SOME BIOLOGICAL ACTIONS OF SILICA: THEIR PART IN THE PATHOGENESIS OF SILICOSIS

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This is not a review article,[1] but an examination of some experimental approaches which might contribute towards a better understanding of the cause of silicosis. The following broad aspects only have been selected for discussion: the surface activity of silica, a possible 'template' action of silica, and a two-stage process comprising macrophage damage by silica with subsequent release of biologically active lipoidal material, which is considered in this paper to be the most plausible explanation for the toxic action of silica in the body.

A reviewer recently wrote that there were '50 theories' of the causes of silicosis.[2] At least nine-tenths of these are not theories but ideas, concepts or hypotheses. Two theories have dominated silicosis research since the 1920s: the silica solubility theory (see Nagelschmidt[3] for an interesting discussion of this), which remained in power for over 25 years, and the immunological theory, which has had a vigorous and interesting life, but is now in failing health. Both have been fully reviewed elsewhere,[1] and it is not intended to devote more than a few lines to them here.

The silica solubility theory[3] proposed that silicic acid, released over long periods of time from a depot of silica in the lung, could lead to the formation of collagen, and although this at one time seemed plausible enough, the theory has now been effectively demolished.[3] The immunological theory, first suggested by the work of Scheel and his colleagues,[4] and by that of Antweiler and Hirsch,[5] was elaborated by the extensive work of Vigliani and Pernis.[6] It can now no longer be regarded as defining the specific cause of silicosis, and what was previously regarded as being a true immunological phenomenon is now considered as a non-specific manifestation,[7] in which silica acts as an adjuvant. Hypotheses based on the surface activity of silica have been moved up to fill the vacuum, and it is perhaps as well, at this point, to remind ourselves of the four postulates given by Nagelschmidt in 1960 for the action of silica in producing silicosis:[3]

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1. Free silica is almost unique in having the specific fibrogenic character.

2. The particles which cause silicosis are about one micron particle diameter.

3. Silicotic nodules can be produced in different species of animals in the absence of tuberculosis.

4. The process is slow and progressive in the absence of further exposure to dust, i.e. the activity of silica is apparently permanent.

THE SURFACE OF SILICA

As a pure, intact mineral, silica occurs as quartz, and as other forms which need not interest us here. Its crystalline structure is of such a form that it exists as 'a lattice of interlinked "infinite" -Si-O-Si-O-spirals'.[8] When fractured, however, such as would happen during drilling or blasting procedures, the freshly-formed surfaces react with water (as moisture) to form a surface of hydroxyl groups which acts as a weak acid, permitting, for example, the exchange of hydrogen ions with other cations, such as sodium.[8] This surface should be regarded not in molecular terms, as single SiO_4 tetrahedral units, but as a relatively large surface of -Si-O-Si-O chains,[8] which at the freshly-fractured surface change to -Si-O-Si-OH after the addition of hydrogen.

It was at one time believed[9] that such freshly-fractured surfaces were responsible for the biological activity of silica, but this concept was demolished by Wright,[10] who pointed out that 'aged' silica and silica recovered from silicotic lungs after death were as fibrogenic as freshly-fractured material. Also, the unsatisfied valencies at the fractured surface should be satisfied almost instantaneously, although not to form oxygen groups but hydroxyl ones, produced after hydration.

The biological activity of such groups in producing fibrosis (collagen deposition) seems, by inference, undisputed. When silica was coated with iron hydroxide,[11] or with aluminium (as metallic aluminium),[12] its solubility in water was almost completely prevented, and it was no longer fibrogenic in animals. The work of Denny *et al.*[12] on aluminium heralded its use as a prophylactic measure whose effectiveness is still undecided.[13] Whatever the result of this application may be, the physical and biological actions of aluminium or silica have turned out to be very useful indicators of what crucial groupings on the silica surface are responsible for the biological activity of silica.

