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STUDIES ON THE NATURE OF SILICOSIS

THE EFFECT OF SILICIC ACID ON CONNECTIVE TISSUE

BY

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Contemporary studies indicate that connective tissue is a coarse network of collagen on which is superimposed a finer network of fibres visible only in the electron microscope (Wassermann, 1951). An amorphous ground substance, which is responsible for the high resistance of the tissue to the passage of fluids, appears to consist of collagen fibrils which are just visible in the electron microscope and are embedded in mucopolysaccharides of high molecular weight.

Much of the evidence for this conception of the structure of connective tissue has been derived from light- and electron-microscopic studies. The sieve-like structure of the collagen network has also been demonstrated by comparing the rate at which saline will flow through animal membranes before and after treatment with hyaluronidase (Day, 1952). Hyaluronidase, by removing the polysaccharides, largely increases the tissue permeability but the permeability is again reduced if the tissue is treated with mucopolysaccharides, such as chondroitin sulphate, or with certain other macromolecules.

The function of the polysaccharides in connective tissue is not understood with certainty, but it is thought that they behave as a filling material packed into the collagen network and that they orientate and perhaps cement together the collagen fibrils to form fibres (Partridge, 1948). Some similarity is suggested between the function of chondroitin sulphate in the production of normal tissue fibres and that of silicic acid in the production of pathological fibrous tissue. Silicic acid, in its polymerized form, may orientate collagen fibrils already present in the tissue with the production of the long, thick, collagen fibres characteristic of silicotic tissue. Consequently, an examination has been made of the effects of dilute silicic acid sols on hyaluronidase-treated membranes to determine whether their action is similar to that reported by Day (1950) for chondroitin sulphate.

Methods

Preparation of Silicic Acid Sols.—The sols were prepared by fusing pure powdered silica with sodium carbonate, the quantity of the latter being calculated to give a 0·85% saline solution when the melt was subsequently dissolved in water and neutralized with hydrochloric acid. When more dilute solutions were prepared these stock solutions were diluted with 0·85% saline. Solutions were brought to *p*H 5–5·5 or *p*H 7–7·5. No difference attributable to *p*H was found between the action of the silicic acid in the two ranges. Viscosities of solutions were measured but differences were too small to affect the rate of flow.

Measurements of Permeability.—The method described by Day was closely followed. A sheet of subcutaneous tissue from the flank of a rat is attached to the lower end of a vertical tube of about 5 mm. diameter. The permeability is measured by running saline or other solution into the tube then recording the time required for the meniscus to travel between two marks on the tube, the lower being 4 cm. above the membrane. Hyaluronidase is then added to the saline and the permeability is again measured.

If the original rate of flow is small (e.g. 0·006 ml. per minute) and a large increase in permeability is recorded (e.g. to 0·03 ml. per minute) the preparation is considered suitable. Saline will then flow through the membrane either at a constant or at a steadily decreasing rate.

Experimental Results

A summary of the experimental results is given in the table.

Sol Concentration.—Fresh sols containing 0·04 g. SiO_2 per 100 ml. or more were effective in reducing the permeabilities of membranes. Fresh sols containing 0·008 g. SiO_2 per 100 ml. or less had no

TABLE
EFFECT OF SILICIC ACID ON MEMBRANE PERMEABILITY

Silicic Acid Concentration (g./100 ml.)	Positive (No. of Experiments)	No Effect (No. of Experiments)
Fresh sol		
0·2	11	4
0·08	7	0
0·04	2	0
0·008	2	8
0·004	0	3
0·002	1	6
Sol aged for 2 hr.		
0·2	10	0
0·08	3	0
Sol aged for 24 hr.		
0·08	7	0
0·04	2	0
0·008	10	0
0·002	4	0

detectable effect. The method is not suitable for the detection of quantitative differences between the effects of different concentrations. Fig. 1 shows the effects of a series of aged sols applied to a single membrane. It is impossible to state positively that the more concentrated sols are more effective in reducing the permeability of the membrane.

Polymer Size.—The effect of polymer size on the ability of sols to reduce the permeability of the membrane was studied by using sols which had been allowed to polymerize for different lengths of time. Polymerization is most rapid at about *p*H 5·5–6 but is very slow in strongly acid or alkaline solutions. The *p*H of alkaline sols was brought to this value. In some cases the sols were used immediately but in others after a measured time interval.

