

## GEL FILTRATION OF ULTRA-MICROSCOPIC AND OTHER PARTICLES: THE ACTION OF ASBESTOS.<sup>1</sup>

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IN 1909 (at the Congress on Hygiene at Leeds) the writer demonstrated that a freshly prepared layer of aluminium hydrate was capable of acting as an efficient filtering or separating medium, not only towards ordinary micro-organisms and other substances microscopically visible in water, but also, within certain modified limits of time and rate of flow, towards rather a wide range of substances, comprising albumin, tetanus toxin, or dye-stuffs such as methylene-blue, when present in watery vehicle (Hayes, 1910<sup>1</sup>).

With regard to efficiency in filtration certain desiderata apply. The hydrate must be freshly prepared and allowed to settle on some supporting layer such as filter paper or fine sand. [In this connection it should be noted that sea-sand is inapplicable because of the tendency to surface caking in the presence of dilute saline solutions.] The hydrate must not be allowed to become dry, and a head of wash-water must be gently maintained for several hours to wash the hydrate comparatively free of the admixed and contaminating electrolyte. The presence of the latter appears to be unavoidable in whatever manner the hydrate is prepared. Throughout the experiments the hydrate is obtained by interaction between solutions of sulphate of alumina, containing 18 per cent. by weight of  $\text{Al}_2\text{O}_3$ , free from uncombined sulphuric acid, and ammonium hydrate. The contaminating electrolyte is therefore ammonium sulphate.

The gelatinous hydrate is collected and placed in a layer 1 inch deep upon filter paper in a 6-inch funnel. Table I. shows the difficulty of freeing the hydrate from electrolyte by means of continuous washing with a stream of low-conductivity tap-water, so maintained that the hydrate remains as an undisturbed and settled layer.

The graphic representation of the results in Table I. plotted with reference to time and electro-conductivity shows a "die-away" curve.

Aluminium hydrate, however, must be regarded as a changing substance showing continual shrinkage (*syn. synæresis*: Graham, 1844) associated with alteration of physical constitution.

In the experiment here recorded the rate of filtration, with constant

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TABLE I.

| Liquid Tested.               | Total<br>Duration of Washing<br>(Minutes). | Electro-conductivity<br>(Reciprocal Megohms,<br>cm. <sup>-1</sup> ). |
|------------------------------|--|--|
| Filtrate, 1st test . . . . . | 15   | 30,000   |
| „ 2nd „ . . . . .            | 30   | 20,000   |
| „ 3rd „ . . . . .            | 45   | 15,000   |
| „ 4th „ . . . . .            | 67   | 9,000  |
| „ 5th „ . . . . .            | 110  | 1,750  |
| „ 6th „ . . . . .            | 130  | 720  |
| „ 7th „ . . . . .            | 153  | 550  |
| „ 8th „ . . . . .            | 210  | 340  |
| Control tap-water . . . . .  | ...  | 95   |

head of wash-water, is constantly diminishing. Sooner or later the rate of filtration increases suddenly and without warning. Deep fissures appear in the layer of hydrate, while the conductivity of the filtering liquid is practically that of the wash-water used: filtration has ceased. If distilled water be used in place of tap-water the onset of fissuring does not appear to be appreciably delayed.

The hydrate of alumina used as a filtering medium between the second and third hours after formation yields excellent results.

With regard to bacteria: 300 c.c. of tap-water artificially contaminated with *B. coli* to the extent of eight millions per c.c. yielded a sterile filtrate.

Dilute saline watery solutions, containing substances such as albumin, tetano-toxin, methylene-blue, yield filtrates apparently free from organic material. In the case of tetano-toxin the whole of the toxin was recovered from the hydrate by maceration with watery alcohol (Table II.).