One of the effective samples used by Denny *et al.*[12] in 1939 was a metallic aluminium powder of 99% purity and of —20 microns (*sic;* mesh?) size,[14] and a more recently prepared sample consisted of 85% aluminium oxide and 15% metallic aluminium, of particle diameter 51% $< \frac{1}{2}$ micron and 47% $< \frac{1}{2}$-2 microns, only the smaller particles being measured because of the very rapid settling of the large ones in the suspension. Particles of aluminium of such sizes, when incubated with silica such as is used in experimental work (a typical size-distribution of 'experimental' silica being 51% $< \frac{1}{2}$ micron, 42% $< \frac{1}{2}$-2 microns and 6% $<$ 2-5 microns particle diameter), are effective solubility depressors and prophylactic agents.[12,14] Adsorption of aluminium particles of such sizes on silica is very unlikely, if not impossible; in fact, what happens is that a gelatinous hydrated oxide of aluminium, aluminium hydroxide, $Al(OH)_3$, is formed on the surface, and later changes on drying into crystalline aluminium monohydrate ($Al_2O_3.H_2O$).[12] All that need interest us at this point is the formation of aluminium hydroxide on the silica surface, a product formed through the interaction of hydroxyl groups (-OH) on the silica surface with aluminium in an ionic form (Al^{3+}). In this connection, for example, 20 mg. of the aluminium mixture used above (85% Al_2O_3; 15% metal) yield 120 μg. (0·12 mg.) and 50 μg. (0·05 mg.) of soluble aluminium after being shaken at room temperature and at pH 7·4, for 24 and 48 hours respectively.[15] There is also other supporting evidence that very little aluminium is required for solubility depression,[13,16] and a recent proposal suggests the use of soluble aluminium preparations for prophylaxis, provided they are not too rapidly drained from the lung after inhalation as aerosols.[15]

THE SURFACE ACTIVITY OF SILICA

The interaction of the positively charged aluminium ions with the negatively charged hydroxyl groups on the silica surface (a process taking place equally well on aged and freshly-fractured silica[16]) implies only one thing, that the anionic groups on the silica surface are responsible for the fibrogenic activity and for the solubility of silica, this last feature being the more easily understood.

Evidence that some sort of reactivity is associated with the silica surface was produced in 1960,[17] when it was shown that silica possesses special oxidative and hydroxylative properties, work reminiscent of earlier findings[18,19] that siliceous dusts and silicates could, under certain conditions, act as oxidation catalysts in the presence of air. It was shown[17] that silica could, for example, hydroxylate proline and several other amino acids and biological materials, very much in a peroxidative capacity such as that shown in a different system by Kalyankar *et al.*[20]

Marasas and Harington[17] concluded that the conversions initiated by silica in their experimental systems were catalytic ones in the presence of air, possibly of the type reported by Johnson *et al.*[21] where reactions involving free radicals *in vitro* simulated certain enzymatic processes. Later work by Marasas[22] showed that quartz under simple laboratory conditions could decarboxylate histidine to form histamine and other biologically active materials. Since such conversions can take place in dry systems as well as wet (for example, dry silica powder shaken vigorously with the test substance), it seems that free radical transfer from the quartz surface to the receiving amino acid (such as, silica + proline $\rightarrow$ hydroxyproline + silica) takes place, and indeed such transfers in dry systems are not unknown. Whether the hydroxylations and oxidations demonstrated for silica and other fibrogenic dusts[17] have any significance in the body is a point which is going to be difficult to prove, since free radical transfer, if it occurs, will be at a molecular level, and difficult to follow at the considerable dilution in which it will probably occur.

Analogy of these changes with X-radiation damage to tissues, and with its mode of action generally, should be profitable. Several similarities between X-ray and silica activity suggest themselves, such as the shared peroxidative property, free radical formation and transfer, and the generally synergistic effect of iron. Furthermore, reduced glutathione can protect tissues from both types of damage, although under different conditions.[23] Anti-oxidant substances have no effect on the development of silicotic lesions.[15]

Among other surface actions of silica of a biological nature which deserve careful consideration are those of the adsorption and precipitation of proteins by silica, and the precipitation of soluble collagen by silicic acid of low molecular weight. Serum globulins are adsorbed on the silica surface; this led Vigliani and Pernis[6] to suggest that the protein adsorbed on the silica could act as an antigen, and so form the appropriate antibodies. Later work[24] showed that γ-globulin was unchanged by adsorption as far as antigenicity was concerned; in other words, that silica did not lead to the production of new antibodies against globulins specifically altered by the silica. Here

again, such features will be difficult to detect in the *in vivo* course of events.

