ON THE TRANSMISSION OF AIR AND MICRO-ORGANISMS THROUGH BERKEFELD FILTERS.

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Plate I and one text figure.

In several former experiments by Bulloch and Craw (1906), Bulloch, Craw and Atkin (1908), and Craw (1908), we determined the relative efficiency of various filters in keeping back micro-organisms. The filters tested were the Doulton ("white" and "brown"), the Chamberland ("F" and "B"), the Berkefeld, and the Slack and Brownlow. Our methods of experimentation embraced filtration of cultures of microbes in bouillon, and the filtration of tap water under constant and variable pressures. The net result was that all the Slack and Brownlow, and nine out of ten of the Berkefeld filters employed gave contaminated filtrates within 15 minutes of the time the filtration experiment was commenced. Seven out of the ten Doulton "white" filters gave sterile filtrates for at least three days.

As the tap water pressure varied, in a few seconds, from 32·5 lbs. per square inch to zero the test was a severe one, for Craw (1906) had previously shown that even the finest gelatine filters, suggested by C. J. Martin, were very permeable under sudden variations of pressure.

All the filters employed in our experiments were subjected to the same treatment before testing, viz. sterilisation in the autoclave at 120° C. for one hour. The results which we obtained with the Berkefeld filter were so divergent from those in the experiments made for the *Lancet* by Professor Sims Woodhead and Dr Cartwright Wood (1894

and 1898) that before the publication of our paper one of us (W. B.) communicated with Professor Woodhead to ascertain whether he could offer any likely explanation of the difference. He was unable to do so.

It would appear however that after January 1902 some alteration took place in the manufacture of the Berkefeld filter and although the "new cylinder" is described as being much finer in grain and firmer and more uniform in texture than the "old one" it is possible that from a bacteriological point of view it is not so good.

Considerable dissatisfaction appears to have arisen in reference to our conclusions on the Berkefeld filter, for in a letter sent to one of us (B.) and dated "Celle, Prov. Hannover, 28. viii. 08" Dr H. Nordtmeyer, the inventor or originator of the Kieselguhr Filtersystem-Nordtmeyer-Berkefeld, pointed out that he had already (1891) drawn attention to the fact that the "filters being very bad conductors of heat must not be sterilised by steam or dry heat but should be placed in a vessel with cold or tepid water and boiled for about an hour." He added that by autoclaving at 120° C. the cylinders were probably cracked, and that although the cracks were not visible, they would allow the passage of test organisms. He avers that the passage of microbes into the filtrates in Professor Pfuhl's (1903) experiments was referable to this cause. In conclusion Dr Nordtmeyer suggested that his partner should call upon us to "fully explain all the points in question."

To Dr Nordtmeyer's letter an answer was directed by one of us (B.) to the effect that if he had doubts as to the accuracy of our experiments or had criticisms to offer, the recognised procedure for him would be to write to the *Journal of Hygiene* or other scientific journal and that when he had done so we should make a suitable reply. We added that we had dealt with the question entirely from a scientific point of view and had no connection whatsoever with any commercial aspects of the case. A letter from the Managing Director of the Berkefeld Filter Co. Ltd., London, dated Sept. 14, 1908, was answered in like strain.

Early in November 1908 we received from Professor Nuttall a letter enclosing another from Dr Andrew Wilson, which letter is published in this number of the *Journal of Hygiene* (p. 33), and it is as an answer to this letter and that of Dr Nordtmeyer that the following new experiments have been undertaken with the Berkefeld filter.

It may be added at once that our previous conclusions have been completely confirmed although the new tests were carried out under conditions enormously *more* favourable to the Berkefeld filter than our former ones, the pressure being about $\frac{1}{40}$ of that previously used.

The objection raised by Drs Nordtmeyer and Wilson seems to be that in our former investigations all the filters were sterilised in an autoclave at 120° C., so that there was a possibility that the difference in coefficients of expansion of the nipples or joints and the filter mass might give rise to cracks in the cement used to unite the two parts of the filter. We have therefore investigated a few more Berkefeld filters sterilised in a manner approved by Dr Nordtmeyer, Dr Andrew Wilson and the Berkefeld Filter Co. Ltd. The filters were placed in a clean vessel with cold or tepid water and boiled for an hour. After being boiled the filter was allowed to cool.