Aged sols were found invariably effective even in concentrations as low as 0·002 g. SiO_2 per 100 ml. Fresh sols were ineffective at this concentration but, as the concentration was increased, showed increasing effectiveness. In a number of cases aged and fresh sols of the same concentration were applied consecutively to the same membrane. The fresh sol was invariably much less effective in reducing permeability than the aged sol (Fig. 2). No difference was detected between sols aged for two hours and others aged for 24 hours.

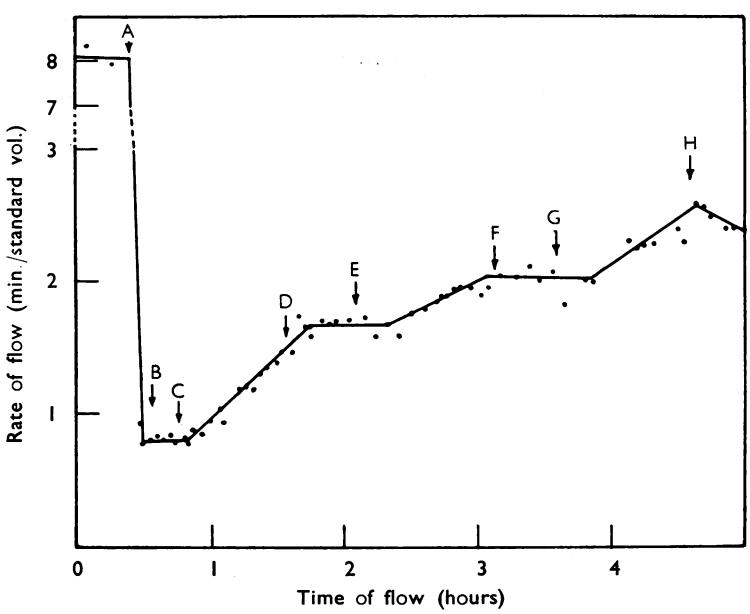


FIG. 1.—Effects of silicic acid on a hyaluronidase-treated membrane.
A=hyaluronidase, B=saline, C=silica sol, 0·002%, aged 24 hours, D=saline, E=silica sol, 0·004%, aged 24 hours, F=saline, G=silica sol, 0·008%, aged 24 hours, H=saline.

Discussion

Silicotic lesions are produced by particles of free silica acting on normal lung tissue. It is still uncertain whether silicates can produce a similar tissue reaction; there is increasing evidence that certain silicates are effective although the reaction they induce is much less intense. Certainly some siliceous dusts which are inhaled in heavy concentrations do not produce silicotic lesions; cement is one example.

It is no longer supposed that silica particles have a direct action on the tissue but rather that silicic acid, produced by the dissolution of the dusts in the tissue fluid, is the direct causative agent. The extensive studies of King (1947) on the solubilities of siliceous materials were largely responsible for the wide acceptance of this solubility theory. King himself emphasized, however, that while the larger part of his data supports such a theory in that more dangerous siliceous dusts are found to be more soluble in water and in physiological fluids, there are many anomalies. Substances which have very high solubilities are apparently harmless but amongst substances in a lower solubility range sandstone, which is known to give a highly dangerous dust, yields less silica in solution than do shales, the toxicity of which is low. Apparently the solubility theory gives a working hypothesis, but other factors may in some instances intervene, and the degree