'TEMPLATE' ACTIONS OF SILICA AND OTHER MATERIALS

What might well be a separate action of silica is that suggested originally for silicic acid by Holt in 1957,[5] and into which might dovetail the earlier finding of Cagliotti *et al.*,[25] that artificial collagen fibres could be produced from collagen solutions by silicic acid of low molecular weight.

For this to be regarded as a 'separate action' suggests two possibilities: (1) that silica damages tissues, or cells such as macrophages, and that the subsequent collagen deposition characteristic of silicosis is a result of this damage; and (2) that silica, in some form or another, intra- or extracellularly, can cause *directly* the laying-down of collagen fibres from a soluble precursor upon itself as template, rather as railway lines are laid down upon sleepers, a biological action first suggested by Meyer[26] for collagen deposition upon mucopolysaccharides, which are later removed. Such possibilities were also proposed by Cohen[27] and Partridge,[28] and Jäger applied this reasoning to silicosis,[29] suggesting that the molecular pattern of silica could complement in some way that of collagen, so that insoluble collagen fibres would be laid down wherever silica was present, and in an orderly fashion. Nagelschmidt[3] found this new view 'almost certainly inadequate' as an explanation of the pathogenicity of silica, but it will nevertheless be examined conjointly with Holt's views,[5] and with the whole subject of the deposition of 'artificial' collagen fibres, and certainly with Glimcher's superb demonstration[30] of the orientation of hydroxyapatite crystals by collagen during osteogenesis. A number of substances of diverse origin and character are capable of precipitating collagen of certain spacing from a solution of soluble collagen[31] such as rat tendon in dilute acetic acid; these include mucoproteins, hyaluronates, sodium chloride at high concentrations,[31] chondroitin sulphate,[31,32] gum arabic,[32] and heparin.[33]

Holt suggested[34] that silicic acid, slowly dissolving from silica particles within the phagocyte, could diffuse from the cells, together with a collagen precursor that would later aggregate as insoluble collagen fibres, which would adsorb the silicic acid molecules; these would then polymerize on the collagen and stabilize the fibre. Such a combination between silicic acid and protein could take place through interaction of the negatively charged groups of the acid with the basic groups of the protein; this suggests that a wider knowledge of collagen tanning processes would be useful in work on the pneumoconioses.[35]

Glimcher's work has shown[30] that reconstituted collagen fibres, prepared from normally uncalcified tissues, were able to nucleate apatite crystals from metastable calcium phosphate solutions, and a regular and periodic arrangement of the dense apatite crystals is evident in electron micrographs of bone in the earliest stages of calcification.

A theoretical study which attempts to correlate the fibrogenicity and chemical structure of a wide range of compounds has recently been undertaken in this Unit,[36] and even if premature, the findings thus far emphasize the importance of negatively charged groups in the fibrogenic molecule. Apart from silica's surface of negatively charged hydroxyl groups, other substances which are fibrogenic may have more highly charged negative groups, such as the sulphate groups of the acid mucopolysaccharide, carageenan, a powerful 'fibrogen'.[36] The fibrogenicity of γ-AlOOH and aluminium phosphate,[37] the latter with its X-ray diffraction pattern very similar to the strongly fibrogenic quartz form, tridmyite,[2] suggests the participation of PO_4^{3-} and OH^- groups. Examination of the fibrosing activity of 26 substances related to the active dicetyl (di-n-hexadecyl) phosphate showed that for fibrosing activity there must be at least one free -OH group, and this was found for dioctadecyl phosphate $[(C_{18}H_{37}O)_2P\lessgtr^{O}_{OH}]$ and di (tri?) cetyl borate, $(C_{16}H_{33}O)_2BOH$.[38]

Other interesting fibrosing agents containing anionic groupings are cellophane,[37] sodium morrhuate (the sodium salts of the fatty acids of cod-liver oil, palmitic, stearic, etc.),[39] and sodium tetradecyl sulphate and cetyl palmitate (spermaceti).[40] Sodium dodecyl sulphate interacts with collagen by an electrovalent reaction of the long chainlike anion of the salt with the cationic protein groups; its fibrogenic properties are as yet unknown. Another such combination is the collagen-polymetaphosphate one.[41]

