SCIENCE

VOL. 75 FRIDAY, MARCH 18, 1932 No. 1942

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

New York City: Grand Central Terminal

Lancaster, Pa. Garrison, N. Y.

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary, in the Smithsonian Institution Building, Washington, D. C.

THE FILTRATION OF BACTERIA[1]

STUDIES IN BACTERIAL METABOLISM CIII

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INTRODUCTION

THE belief exists in some laboratories that bacteria, ordinarily deemed unfilterable, may under certain circumstances become so altered by chemical means, by cultivation for long periods of time in fluid media or through animal inoculation as to find their way through the pores of filters that would ordinarily restrain passage of the corresponding bacteria in their unfiltered state. The literature on this subject has become quite voluminous,[2] and opinion is now rather sharply divided into two groups; the "filtrationists," those who believe that bacteria may be filtered in some manner, and the "non-filtrationists," those who deny this possibility. This separation into two opposing camps is quite natural, because if one admits the possibility of filtration with one typical, ordinarily non-filterable microbe, one would rather logically be forced to admit that under suitable conditions all ordinary bacteria might be made filterable.

The problem of bacterial filterability has more than academic significance. There is a heterogeneous but formidable group of diseases of man and animals to which the term "filterable virus" is applied. The natural history of many of these "filterable virus" diseases—their clinical course, their method of dissemination and of inducing immunity—is generically akin to microbic diseases in which the etiological

[1] De Lamar Lecture, Johns Hopkins University School of Hygiene and Public Health, January 12, 1932.

[2] It is reviewed carefully by Klieneberger, "Bakterienpleomorphismus und Bakterienentwicklungsgänge," Erg. d. Hyg., Bakt., Immunitätsforsch. u. Exp. Therapie, Berlin, 11, 499–555. 1930.

agents are presumably known, and cultivable in appropriate media. Up to this time, however, the incitants of this "filterable virus" group, whatever they may be, have not for the most part been cultivated unequivocally outside their respective hosts upon artificial, laboratory nutritives.

It is surmised, and there is basis for this supposition, that the "viruses" of some of these diseases exist in the infected body in the filterable state, and it is not without some significance that many, if not most of these viruses enter, and probably leave the body, through the respiratory tract and its appendages. The possible import of this will be commented upon later. It is not proposed to discuss here the diseases allocated to the "filterable virus" group, but to present in detail some rather extensive studies upon the filterability of bacteria which may be relevant. These will be recounted at some length, as certain conclusions drawn from them would seem to indicate that the older division of microorganisms into "filterable viruses" and non-filterable bacteria may perhaps require some revision.

The experiments which led to the studies reported here have been described elsewhere in detail,[3] and will not be further referred to here, except for the statement that the first indication of the dual existence of an organism in a filterable and a non-filterable state was obtained from a study of blood from a small series of sporadic cases diagnosed clinically as influenza. A coccus was isolated from three of these cases in a special, protein-rich, peptone-poor medium (K medium);[4] at first in a non-visible, filterable state, and later apparently recovered after suitable procedure, as a visible, cultivable, non-filterable organism. From this wholly unexpected event, it was surmised that an actual, fundamental bacteriological principle was involved. If this be true, then other, well-established bacteria should also be rendered filterable experimentally, using the same general procedure, and recovered again in the non-filterable state.

Preparation of Culture

The organism chosen to test this premise was *B. typhosus*. The strain (Rawlin) was an old one, long in the laboratory. It has been used for many years in the customary manner for diagnostic purposes. It grows readily in plain, nutrient broth containing peptone and meat extractives. It also grows quite readily in the protein-rich, peptone-free K medium, prepared from hog intestine.[4] This is important. If the organism had failed to grow well in K medium, it would obviously have been less suited for these filtration experiments. The recital of an actual experiment with this strain of *B. typhosus* will make the procedure followed clear. Comments at the proper places will indicate the phenomena elicited. To insure purity in the usual bacteriological manner, this Rawlin strain of *B. typhosus* was replated three times upon agar. Between platings, growth from representative colonies was obtained in plain, nutrient agar. A colony from the last (third) plating was cultivated upon agar on October 29.

Filtration of Culture

Procedure

November 2. At 4 P. M. a generous loopful of this agar culture was introduced into 6 cc of K medium.[5] This was incubated at 37° C. over night.

November 3. Filtration I. At 10 A. M., growth being satisfactory, as estimated by a very distinct increase in turbidity above that of an uninoculated control, incubated in parallel, this 6 cc of K medium culture, after dilution with four volumes of sterile salt solution, was filtered through a Berkefeld "N" filter. (This filter did not pass a broth culture of *B. typhosus*). The vacuum used was less than 4 inches, water pressure, and the time of filtration was less than 10 minutes. The filtrate was clear. The filtrate was distributed at once, with sterile precautions, to the following media: to plain, nutrient broth; 1 cc, 1/2 cc, 1 drop; to K medium;[6] 1 cc, 1/2 cc, 1/4 cc, 1/10 cc, 1 drop. Incubation of these cultures together with uninoculated controls, was practiced at 37° C.