TABLE II.

|   | Minimum<br>Lethal<br>Dose. | Biuret<br>Reaction<br>Albumose. | Comparative<br>Electro-<br>Conductivity. |
|---|----------------------------|---------------------------------|--|
|   | c.c.                       |                                 |  |
| Control: Tetano-toxin in saline solution—<br>Before filtration through $\text{Al}(\text{OH})_3$ . . . . . | 0.1                        | Marked                          | 15.5                                     |
| First portion of filtrate . . . . .   | >5.0                       | Nil                             | 30.0                                     |
| Second portion of filtrate . . . . .  | >5.0                       | Nil                             | 29.5                                     |
| Material obtained by subsequent treat-<br>ment of the $\text{Al}(\text{OH})_3$ . . . . .                  | 0.1                        | Marked                          | 19.5                                     |

It is obvious that in the foregoing experiment the hydrate had been but imperfectly freed from the contaminating electrolyte ( $\text{Am}_2\text{SO}_4$ ).

The effect of transuding quantities of normal urine through undisturbed and partially washed layers of the hydrate was next

tested. In Table III. results of three experiments, A, B, and C, are shown. A and B are approximately parallel tests, the urine being divided into two parts, and each portion filtered through filters A and B respectively.

The filtrate from A was collected and tested whole, while from B the fractions were collected in equal volume and tested serially. The periods stated against the several fractions illustrate the increasing difficulty in permeation. The experiments were concluded before fissuring appeared.

In Experiment C the hydrate from funnel B was collected, distilled water added to make up the volume equal to that of the combined filtrate, and shaken up. Thereafter the turbid liquid was poured into a cylinder, and after settlement the clear top and turbid bottom layers were separately tested.

TABLE III.

|   | Colour.          | Reaction. | Specific Gravity. | Urea per cent. | Phosphates per cent. | Chlorides per cent. | Electro - Conductivity (Reciprocal Megohms cm. <sup>-1</sup> ). | Time (Minutes). |
|---|------------------|-----------|-------------------|----------------|----------------------|---------------------|---|-----------------|
| Control urine . . . . .   | Straw            | Acid      | 1019.0            | 2.02           | 0.145                | 0.7                 | 11,000  | ..              |
| A. Combined filtrate through funnel A                                 | Pale straw       | Neutral   | 1014.0            | 1.63           | <0.01                | 0.4                 | 9,000   | ..              |
| B. Fractions of filtrate through funnel B—                            |                  |           |                   |                |                      |                     |   |                 |
| First fraction . . . . .  | Colourless       | Neutral   | 1001.0            | 0.0            | Trace                | Trace               | 380   | 73              |
| Second " . . . . .  | "                | "         | 1003.5            | 0.4            | "                    | "                   | 2,520   | 30              |
| Third " . . . . .   | "                | "         | 1008.0            | 1.05           | <0.01                | 0.28                | 6,500   | 34              |
| Fourth " . . . . .  | Very faint straw | "         | 1010.0            | 1.3            | <0.01                | 0.34                | 8,000   | 35              |
| Fifth " . . . . .   | Faint straw      | "         | 1010.0            | 1.5            | <0.01                | 0.4                 | 9,000   | 45              |
| Sixth " . . . . .   | Pale straw       | "         | 1010.0            | 1.55           | <0.01                | 0.4                 | 9,000   | 45              |
| Seventh " . . . . .   | "                | "         | 1010.0            | 1.55           | <0.01                | 0.4                 | 9,000   | 45              |
| C. Al(OH) <sub>3</sub> . Ex-funnel B, shaken up with distilled water— |                  |           |                   |                |                      |                     |   |                 |
| Top clear layer . . . . .   | ..               | ..        | 1002.0            | 0.375          | ..                   | ..                  | 2,900   | ..              |
| Bottom turbid layer, containing settled Al(OH) <sub>3</sub> }         | ..               | ..        | 1002.0            | 0.61           | ..                   | ..                  | 3,500   | ..              |

The transuding urine must necessarily displace all liquid mechanically retained in the hydrate, and the explanation of the analysis, more particularly the electro-conductivity of the first fraction through funnel B, may be found along these lines.

The differential rate of increase of the various saline and organic constituents of the urine in the subsequent fractions, however, cannot be so explained. There would appear to be a loose combination between the hydrate and urea, as appears from the results of Experiment C, and similarly with the chlorides. Pigmentation of the hydrate occurs, which is most marked in the superficial layers. The practically complete disappearance of the phosphates may be due to

the formation of insoluble aluminium phosphate by interaction of the acid urine upon the hydrate, the transudate becoming neutral.