Space limitations preclude further discussion on the possible theoretical significance of correlations such as those suggested above, but one last requirement should be proposed: if structure (such as possession of conveniently situated or spaced anionic groups) is a pre-requisite of direct collagen formation, that is, not by injury, and occurring either *in vitro* or *in vivo*, the solubility of the fibrogenic agent must be considered. Dahl *et al.*[38] found that a sharp cut-off in chain length of their long-chain compounds corresponded with a resultant loss of fibrogenic activity. They attributed this to a possibly greater solubility of compounds with shorter chain lengths, resulting in subsequent diminution of fibrogenic activity. They found that an essential requirement for fibrogenic activity was that the molecule should possess at least one free -OH group for activity, and that chain length must be more than 14 carbons long. This cannot be the sole criterion, though, for there are some of Dahl's compounds with chain lengths greater than C_{14} and containing one -OH group, which are not active, e.g. di(or pyro)-cholesterol phosphate and diphenoxyethyl phosphate. Gerstl and Tennant[42] found that the necrotizing action of long-chain α-methyl fatty acids was reduced with increasing length of the carbon chain, finally disappearing when the chain length reached C_{27}.

On the basis of Dahl and his colleagues' findings, any compound, to be fibrogenic, should be relatively insoluble and have a correctly spaced or orientated, negatively charged group arrangement for binding with cationic protein (?) sites. A case in point is carageenan's fibrogenicity, and the non-fibrogenicity of its very close but much more soluble relative, heparin. It is probably not a barren prediction that relatively insoluble forms of sulphated mucopolysaccharides, such as less soluble heparins, should be fibrogenic; so too with complex materials such as shellac, which at first sight should be actively fibrogenic, consisting of a mixture of aleuritic acid (8:9:15 -trihydroxypalmitic acid) and shellolic acid,[43] and which produces pronounced granulomata after subcutaneous inoculation in rats.[36] Other compounds which would be of more than passing interest in studies of fibrogenesis are the dextran sulphates (with varying degrees of sulphation) and other anionic macromolecules such as polysulphonic acids, e.g. lignosulphonic acid. The important criterion in the use of such molecules is that they must be large enough to function as cross-linking agents by multipoint attachment to adjacent (soluble) collagen chains, or perhaps to other proteins such as lipoproteins and fibrinogen.

MACROPHAGE DAMAGE IN SILICOSIS: A PRIMARY FEATURE

It is now quite clear that an essential feature in the pathogenesis of silicosis is that tissue damage must take place, and that the subsequent fibrosis must arise from this, either primarily or secondarily. Such damage is almost certainly caused through the agency of some sort of surface activity of the inhaled silica particles, which are later engulfed by macrophages, and could be caused by protein adsorption or denaturation,[44] by oxidative changes,[17] by interference with intracellular enzyme activity,[45] such as esterases in the macrophage,[23] or by the oxidation of glutathione caused by surface metals, especially iron, an action shared by the fibrogenic asbestos materials, crocidolite, amosite and chrysotile.[46]

Whatever happens, it seems beyond doubt that the first critical action of silica occurs in the macrophage, and that macrophage damage is a pre-requisite for fibrosis,[47] and even a function of the extent of a dust's fibrogenic potential, although the prediction of the behaviour of a dust in Man from its toxicity in cell cultures is still hazardous.[48] This may now be termed the *1st stage process* in the pathogenesis of silicosis. The *2nd stage process* is the release, from damaged or dead macrophages, of tissue

materials probably responsible for the ensuing fibrosis. These are not revolutionary views, and they have been put forward in different form from time to time.[47] But having accepted the likelihood of this two-stage process, it now becomes necessary to determine the nature of the substances liberated by the disintegrating macrophages, and the extent of their fibrogenic activity.

LIPID RELEASE FROM THE MACROPHAGE – A SECONDARY FEATURE?

The use of macrophages as experimental material should now, for biochemical studies at least, supersede the use of animals, and a full-scale attack on the interaction of silica on components of these cells is a priority, if we are to get any further in understanding the cause of silicosis. It is possible that released proteins, polysaccharides or other carbohydrates, or the complexes of these materials with each other, could be biologically active after their release from the macrophage into the ground substance or extracellular spaces of the tissue. The action of macrophage lipids or lipoproteins is probably the most attractive horizon to contemplate at present.