In order to ascertain whether there is any evidence that cracks are produced in the filter while being sterilised in the autoclave at 120° C. we applied a method of testing by air pressure, and we subjoin the results which we obtained with the old filters used in our previous experiments (and which had again been autoclaved at 120° C.) and a series of new filters never previously used for any purpose. For the most part these were purchased from Messrs Baird and Tatlock who obtained them from the Berkefeld Filter Co. Ltd., Oxford St., London. A few were obtained from the Army and Navy Stores.

Whether Berkefeld filters are tested in any way before being placed on the market we do not know, but the air pressure test is frequently used in laboratories to determine the existence of cracks, the air coming streaming out as the pressure is applied.

Technique. (Fig. 1.) The pressure applied to the filters was obtained by means of an ordinary bicycle pump *P*, connected to a pressure gauge *G* and also to the filter to be tested. The filters were first tested for perfection of joint (position *J*, fig. 1) and subsequently for permeability of wall (position *W*, fig. 1). In the latter case the joint or neck of the filter was maintained at least half an inch above the surface of the water. The pressure was raised to about 8 lbs. per square inch and allowed to fall gradually till leakage of air ceased. The data as regards leakage given below (Table I) refer to the pressures at which the *cessation* of marked air bubbles took place from joint and wall. The reasons for testing the joint first were (*a*) that it allowed transmission of air first and (*b*) that the inverted position (*J*) eliminated the chance that bubbles apparently emanating from the neck were due to a collection of air bubbles from the wall. From Table I it is evident that there is no very striking differences between the unused and used, i.e. sterilised filters, except perhaps that in general the used filters leaked under lower pressures. The worst of all however was Filter No. 1 which transmitted

air freely at the neck at ½ lb. of pressure. This filter had never been used. In no filter autoclaved or not-autoclaved could any evidence be found of fissure or fracture as judged by the streaming of air from one point. A series of six new Doulton filters tested under the same conditions and at the same time withstood pressures of 10, 11, 10½, 14, 13½, and 13 lbs. air pressure without a single bubble being detected coming through the filter.

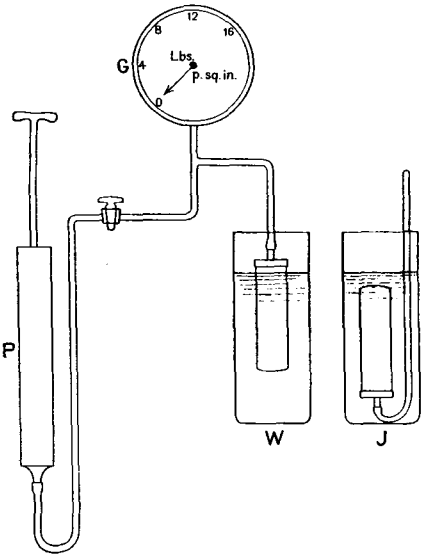

Fig. 1.

Initial Capillary Pressure.

During the application of the air pressure tests an interesting and important phenomenon was observed on immersing vertically in water at room temperature a new, perfectly dry Berkefeld filter. The filters being connected with the pressure gauge the indicator rose to 5—6¼ (see fig. 2) lbs. per square inch *although no pressure was applied from the pump.* This phenomenon we can only ascribe to the displacement of the air within the pores of the filter mass by the capillary attraction the latter exerts upon the water in which it is immersed. A similar rise of pressure was indicated on the gauge even when the filter was immersed horizontally, indicating that the rise of pressure is not hydrostatic in nature.

Autoclaved filters on horizontal immersion in water at room temperature gave an initial or auto-pressure of 2½—3 lbs. per square inch and in two cases out of three showed leakage at the metal joint without applied pressure, a result which was observed to an equal extent with new filters which had never even been boiled.

TABLE I.

Examination of Berkefeld Filters for air transmission.

(Pressure in lbs. per square inch.)

	New unused Filters		Used Filters	
No.	Joint lbs.	Wall lbs.	Joint lbs.	Wall lbs.
1	½	—	—	—
2	3½	4	—	—
3	4	4	—	—
4	—	—	2	3½
5	—	—	2	4
6	—	—	3	4
7	—	—	2½	3
8	—	—	4½	6
9	—	—	4	5
10	—	—	1	2
11	5	7	—	—
12	5	5	—	—
13	6	6	—	—
14	2½	4½	—	—
15	3½	5¼	—	—
16	4	6	—	—
17	6	6	—	—
18	6	6	—	—
19	—	—	4½	4½
20	—	—	2½	2½

Filters 1, 2, 3, 4, 5, 6, 7, 8, 9, 16, 17, 18 had metal tops and were of the kind known as "House Filter F" measuring 5 × 2 in. or 13 × 5 cm.