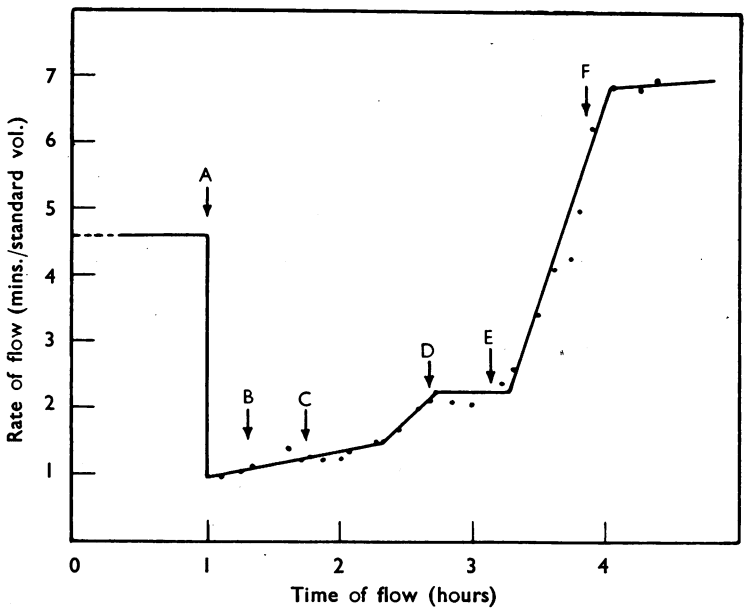
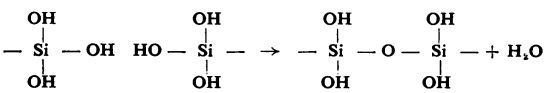


FIG. 2.—Effects of silicic acid on a hyaluronidase-treated membrane. A=hyaluronidase, B=saline, C=fresh silica sol, 0·2%, D = saline, E=silica sol, 0·2%, aged 2 hours, F=saline.

of pathogenicity of a dust may then be completely different from that which would be deduced from its solubility alone.

Our experimental work has shown that silicic acid sols, so dilute that they have viscosities little different from that of saline, contain particles capable of reducing the permeability of tissue which has been treated with hyaluronidase. This indicates that in forming fibrotic tissue silicic acid may act as a filling material in the collagen network and may organize the fibrils, acting in a manner analogous to mucopolysaccharides. The inactivity of sols which had been allowed to polymerize for only a short time would then mean that silicic acid polymers below a limiting size are incapable of initiating fibrosis. This hypothesis of the formation of silicotic tissue requires not only the solution of the dust but also subsequent polymerization of the silicic acid which is formed.

During the last 25 years knowledge of the chemistry of silicic acid has increased rapidly. The statement made in Gye and Purdy's (1922) classic contribution to the silicosis problem that two distinct types of silicic acid exist is now known to be erroneous. Silica dissolves in water to produce orthosilicic acid, $Si(OH)_4$, a substance which begins immediately to polymerize (Willstätter, Kraut, and Lobinger, 1929). It is generally believed (Carman, 1940), that the polymerization is a condensation, proceeding thus :



Polymerization, which can continue until very large molecules are formed, is most rapid at *p*H 5·5–6 and the rate quickly decreases either side of this value. This was shown by Treadwell (1935) who measured the time necessary for sols of silicic

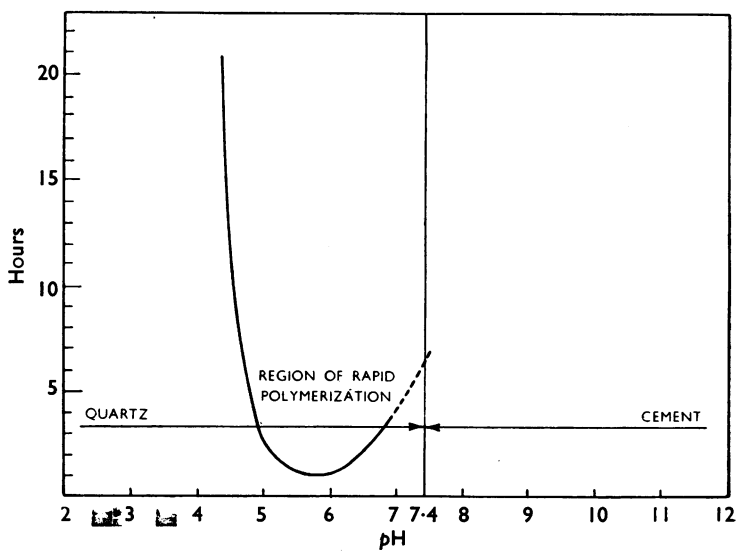


FIG. 3.—Variation with *p*H of the rate of gelling of silicic acid (Treadwell, 1935).

acid at different *p*H values to polymerize to gels. In the light of present knowledge of the chemistry of silicic acid, the probable fate of quartz and cement dusts in the lung will be considered. The effects are represented diagrammatically in Fig. 3, which includes Treadwell's measurements on the polymerization of silicic acid.