This aspect has been reviewed recently,[2] and, very briefly, the history of lipid participation in the development of silicosis is as follows: In 1931 Strachan and Simson showed an increase in pulmonary lipid with increasing severity of silicosis in miners.[49] In 1937 Fallon suggested[50] that the release of phospholipids from dying macrophages could cause the silicotic fibrosis, just as the phospholipids of the tubercle bacillus caused its granulomata.[51] Phospholipids recovered from rabbits, which had had intratracheal injections of silica 1 - 16 weeks before, gave rise to peritoneal granulomata. Clerici and Pernis[52] found more total lipid in silicotic masses than in other hyaline tissues, and suspected precipitation of lipoproteins; fatty acids bound to reticulin were also found in greater amounts than in normal tissue.

A lipoid material has also been demonstrated in all stages of the development of the silicotic nodule in human cases of silicosis.[49] Marks and Marasas in 1960 found that phospholipids, lecithin, cholesterol and free cholesterol in the guinea-pig lung increased with increased exposure of the animals to silica dust,[53] although analysis of the results shows that on a percentage basis only phospholipids, including lecithin, increase to any significant extent, such increases taking place after a period of 28 weeks' exposure to dust. Marks and Marasas found no significant changes in rabbits exposed to dust, in contrast to Fallon's results.[50]

With this information in mind, a further search for information on the activity of lipids or lipoidal fractions in Man or animals produced encouraging results.

THE BIOLOGICAL ACTIVITY OF LIPIDS AND LIPID FRACTIONS

In 1928 Pinkerton[54] injected a number of different oils intratracheally into rabbits. He found that simple neutral vegetable oils (iodized sesame, poppyseed oil and olive oil) produced hardly any reaction in the lung, whereas animal oils (milk fat, rabbit fat, cod-liver oil and lard oil) caused marked fibrosis in the lungs in a few days. Mineral oil gave rise to slight fibrosis at the end of the second or third month. Pinkerton considered that the difference in activity shown by vegetable and plant oils was due to the amount of free fatty acid originally present, and the rapidity with which hydrolysis of fat had progressed. Free fatty acids from certain animal oils produced caseation necrosis in lung tissue. The failure of vegetable oils to cause any reaction was ascribed to the absence of hydrolytic enzymes in the tissue for this type of fat. Chaulmoogra oil, however, produced necrosis and extensive fibrosis, which Pinkerton considered the result of its high fatty-acid content.

An interesting finding was that rabbit fat (from adipose tissue), inoculated into a second rabbit, was 'organized' in some way, and stored locally with no pronounced adverse effect. In 1937, Gardner[55] stressed the fact that silica could mimic the entire histological pattern of tuberculosis in every way, and that it called forth the same phagocytic cells as the tubercle bacillus. Also, in both instances, the phagocytes could show fat droplets.[55]

Fifteen years later, Gross *et al.*[56] produced evidence which fits in very well with the macrophage damage - lipid release hypothesis under discussion here. In studies on experimental endogenous lipoid pneumonia associated with the inhalation of antimony trioxide by rats, it was found that these particles caused metabolic disturbances within the macrophages which led to their fatty degeneration and necrosis. After accumulation of intracellular lipids, the alveolar macrophages ultimately ruptured, and much, if not most, of the fibrosis within the lung appeared to be secondary to the lipids so liberated, which then act as irritants. The absence of fibrosis in lymph nodes, where heavy deposits of antimony trioxide were present, was considered as evidence for the assumption that antimony trioxide alone did not directly cause pulmonary fibrosis. Gross *et al.*[56] saw the similarity of their findings with those of Fallon,[50] that silica also was acting indirectly, and that released lipids could well be the true fibrogenic agents. 'There is the possibility that lipidic substances may be associated with other forms of pneumoconiosis which are characterized by pulmonary fibrosis', wrote Gross and his colleagues.[56]

A progressive accumulation of lipids within pulmonary macrophages in experiments with submicroscopic, amorphous silica, and with beryllium salts, has also been reported.[57] Schepers has stated (unpublished, see ref. 2) that 'certain lipids artificially introduced into the lung are capable of producing extensive fibrosis', findings which will be awaited with great interest, especially in view of Pinkerton's earlier work. In fact, a re-examination of Pinkerton's findings in the light of modern knowledge, especially of lipid chemistry and technique, seems to be a pre-requisite for further studies on the fibrogenic properties of lipids and of lipid participation in disease.