Filters 10, 11, 12, 13, 14, 15, 19, 20 had porcelain nipples and measured 18 cm. in length and 2½ cm. diameter, i.e. 7¼ × 1 inch.

Direct transmission of microbes in water through Berkefeld Filters—new experiments.

The new filters chosen for experiment were those marked Nos. 13, 14, 16, 17, 18 (Table I).

Nos. 13 and 14 had porcelain ends.

Nos. 16, 17, 18 had metal ends.

TABLE II.

Re-examination of Berkefeld Filters for air transmission, after steeping 24 hours in water at air temperature.

	New Filters		Used Filters	
No.	Joint lbs.	Wall lbs.	Joint lbs.	Wall lbs.
1	½	—	—	—
2	3	3	—	—
3	4	4	—	—
4	—	—	2	3½
5	—	—	1½	4
6	—	—	3	4
7	—	—	3	3
8	—	—	3¾	4½
9	—	—	2	4¼
10	—	—	2	1

The numbers of these filters correspond to those in the previous table and the results give perfectly unbiassed information as to air transmission, the notes of Table I not having been compared until the following day.

The filters, with the neck of the metal case and an indiarubber connecting tube with a hooded pipette (as described in our previous communication), were boiled in a large clean vessel for one hour and allowed to cool. With the greatest care the filter was then inserted into the metal case attached to the tap and screwed home. In order that the tests should be less severe than in our previous communications they were carried out in a perfectly quiet laboratory in which the pressure of water was only ·9 lbs. per square inch (!) (24·5 inches) in comparison with 32·5 lbs. applied in our former experiments. The supply of water was controlled by a cock. In the course of the experiments the water was plated out on gelatine twice and showed from 100—200 colonies per c.c. After the filtration was started, samples of the filtrate measuring 70 c.c. were run into a peptone salt solution so that the whole was ultimately 1 % peptone and ½ % salt solution. The samples were then incubated for several days to determine whether they were sterile or not. During the intervals between the collection of the samples the dropping water from the hooded pipette was carefully guarded from contamination by a long sterile glass tube open at the bottom. The results obtained were as follows.

Examination of Berkefeld Filters for transmission of micro-organisms.

FILTER No. 13. (Porcelain nipple.)

Sterilised by boiling in water for 1 hour at 100° C.
Connected with tap at 2.30 p.m. Dec. 16th, 1908. Pressure ·87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	3 p.m. (16. xii. 08)	9 c.c. per min.	Growth
II	4 p.m. ,,	8 ,,	,,
III	12 noon (17. xii. 08)	7 ,,	,,
IV	4 p.m. ,,	6 ,,	,,
V	11 a.m. (18. xii. 08)	5 ,,	,,

Result: Filtrate contained bacteria within ½ an hour.

FILTER No. 14. (Porcelain nipple.)

Sterilised by boiling in water for 1 hour at 100° C.
Connected with tap at 2.10 p.m. (13. xii. 08). Pressure ·87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	2.15 p.m. (13. xii. 08)	16 c.c. per min.	Sterile
II	5.50 p.m. ,,	10 ,,	Growth
III	11.30 p.m. (14. xii. 08)	10 ,,	,,
IV	4 p.m. ,,	6 ,,	,,
V	11 a.m. (15. xii. 08)	5 ,,	,,

Result: Filtrate contained bacteria within 3 hours.

FILTER No. 16. (Metal end.)

Sterilised by boiling in water for 1 hour at 100° C.
Connected with tap at 2.30 p.m. (7. xii. 08). Pressure ·87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	2.40 p.m. (7. xii. 08)	30 c.c. per min.	Growth
IA	2.50 p.m. ,,	30 ,,	,,
II	3.30 p.m. ,,	28 ,,	,,
III	5 p.m. ,,	27 ,,	,,
IV	2 p.m. (8. xii. 08)	24 ,,	,,

Result: Filtrate contained bacteria within 10 and 20 minutes.

FILTER No. 17. (Metal end.)