When a silica particle makes contact with tissue fluid in the lung the particle dissolves and orthosilicic acid is formed at a very low *p*H. The *p*H has been studied (Elton and Benton, 1953) by measuring the rise in the conductivity of conductivity water when in contact with powdered vitreous silica. The bulk solution has a *p*H of 4·8–5·1 corresponding to a surface *p*H of 1·8–2·1, assuming an electrokinetic potential at the surface of 177 mv. (Wood, 1946). In pure water the change in *p*H from about 2 at the surface of the particle to that of the bulk solution occurs over a distance of about 0·1 to 0·2 microns, but over a smaller distance in salt solutions. At the low *p*H silicic acid polymerizes extremely slowly. The small orthosilicic acid molecules immediately formed by dissolution of silica will diffuse rapidly from the silica particle. In the heavily buffered tissue fluid the *p*H is brought rapidly to 7·4, the silicic acid passing through the region of rapid polymerization. Large polymers will then occur a short distance from and circumferentially around the silica particles, but as the rate of diffusion of a particle in solution is inversely as its size, only the smaller polymers will diffuse beyond this region. The expected distribution of the silicic acid in the zones around the dust particle will then be (Fig. 4) a central zone, B, immediately surrounding the silica particles containing orthosilicic acid and low polymers, a surrounding area, C, containing highly polymerized silicic acid and an outer zone, D, in which smaller polymers exist in lower concentration.

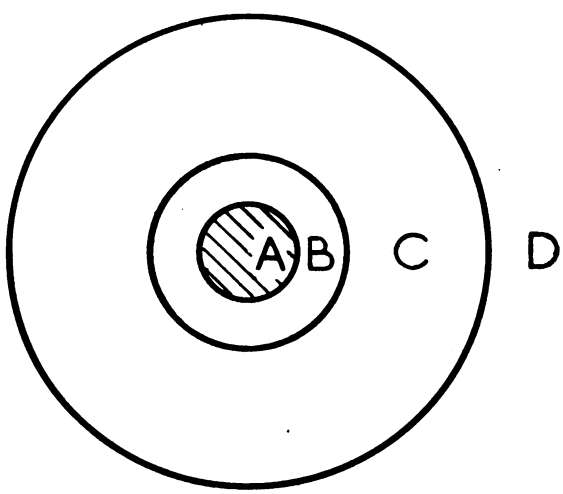


FIG. 4.—Distribution of silicic acid around a silica particle.
A=silica particle, B=small polymers of silicic acid, C=complex polymers of silicic acid, D=low concentration of smaller polymers.

These zones of silicic acid exactly follow the arrangement of the collagen fibres in the silicotic nodule. Middleton (1921) described the concentric arrangement of fibres around the dust particle. "The densest fibrous tissue is near to the centre (zone C) and is surrounded by a less dense region of younger fibrous tissue (zone D). The centre of the nodule (zone B) is looser in texture, less definite in structure and has the appearance of breaking down in a way which suggests what occurs in tumours." Middleton suggested that the centre was necrosing.

Cement dust has not been shown to cause fibrosis but it dissolves *in vivo* (Holt, 1950) and yields a high concentration of silicic acid. Cement is an alkaline dust. The silicic acid which it releases in the tissue is brought to *p*H 7·4 from a higher *p*H value. At no time does the silicic acid enter the *p*H range in which polymerization is rapid, and consequently the polymers necessary for the production of collagen fibres are not formed. The high concentration of low molecular weight silicic acid is eliminated through the kidneys or is detoxicated by mechanisms discussed earlier (Holt and Yates, 1953).

By considering polymerization as a factor in the process of fibrogenesis initiated by silicic acid, it is possible then to explain the contrast between the pathogenic nature of silicic acid formed *in vivo* from dissolving silica and the inertness of silicic acid formed by the dissolution of cement. The extension of this theory to other dusts will require an examination of the surface properties of other siliceous substances.

Summary

Silicic acid is capable of reducing the permeability of membranes treated with hyaluronidase. Its function in fibrogenesis is considered to be analogous to that of the mucopolysaccharides. By considering that the formation of fibrotic tissue requires (1) the dissolution of dust in the tissue fluid, and (2) the polymerization of the silicic acid formed, an explanation is given of the pathogenic effects of free silica dust and the harmless nature of cement dust.

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