The amount of total lipids extractable from silicotic nodules is several times higher than that contained in other hyaline tissues.[52] There is also the suggestion that lipoproteins could have been precipitated by the silica, a finding in keeping with the propensity of other anionic macromolecules such as dextran sulphate and carageenan to precipitate serum lipoproteins.[58] Fatty acids were also found,[52] similar to those bound to reticulin fibres,[59] and in a greater proportion in silicotic masses than in normal lung tissue.

Shelley and Hurley[60] considered that the constant distinguishing feature of the silica granuloma (intradermal) is that it is a foreign-body granuloma which ultimately disappears. They accepted from their earlier work[61] that the silica granuloma is a primary granuloma formation dissociated with difficulty from the secondary granuloma following the release of fatty acids and soaps from the fat.[61] Furthermore, the action of both silica and lipids is very similar, the intradermally trapped silica acting exactly like the stearate mass in the earlier work.[60]

In the formation of atheromatous deposits (a field offering much stimulation to workers in other related

fields, such as silicosis), a possible stage at which biochemical failure or abnormality could occur is in the building-up or breakdown of lipoprotein. A heparin-activated enzyme has been described[63] which can quickly attack the neutral fat of low-density lipoproteins and so liberate the constituent fatty acids. Morton[62] considered that the importance of this work lies in the fact that it reveals a process whereby lipoproteins of very high lipid content could shed the products of triglyceride hydrolysis; any failure in enzyme activity could possibly lead to atheromata. Precipitation of lipoproteins by silica, a possibility evident from the work of Clerici and Pernis,[52] is not unlikely, nor is the expulsion of lipoprotein and lipid from the disintegrating macrophage, both materials serving as potential depots of fatty acids after their hydrolysis in the tissues.

Hill and his colleagues,[64] in studies on atherosclerosis, suggested that there is a progression of events from fatty deposition to fibrosis and then to complicated lesions. The fat deposition seemed to occur *de novo*, and is possibly not reversible. In the early stages it produces very little reaction, but from about 33 to 45 years of age it leads to fibrosis and, after this, to complicated lesions. Gresham and Howard[65] supported this view from evidence obtained from animal studies, but considered that the presence of histologically demonstrable lipid as a trigger for intimal fibrosis is still a debatable question.

THE ROLE OF LIPIDS IN SILICOSIS

The evidence reviewed above seems more than circumstantial as far as the incrimination of lipids in fibrogenic action is concerned, and before concluding this part of the work, there remain two aspects to consider. What is it in the lipid fraction which could cause fibrosis, and are lipids in any way altered by silica so that an unnatural fibrogenic agent could be produced?

Firstly, Pinkerton's results and those of others already reported, do not suggest that an altered lipid component is necessary for fibrogenic action, and work to be described below will show that synthetic fatty acids and pure fatty acid esters may be fibrogenic. Fallon unfortunately did not inoculate rabbits with 'control lipid', i.e. lipid extracted from animals which had not been treated with silica;[50] a crucial omission which, had it given positive results, might have greatly clarified the issue.

Rabbit adipose fat inoculated into the lung is not fibrogenic, but is 'stored locally',[54] so it is not yet known whether lung lipids are toxic to untreated lungs of the same species. We have found in preliminary experiments[66] that adipose guinea-pig lipid (extracted from the fat according to the method of Folch *et al.*[67]) is not in any way active (no foreign-body reaction or fibrosis) when inoculated subcutaneously into those guinea-pigs from which the fat had been excised (homologous transfer), nor is it active when cross-inoculated into different guinea-pigs (heterologous transfer). Total lipids extracted from untreated alveolar macrophages of guinea-pigs, however, when inoculated subcutaneously into other guinea-pigs, in two months produced discrete granulomata twice the size of those produced by 25 mg. of silica over a period of three months. The nodules induced by macrophage lipid were composed of non-granular cells (histiocytes mainly, and lymphocytes), with a loose reticulin network, scant in one nodule.[68] This work is now being repeated and extended, and if it is confirmed and shown to be a true property of macrophage lipid, it may provide a link in the chain of events leading to the development of silicosis.