Sterilised by boiling in water for 1 hour at 100° C.
Connected with tap at 12.10 p.m. (2. xii. 08). Pressure ·87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	12.15 p.m. (2. xii. 08)	30 c.c. per min.	Growth
II	12.25 p.m. ,,	30 ,,	,,
III	1 p.m. ,,	29 ,,	,,
IV	2 p.m. ,,	30 ,,	,,
V	4 p.m. ,,	29 ,,	,,
	Filter stopped over night, started 12.30, 3. xii. 08.		
VI	12.30 p.m. (3. xii. 08)	29 c.c. per min.	Growth
VII	2 p.m. ,,	29 ,,	,,
VIII	3.30 p.m. ,,	29 ,,	,,
IX	12 noon (4. xii. 08)	24 ,,	,,
X	2.40 p.m. ,,	23 ,,	,,

Result: Filtrate contained bacteria within 5 minutes.

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FILTER No. 18. (Metal end.)

Sterilised by boiling in water for 1 hour at 100° C.
Connected with tap at 2.20 p.m. (10. xii. 08). Pressure ·87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	2.32 p.m. (10. xii. 08)	26 c.c. per min.	Sterile
II	5 p.m. ,,	27 ,,	Growth
III	5 p.m. (11. xii. 08)	17 ,,	,,
IV	12 noon (12. xii. 08)	15 ,,	,,

Result: Filtrate contained bacteria within 2½ hours.

All these five new filters showed contaminated filtrates between 5 minutes and 3 hours of the time filtration was started. These results although carried out under conditions extraordinarily favourable to the Berkefeld bougie were so disastrous to its reputation as a filter that we decided to carry out a still more lenient test—one that we may describe as autofiltration. Under the term "initial capillary pressure" we have already described (p. 38) the peculiar fact that when a dry Berkefeld filter is immersed vertically in water a pressure of 5—6¼ lbs. may be registered on the indicator of the gauge. This led us to observe what is going on inside the filter during the period of immersion by sawing off the metal or porcelain end. On dipping one of these cut filters into water containing a solution of acid fuchsin a large quantity of the fuchsin appeared in the lumen of the filter within a few minutes, and it suggested itself to us that possibly bacteria might come through by "autofiltration" alone. Two filters were chosen, one No. 16 used in the above experiments (p. 41) and No. 15 which had never been used. The outside of No. 16 was carefully brushed and both filters were then dried in a hot air chamber at 54° C. At the end of 24 hours both filters held in vertical position on stands were slowly and carefully lowered into a vessel of water which had been previously inoculated with a culture of *Bacillus prodigiosus* and incubated at 25° C. for two days. The utmost care was taken to prevent the water coming into contact with the cement of the joint which stood nearly 1 inch above the level of the water. The hydrostatic pressure amounted to only 9 cm. of water (0·12 lbs. per sq. inch for the base of the filter and a mean pressure of ·06 lbs. per sq. inch). By means of a series of long, sterile Pasteur pipettes the water which passed into the lumen of the filter was removed and inseminated into bouillon and on agar with the following result.

	Sample	Time after starting	Volume of filtrate	Result
Filter 16.	1	6 mins.	1 c.c.	Sterile
	2	7 ,,	1 ,,	,,
	3	8 ,,	1 ,,	,,
	4	10 ,,	6 ,,	,,
	5	11 ,,	2 ,,	,,

After the removal of the 5th sample 5 c.c. of bouillon was introduced by means of a sterile pipette into the lumen of the filter, the metal end being well flamed by a bunsen, a sterile cotton wool plug was placed in the opening and the filter left in the prodigiosus water over night. Next day a sample of the contents of the filter lumen was plated out on agar and showed numerous colonies of *B. prodigiosus*. Thus while we were unable to demonstrate the *B. prodigiosus* directly in samples as large as 6 c.c. the microbe had apparently passed through and we demonstrated this indirectly. In order to get a large initial volume of filtrate for inoculation we allowed Filter 15—the new filter—to stand in the prodigiosus water for 20 minutes and at the end of this time we abstracted about 35 c.c. from the interior of the filter, inseminating the whole in peptone water. After incubation for three days abundant growth of prodigiosus was proved by plating on agar. Thus the microbe had passed from the water through the wall of a perfectly new Berkefeld filter.

On Leakage at Joint of Filters.