Fissuring of the hydrate in funnels A and B respectively commenced *immediately after the cessation of the experiments*.

To prevent fissuring the following methods were attempted:—

(a) The hydrate was confined in a filter funnel between two folded cones of filter paper, in the form of an interlamellar layer. This method has the advantage that the layer of hydrate is not readily disturbed by alteration of flow of liquid into the funnel. It was thought that contraction of the hydrate would result simply in an approximation of the upper towards the lower parallel cone of filter paper. Nevertheless fissures continued to develop and the experiments proved abortive.

(b) By admixture of the hydrate with alizarin paste. This method appeared to increase rather than diminish the difficulties.

(c) By admixture of the hydrate with comparatively inert fibrous substances. In this connection the material used was a floss asbestos obtained from the Styrian and Tyrolese districts.

This method apparently yielded all the desired results, save that, as time proceeded, there was an enormous diminution in the rate of filtration.

It became necessary in this connection to test the action of the fibre independently, on the various materials included in the scope of these experiments, and, as an outcome, a number of observations were made, which go to show that asbestos fibre possesses a number of activities which stamp it as anything but an indifferent and inert substance.

#### THE PROPERTIES AND ACTIONS OF ASBESTOS.

Attempts were made to obtain the fibre in a pure form. It was found, however, that distilled water, even after treatment prolonged over several months, continues to act readily upon the fibre, as may be easily shown by electro-conductivity tests of the liquid in which the fibre is boiled or steeped at temperatures below 20° C.

To treat asbestos fibre, which is mainly a hydrated silicate of magnesia, lime, or iron, with boiling mineral acids is to produce a material consisting mainly of silica, and the result is scarcely entitled to the appellation "asbestos."

Similarly, to heat the silicate to a cherry-red heat is to convert a fibrous material of silken lustre to an anhydrous, dull, readily friable, amorphous mass, which, as subsequent tests showed, possesses qualities distinguishing it from the original untreated fibre.

Unless otherwise stated, the raw mineral, finely divided or carded, has been used, and the investigation has been extended so as to include the activities of the main varieties of the "asbestos" group of minerals (see Appendix): Ural, Italian, or Cape (or blue) asbestos, chrysotile-asbestos of Quebec, and, in addition, the steatites, meerschauum, and animal charcoal, as exhibited towards toxins, including those derived from *B. diphtheriæ*; *B. tetani*, *B. tuberculosis*, and *B. mallei*, certain antitoxic sera, specific agglutinins, certain proteins, pigments and colloids including starch solution, diastase, cobra venom, complement and amboceptor of a hæmolytic system.

All these substances apparently readily disappear from dilute watery solution, or are rapidly changed in character when brought into intimate contact with finely divided minerals of the asbestos group, while watery agar,

watery gelatin, and watery isinglass, after similar treatment lose the power of setting: the interaction with certain varieties of finely divided meerschaum is rapid and intense.

In the case of the ferment diastase, the liquid, after being steeped with the fibre, appears to be inert, and the biuret reaction becomes negative. The separated fibre on being steeped in starch solution, and incubated at 37° C., appears to convert the starch rapidly to sugar, and presumably the fibre has retained the ferment in active form.

With toxins of such a strength that 0.1 c.c. contains a minimum lethal dose, the liquid after being steeped with asbestos fibre for but a few seconds appears to be quite inert and the biuret reaction disappears. In the case of more concentrated toxins, such as diphtheria and tetanus, the latter disappears from the solution more readily than the former in parallel conditions of experiment.

A hæmolytic immune serum placed in contact with finely divided asbestos fibre loses its hæmolytic principle: normal serum similarly treated becomes inactivated, but the former action is slower than the latter. The differential rate of action is better marked in the case of asbestos fibre which has been previously heated to a dull red heat.