An important aspect not yet resolved is whether the granulomata produced by macrophage lipids are simply foreign-body manifestations, or whether they might ultimately develop into a truly collagenous entity, such as is produced by silica in the lung. Intratracheal administration of macrophage lipid as an aerosol would throw further light on its mode of action.

It is important to remember that the granulomata were produced by lipids from macrophages which had had no contact with silica whatever, and were, to all extents, normal alveolar cells.[66] Thus, we might be able to consider an approach to silicosis *without silica*, i.e. by examining the biological activity of different fractions of macrophage lipids after chemical analysis (e.g. for saturated and unsaturated fatty acids), and of course, after the rigorous exclusion of such artefacts as impurities in the preparations. Whether silica modifies macrophage lipids in any way after incubation in cell culture, or in the body, is yet another important aspect to be studied. The use of alveolar macrophages is recommended,[69] rather than peritoneal cells collected by the use of irritants such as liquid paraffin, which leaves the macrophage filled with globules of a mixture of liquid hydrocarbons, not in themselves entirely inactive.[70]

A recent article by Arean *et al.*[71] on the pathogenesis of the ascaridic granuloma, showed that granulomata were produced by lipids when given in a poorly 'resorbable' form, but none when the lipids were readily resorbed (in saline and Tween 80). The authors referred to Rich's suggestion[72] that the mode of action of tuberculolipids in the development of the granulomatous response may be related more to their being insoluble (that is, less easily resorbed) in watery tissue fluids, than to a specific chemical action, although each individual lipid should determine its own specific irritative power, and the intensity and character of its action.[54] The lipid which we isolated from macrophages,[66] unlike the oil from the adipose fat, was a waxy, solid material requiring emulsification before it could be administered.

There is a certain amount of evidence which permits us to make 'an educated guess' as to what part of an active lipid fraction might be responsible for the fibrogenic activity. The granuloma-producing properties of synthetic fatty acids from intact tubercle bacilli have been described by Ungar,[73] the necrosis caused by such materials being a very early event occurring soon after injection. Ungar regarded the lesion produced by synthetic fatty acids as being a foreign-body reaction. His paper should be consulted for references to other work on the production of granulomatous lesions by α,α-dimethylstearic acid, phthoic acid, trimethyl-3 : 12 : 15-docosanoic acid and other fatty acids. Hurley and Shelley[61] found that the saturated fatty acids, palmitic and stearic, did not cause granulomata, but that their sodium salts did, the granulomata being resolved in a few weeks. Zinc stearate has been shown to produce extensive fibrosis of the lungs of a worker in a rubber

factory.[74] The fibrosing action of sodium morrhuate[39] and cetyl palmitate[40] has already been mentioned. There are many references to the tuberculocidal effects of long-chain fatty acids (at low pH), such activity being related to the length of the carbon chain in the saturated fatty acid series.[75] In the work of Hart and his colleagues,[75] lethal effect to tubercle bacilli increased with increasing chain length, palmitic and oleic acids being the most active.

Any involvement of fatty acids in tissue reactions presupposes their release from lipoidal material unless, of course, they are used as pure substances. Such release is a hydrolytic process effected by lipases, the injection of which can, by the production of fatty acids, produce fat necrosis.[76] It will be recalled that Pinkerton in 1928 considered that the degree of damage and resulting fibrosis produced by animal oil (fat) in the rabbit lung depended largely on the amount of free fatty acid originally present, and on the rapidity with which hydrolysis progressed.[54] Therefore the process:

silica + macrophage → lipid → fibrosis

might be further, but tentatively, restated as follows:

silica + macrophage → $\left.\begin{matrix}\text{lipid}\\ \text{lipoprotein}\end{matrix}\right\}$ → gradual hydrolysis by lipase → fatty acids (?) → fibrosis.

CONCLUSION AND SUMMARY

An attempt has been made in this paper to define more clearly the part which the surface activity of the silica particle might play in the pathogenesis of silicosis, and analogies have been drawn with the structure and biological action of other anionic macromolecules. In particular, the role played by the macrophage is emphasized, it being once more suggested that a two-stage process is probably responsible for silicotic fibrosis, beginning with damage to the macrophage by the silica particle, and followed by the release of lipids (possibly containing the fibrogenic agent or agents) into the ground substance.

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