An important point, which seems to have been generally overlooked, is the relative efficiency of the joint and the wall of filters. The large Berkefeld filter has a metal neck or socket into which the Kieselguhr mass is cemented. It would be strange if a composite filter of this description could with certainty withstand boiling when one considers the relative coefficients of expansion of the majority of the metals and the majority of siliceous materials. It appears to us that the metal socket consists of brass which has been nickel-plated. Now the linear coefficients of expansion of these substances on heating from the freezing point to the boiling point of water are as follows:—brass expands ·188 %, siliceous materials ·06—·1 % of their lengths, that is to say, the metal expands from 1·88 to 3·13 times more than the siliceous material. The superficial or planar and the cubic coefficients show a greater divergence. It seems to us improbable that any cement has yet been discovered which could resist the strain thus implied if it were used to unite metal and siliceous matter. Any filter therefore of this composite character, such as for example the large Berkefeld bougie, may show leakage at the neck or joint after heating in boiling water, and might possibly show such leakage before any attempt has been made at sterilisation, owing to variations in room temperature during storage. Whether this is the true explanation or not, the fact remains that in

the great majority of Berkefeld filters tested by us the neck or joint leaked at much lower pressures than the wall. On the other hand the Doulton filters consisting of porcelain nipple and porcelain wall did not show this effect.

Conclusions.

Transmission of Air.

1. The passage of air through the joint and wall of the Berkefeld filter does not seem to be materially affected by (*a*) boiling in water, or (*b*) autoclaving at 120° C., or (*c*) soaking new and used filters in water for 24 hours.

2. These filters leaked, in general, at the neck or joint at lower pressures than at the wall, the joint from ½ to 6, the wall from 4 to 7 lbs. per square inch with new filters. Used filters showed leakage at the joint from 1 to 4½, and wall 2 to 6 lbs. per square inch.

3. Simple immersion of a dry Berkefeld filter may give rise to a pressure of over 6 lbs. per square inch and this caused immediate leaking at the joint. With autoclaved, comparatively moist filters a pressure of 3 lbs. per square inch was obtained with leakage at joint. We have thus an auto-transmission of air.

4. At the pressure used in our experiments, viz. 0·9 lbs. per square inch, the large Berkefeld filters gave a yield of filtrate approximating to 0·4 gallons per hour. This is an exceptionally lenient test for a filter which the Berkefeld Company consider should give about 6 gallons an hour, i.e. 15 times as much.

Transmission of Micro-organisms.

5. Of two Berkefeld filters with porcelain nipples, one gave a contaminated filtrate from ordinary London tap water immediately, the other likewise after 3 hrs. 40 mins. These were new filters which had not been autoclaved but merely boiled one hour.

6. Of three Berkefeld filters with metal nipples, two gave immediate contamination with tap water and the third after 2 hrs. 40 mins. These filters were likewise new and had only been boiled one hour.

7. Two dried Berkefeld filters, one with metal and one with porcelain ends, on immersion of a portion of their walls in a water culture of *B. prodigiosus* allowed this organism to pass into the interior of the filters.

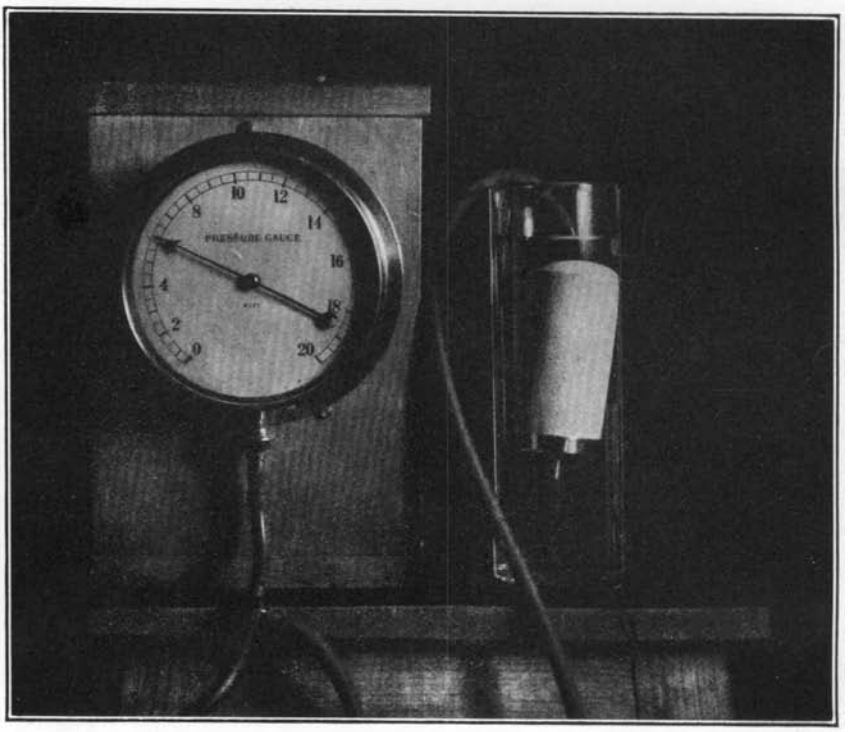

Fig. 1.