A differential rate of action is seen in the behaviour of asbestos fibre, which was subjected to no treatment other than that of simple carding towards Koch's old unconcentrated tuberculin. In this instance, proteose is removed entirely, the expressed liquid, which yields a negative biuret reaction, producing typical cutaneous reactions in tuberculous subjects. This appears to offer a comparatively simple process for obtaining proteose-free tuberculin. A differential action is readily exhibited towards mixtures of dye-stuffs, of which eosin and methylene-blue form a convenient example, the latter readily disappearing from the solution.

The writer does not consider that the term "simple adsorption" suffices to explain adequately the phenomena under consideration.

In the presence of distilled water asbestos fibre is continually being acted upon, and substances in the form of electrolytes pass into solution, while the electro-conductivity of the liquid constantly rises. If the material passing into solution possessed the composition of asbestos, the process might be regarded as the simple solution of material soluble only with difficulty, or, alternatively, the solution might even be regarded as the sol or colloidal form of a solid gel, otherwise "asbestos." The electrolytes present in solution have neither the composition nor the proportions to justify either of these assumptions, and it appears to be more reasonable to regard the action of distilled water upon finely divided asbestos as one of simple chemical degradation.

The rate of degradation appears to be greatly altered when the degrading liquid contains substances in suspension or solution, which, as a result of contact of the liquid with the asbestos, either disappear from the liquid or are altered in form; in short, "simple adsorption"

is a phenomenon associated with some alteration in the rate of degradation or discharge of energy on the surface of the "adsorbing" material.

Table IV. gives the results in a specific test.

TABLE IV.

| Material.  | Electro-conductivity<br>(Reciprocal Megohms<br>cm. <sup>-1</sup> after Twenty-<br>four Hours' Contact). | Comparative Intensity<br>of Biuret Reaction<br>of expressed Liquid. |
|--|---|---|
| (a) Distilled water . . . . .                                  | 3.2   | Nil   |
| (b) Witte's peptone 0.2 per cent. in (a) .                     | 102.4   | 100   |
| (c)=(a) + 50 per cent. by weight of Ural<br>asbestos . . . . . | 336.0   | Nil   |
| (d)=(b) + 50 per cent. by weight of Ural<br>asbestos . . . . . |   |   |
|  | 620.8   | < 10  |

The results obtained with dialysed iron and methylene-blue respectively are of a different order (Table V.), which again differ from those obtained with colloidal gold.

TABLE V.

| Material.                               | Electro-conductivities. |  |
|---|-------------------------|--|
|   | Control.                | After 15 Minutes'<br>Contact with Half<br>Weight Asbestos. |
| Distilled water . . . . .               | 3.2                     | 317.8  |
| Dialysed iron (B.P.), 2 per cent. . . . | 501.8                   | 400.2  |
| Methylene-blue, 0.01 per cent. . . .    | 230.4                   | 134.4  |
| Colloidal gold . . . . .                | 258.8                   | 345.2  |

In each of the above tests the colloid appears to be entirely deposited upon the fibre, forming perhaps a protective coating and so altering the rate of degradation.

In the case of solutions containing salts of radium, thorium, uranium, and lead, placed in contact with asbestos fibre, the metals readily disappear from the solution and are apparently replaced by bases derived from the fibre, which, in the case of radium and thorium, become radio-active to the degree corresponding with the amount removed from the solution. The action of the salts of other metals is under investigation.

Dilute mineral acids or alkalis appear to be neutralised when placed in contact with asbestos, presumably by interaction respectively with the basic and acid substances present in the fibre.

Further toxin-producing organisms grown in suitable media, to which asbestos fibre has been added, yield fluids comparatively toxin-free, and particularly is this the case with *B. diphtheriae*. In a series of experiments conducted with a variety of this organism which in eight days at 37° C. produces a toxin containing 200 M.L.D. per c.c. in broth culture, corresponding broth cultures to which 7½ per cent. weight of asbestos had been added yielded a fluid 5 c.c. of which was without toxic effect in an appropriate biological test. The amount of toxin produced in the presence of asbestos is therefore diminished by over 99·9 per cent., and it has been found that the organism when transplanted to a secondary simple broth medium has had its toxin-producing power impaired by over 90 per cent.

