suggesting that fibres are essentially promoters. There is a strong association between asbestos exposure and cigarette smoking in cancer production in humans, with a roughly 200-fold increase in lung tumours where the two are combined. Alcohol too has an important effect on oesophageal cancer. Could these be the initiating factors in the populations you were studying, or do you feel that silica fibres are a complete carcinogen, rather than just a promoter?

O'Neill: All the evidence from our animal studies is that biogenic silica fibres act solely as promoters. We therefore have to postulate some additional initiating factor to explain the incidences of oesophageal cancer in humans. Small concentrations of nitrosamines and tar from opium pipes have been identified in Lin Xian and N.E. Iran, respectively, but the concentrations found are far below those which have been used to cause cancer experimentally (Ching et al 1982). However, it must be borne in mind that we still do not know the relative importance of initiation and promotion in human cancer. The human disease can take more than 40 years to develop. Mutations might be accumulating during the whole of that time.

Dobbie: Talc has been detected in gynaecological tumours (Henderson et al 1979). What shape are talc particles?

O'Neill: Talc is often associated with asbestiform minerals and it is difficult to prove that a person exposed to talc has not also been exposed to asbestos particles.

Newman: Talc is basically magnesium silicate but it can be contaminated with tremolite, which is a form of asbestos. In the UK this should no longer happen, with stricter regulation of talc manufacture.

O'Neill: Talc comes in little plates, not very different in size from the islands of adhesive substratum that we used (see Fig. 1). So talc fits into our hypothesis about cell spreading.

Williams: Do I gather that the composition of the fibres is not important, Dr

O'Neill, and that any material will be effective, provided it is of the appropriate size, and persists?

O'Neill: Yes, that certainly sums up what we know so far. However, we have only done tissue culture studies on fibres of *Phalaris* and glass, and animal work with *Phalaris* alone.

Volcani: There are studies on the size distribution of synthetic organic fibres and their effects, and the results are inconsistent. Some fibres will produce proliferative effects and others will not. There is not a perfect correlation.

Birchall: The work of Stanton et al (1977) and others shows that the geometry of the fibre is important and not its chemical constitution. But it has to persist in the body. So if the fibre is made up of a polyaromatic compound and is very stiff, presumably it would give the same problems as an inorganic fibre.

Carlisle: In one study of a large number of fibres that were carcinogenic because of their size and shape, when they were aggregated none of them had any effect, whatever they were made of (Selikoff 1978).

O'Neill: This eliminated fibres over 10 μm in length?

Carlisle: Yes. I would like to add something that may be relevant from our feeding studies in animals (unpublished work, 1985). In one study in rats over many generations, it seemed that the rats on silicon-free diets had a much higher incidence of tumours than the silicon-supplemented animals. In a recent two-year study in rats on low silicon and silicon-supplemented diets, 60% of rats on the low silicon diet had an identical and very large tumour in the brain, so far not characterized, whereas there were no tumours in the animals on the silicon-supplemented diet.

Dobbie: Diatoms have siliceous skeletons, as we have heard. Have they been incriminated in the pathogenesis of carcinoma?

Volcani: Not to my knowledge, nor does natural diatomaceous earth, known as diatomite, cause lung fibrosis in humans. Diatom shells (frustule) commonly have no fibres. They vary greatly in shape (e.g. oval, circular, triangular) and in size (2–300 μm).

Dobbie: The finding of diatoms in the lung and bloodstream is used forensically in establishing drowning as a cause of death. If spicules from diatoms gain entry to the body via the gut, is it naive to consider these as a possible carcinogen?

Volcani: The geometry of diatoms doesn't seem to be right; the shells don't have the needle shape which the fibre theory seems to require, and they are too large, as I said.

Werner: This is true for many frustules, but there are diatom species such as many *Chaetoceros* spp. and *Rhizosolenia* spp., abundant in many areas of the oceans, which have very long spines. The spines are longer than 20 μm. Such material could come in contact with humans via products made from infusorial earth.

O'Neill: This is obviously a fascinating area, but the point to make is that we

haven't yet found a fibre of the 'wrong' length (less than 50 μm) that is associated with cancer, or a fibre of the 'right' length (more than 250 μm) that when ingested does not cause cancer, despite much searching. The *Phalaris* fibre is also quite remarkable in its sharpness. The other known fibre that produces cancer is asbestos, which is not pointed but is very narrow, and also is so light that it can penetrate to the bottom of the lung. These seem to be two rather special things. It is not just the presence of a silica particle of about the right size but the question of its entry to the body. Diatoms enter the lung when you breathe in water, but I am not sure that they would enter from the air. I don't know whether siliceous sponges get eaten.

Carlisle: Diatoms have been found in stillborn babies.

Williams: You could perhaps attack these problems by radiocarbon labelling of the hairs, by growing them in ^{14}C-glucose and then doing autoradiography. Carole Perry used this approach when she was following the biosynthesis of the polysaccharides of the cell wall from the silicified macrohairs from *Phalaris canariensis* L.

Perry: The extent of labelling which occurs will depend on when the plant is labelled, as the rate of synthesis of the cell wall and therefore the incorporation of radioactive label varies significantly with the age of the inflorescence. Maximal incorporation of radioactive label into the cell wall occurred at about 14 days after emergence of the inflorescence.

Sangster: Is it possible that the heat from the baking process of the bread in Iran would change the silica fibres chemically?

O'Neill: I don't know. The temperature must reach well over 100°C.

Perry: We have done solid state ^{29}Si NMR experiments of samples of plant silica dried in an oven at 140°C. The spectrum is unchanged from that of the non-heat-treated samples.

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