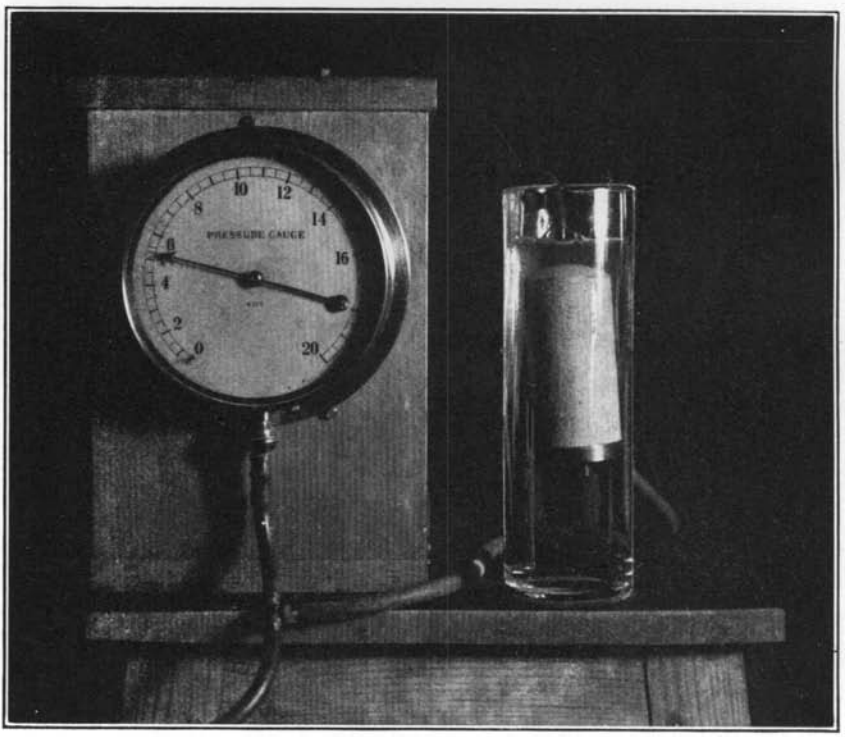

Fig. 2.

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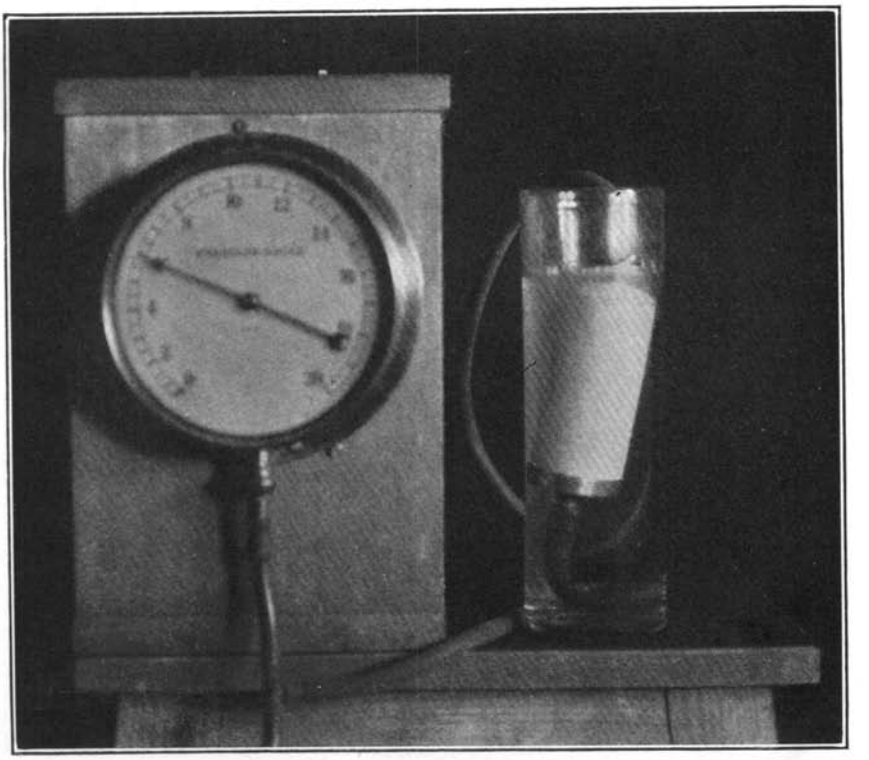

Fig. 3.