*B. pestis*, *B. anthracis*, and *B. tuberculosis* have, when grown in fluid media to which asbestos has been added, been found to have a diminished virulence. With such a culture of *B. tuberculosis* (human), the expressed liquid, while proteose-free, contains marked quantities of the specific anaphylactic substance (Lyons, 1913<sup>2</sup>).

The action of finely powdered and fibrous substances such as kaolinite, spun glass, the vegetable fibres (jute, flax, cotton, and ramie), silk and wool, and in addition animal charcoal, has been under investigation in a parallel series of experiments which, in many instances, have yielded somewhat comparable results, although the writer does not think that, for convenience of handling, any of these substances offer the advantages of asbestos fibre, which readily exposes so much surface and requires no shaking apparatus. If the asbestos fibre be sufficiently finely divided and of the appearance of floss silk, the results obtained appear to excel those yielded by other substances.

As an outcome of the results following the interaction between certain radio-active substances in solution, and members of the asbestos group of minerals, the investigation is being extended to include the felspathic and plagioclasic granites, which, on weathering, give rise to complex hydrated silicates known as Zeolites (Coplans, 1913<sup>3</sup>).

The virulence of *B. anthracis* becomes markedly impaired, after a variable period, when grown in liquid nutrient medium to which artificial sodium zeolite has been added.

Both the artificial sodium and manganese zeolites offer the opportunity of studying the effect of nutrient liquid deprived of salts of calcium and magnesium or of iron upon the general activity of organisms, the calcium and magnesium salts being replaced by salts of sodium, while the iron, by a process of oxidation, is precipitated as colloidal ferric hydroxide.

Since the foregoing experiments were carried out, the writer has, in conjunction with W. Gibbs Lloyd (1913<sup>4</sup>), extended the observa-

tions to a number of animal and plant physiological substances. Dilute solutions of alkaloids such as curare and strychnine, hormones such as adrenalin, and various ferments readily lose their active principle in the presence of asbestos fibre.

In like condition of experiment a differential action has been noted with pancreatic ferment, for, after treatment, trypsin alone remains in solution in reduced quantities.

In connection with dilute peptone solution (Witte's peptone, 0.2 per cent. in distilled water), a peculiar oscillatory phenomenon has been noted. At one time, in the presence of Ural asbestos, the expressed liquid has yielded an entirely negative result with the biuret test, to be followed a few hours later by a slightly positive test when the newly expressed liquid is again tested. Still later, with a third test the result is again entirely negative. We are unable to offer any explanation at present of this phenomenon, which does not appear to be constant.

It was thought, with reference to starch solutions, that asbestos fibre exercised a slow catalytic action, converting the starch by easy stages to erythro-dextrin, achroo-dextrin, and later, reducing sugar, but further experiments conducted with a rigid regard for sterility have not confirmed these early observations.

In this connection the writer has noted that on account of the low heat conductivity of asbestos unsatisfactory results must be expected, if intermittent sterilisation at 100° C. be conducted for any period less than eight hours in connection with liquids containing 5 per cent. or more by weight of asbestos fibre in suspension.

S. Wyard (1914<sup>5</sup>) has since noted, while investigating the action of asbestos fibre on the components of a simple hæmolytic system, that the fibre itself is capable of acting as a rapid hæmolytic agent towards a suspension of sheep's corpuscles in normal saline. His remaining results confirm generally the early observations made by the writer.

The investigation upon the action of asbestos upon radium and thorium in solutions of their salts has been extended by S. A. Edmonds (1913<sup>6</sup>).

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APPENDIX.

CHEMICAL RELATIONSHIPS OF "ASBESTOS" MINERALS.

"Asbestos" = Following Three Groups.