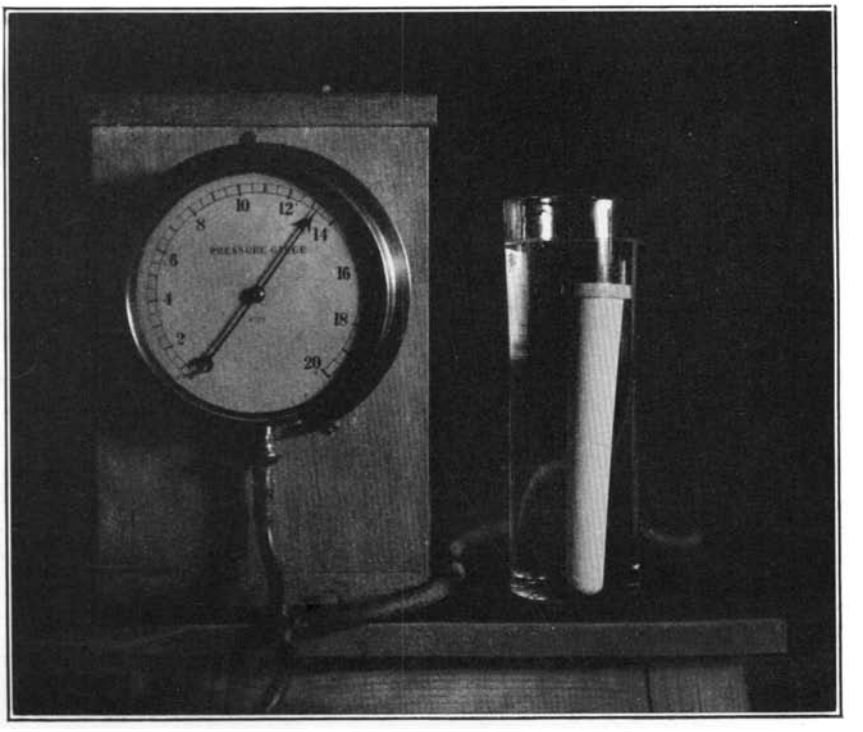

Fig. 4.

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8. These results are entirely confirmatory of our former work and effectually dispose of the objections raised in the private communications received from Messrs Nordtmeyer and Andrew Wilson.

REFERENCES.

BULLOCH and CRAW (1906). *Journ. of Hygiene*, VI. p. 409.
BULLOCH, CRAW and ATKIN (1908). *Journ. of Hygiene*, VIII. p. 63.
CRAW (1906). *Proc. Roy. Soc.* London, 77 B. p. 311.
NORDTMEYER (1891). *Zeitschr. f. Hygiene*, X. p. 145.
PFUHL (1903). *Festschr. z. 60sten Geburtstag von R. Koch*, Jena, p. 75.
WOODHEAD and WOOD (1894). *Brit. Med. Journ.* II. pp. 1053, 1118, 1182, 1375, 1486.
—— (1898). *Ibid.* I. p. 261.

EXPLANATION OF PLATE I.

Fig. 1 shows an initial capillary pressure of 6 lbs. per square inch by simple immersion of a new Berkefeld filter without applied pressure.

Fig. 2 shows bubbles of air accumulating on the surface of water in which is immersed a new Berkefeld filter under an air pressure of $5\frac{1}{4}$ lbs.

Fig. 3 shows the same filter (as in Fig. 2) under an air pressure of 6 lbs. per square inch. The extra $\frac{3}{4}$ lb. of pressure causing streaming of air bubbles from every pore of the wall, as is indicated in the photograph by the fact that the water above the joint of the filter is opalescent.

Fig. 4. Doulton white filter under same conditions as Figs. 2 and 3, with gauge showing pressure of $13\frac{1}{2}$ lbs. per square inch. No bubbles: water perfectly transparent.