1. Anthophyllite Group [(MgFe)SiO<sub>2</sub>].
2. Amphibole or Hornblende [RO.SiO<sub>2</sub>], where RO may represent CaO, MgO, FeO, etc.
- (a) TREMOLITE [3MgO.CaO.2SiO<sub>2</sub>].
  - (b) ACTINOLITE [3(MgFe)O.CaO.2SiO<sub>2</sub>].
  - (c) HORNBLende ASBESTOS, HYDRATED. (ITALIAN ASBESTOS.)
  - (d) MOUNTAIN LEATHER, MOUNTAIN WOOD, MOUNTAIN CORK.
  - (e) CROCIDOLITE (BLUE OR AFRICAN, WEST GRIQUALAND) [NaFe(SiO<sub>3</sub>).FeSiO<sub>3</sub>].
3. The Serpentine Group (3MgO.2SiO<sub>2</sub>.2H<sub>2</sub>O).
- (a) PICROLITE (CANADA).
  - (b) CHRYSOTILE-ASBESTOS (CANADA-QUEBEC).
  - (c) TALC.

Meerschaum = H<sub>2</sub>Mg<sub>2</sub>(SiO<sub>3</sub>)<sub>3</sub>.H<sub>2</sub>O.

Natural Zeolite : Natrolite = Na<sub>2</sub>O.Al<sub>2</sub>O<sub>3</sub>.3SiO<sub>2</sub>.2H<sub>2</sub>O.

Artificial Zeolites = (Trade Synonym : "Permutit").

GENERAL FORMULA = X<sub>2</sub>O.Al<sub>2</sub>O<sub>3</sub>.3-4SiO<sub>2</sub>.YH<sub>2</sub>O.

Sodium Zeolite = Na<sub>2</sub>O.Al<sub>2</sub>O<sub>3</sub>.2SiO<sub>2</sub>.6H<sub>2</sub>O.

N.B.—Kaolinite = Al<sub>2</sub>O<sub>3</sub>.2SiO<sub>2</sub>.2H<sub>2</sub>O.

Proximate Composition of "Asbestos" Minerals (Dana).

|  | TREMOLITE. | ACTINOLITE. | CROCIDOLITE. | CHRYSOTILE. | TALC. | PICROLITE. |
|--|------------|-------------|--------------|-------------|-------|------------|
| SiO <sub>2</sub> . . . . .               | 57.7       | 61.82       | 49.6         | 40.87       | 59.59 | 37.88      |
| Al <sub>2</sub> O <sub>3</sub> . . . . . | ...        | 1.12        | ...          | 0.9         | 1.76  | 1.10       |
| FeO . . . . .                            | ...        | 6.55        | 19.8         | 2.81        | 0.79  | 0.36       |
| Fe <sub>2</sub> O <sub>3</sub> . . . . . | ...        | ...         | 22.0         | 2.81        | ...   | 2.7        |
| MgO . . . . .                            | 28.9       | 23.98       | ...          | 41.5        | 32.92 | 43.29      |
| CaO . . . . .                            | 13.4       | 1.63        | ...          | ...         | 0.59  | 0.82       |
| Na <sub>2</sub> O . . . . .              | ...        | ...         | 8.6          | ...         | 0.56  | ...        |
| H <sub>2</sub> O . . . . .               | ...        | 5.45        | ...          | 13.55       | 3.79  | 14.52      |

*Humidity Tests.*

FOR RELATIVE ABSORPTION, AS COMPARED WITH CONDITION AT 103° C.

| PROCESS.  | TEMP. (° C.). | TIME (Hours). | CHANGE<br>(per cent.). | CHRYSOILE. | RUSSIAN. | CROCIDOLITE. |
|---|---------------|---------------|------------------------|------------|----------|--------------|
| (1) Drying . . . . .  | 120           | 3             | Loss . . . . .         | 0·49       | 0·48     | 0·19         |
| (2) Exposure to saturated at-<br>mosphere. Moisture = 50<br>grms. per cubic metre } | 40            | 3             | GAIN . . . . .         | 17·13      | 28·86    | 12·12        |
| (3) Drying . . . . .  | 40            | 3             | Water retained .       | 0·33       | 0·34     | Nil          |
| (4) Like (2). Moisture = 10<br>grms. per cubic metre }                              | 13            | 12            | Water absorbed .       | 2·47       | 19·28    | 0·24         |