November 4. 10 A. M. Growth, as indicated by turbidity above parallel controls, occurred in all the tubes, both those in plain broth, and those in K medium. From the broth culture containing 1 drop of K medium filtrate, sugar fermentation reactions in glucose, lactose, saccharose and mannitol were made. After 24 hours, acid appeared in glucose and mannitol; saccharose and lactose were not acidified. An agar plate was also made. At the end of 24 hours, the colonies were typical and the culture appeared to be pure. Finally, after the sugar fermentation tests and agar plate had been started, a macroscopic agglutination test (dilution 1/500) was performed with this same broth culture. The culture agglutinated after 40 minutes' incubation at 37° C. An agar plate

[3] Patten Lecture, "Observations upon the Filterability of Bacteria, Including a Filterable Organism Obtained from Cases of Influenza," *Northwestern University Bulletin*, Vol. 32, No. 5, 1931; SCIENCE, 74, 129–139, 1931.

[4] "Mediums for the Isolation and Cultivation of Bacteria in the Filterable State," *Northwestern University Bulletin*, Vol. 32, No. 8, 1931.

[5] 100 mg. dried intestine, 6 cc Tyrode solution, containing neither glucose nor glycerin. This mixture is thoroughly shaken to wet the dried intestine powder, and then sterilized in the autoclave, 15 pounds for 15 minutes. *This medium should be slightly turbid when cool.*

[6] 6 cc, prepared as indicated in footnote 5, was used.

was also inoculated from the K medium tube which had received one drop of the K medium filtrate. The next day (November 5), typical colonies of *B. typhosus* were found in apparently pure culture. After transfer to broth, and proper incubation, they agglutinated with specific typhoid antiserum. Hence, from this first filtration, both the broth and the K medium tubes inoculated therefrom contained viable organisms which subsequently were identified as *B. typhosus* by plating, by fermentation reactions and by agglutination tests. The remainder of the tubes were reincubated for another 24 hours, together with their proper controls at 37° C.

November 5. Filtration II. 10 A. M. The tube of K medium which received 1/10 cc of the first filtrate (November 3), having a very noticeable turbidity after these 48 hours of incubation, was diluted with 4 volumes of salt solution and filtered through a Berkefeld "N" filter. The vacuum used was less than 4 inches, on the water-gauge, and the time required was somewhat less than 10 minutes. This filtrate was distributed as follows: to plain broth; 1 cc, 1/2 cc, 1 drop: to K medium; 1 cc, 1/2 cc, 1/4 cc, 1/10 cc, 1 drop. Incubation was practiced as before at 37° C.

November 6. 10 A. M. All cultures made November 5 grew; the plain broth cultures were distinctly less turbid than the corresponding ones of November 4. The K medium cultures, on the contrary, were rather more cloudy than the corresponding ones of November 4. Fermentation reactions were set up from the plain broth culture (that one inoculated with 1 drop of K medium filtrate of November 5); also an agar plate was inoculated. On November 7 the fermentation reactions were typical. The colonies on the agar plate were somewhat mucoid in appearance. An agglutination test (macroscopic, 1/500) made upon this broth culture was positive in 90 minutes. It will be recalled that the corresponding agglutination test made upon the broth culture recovered from the first filtration (November 3) was complete in 40 minutes. The growth in K medium failed to agglutinate in typical fashion, but a broth culture made from a K medium growth agglutinated characteristically. The significance of this observation, which has been repeated several times, is yet to be elucidated. From the results of this second filtration, it appears that there is some slight tendency both toward a change in the appearance of the colonies developing upon agar from broth inocula, and also a distinct tendency toward slowing of the speed of agglutination. Nevertheless, it is adduced that this second serial filtration of *B. typhosus,* and its recovery in the non-filterable state, was successfully accomplished.

November 6. 11 A. M. *Filtration III.* The K medium culture of November 5, containing one drop of filtrate, was diluted with 4 volumes of salt solution and filtered, this time through a Berkefeld "W" filter. The vacuum used was less than 5 inches on the water-gauge, and the time required was about 15 minutes. The filtrate was distributed as described above, in the following media: to plain broth; 1 cc, 1/2 cc, 1 drop: to K medium; 1 cc, 1/2 cc, 1/4 cc, 1/10 cc, 1 drop. Incubation was practiced at 37° C.

November 7. 9:30 A. M. The cultures in K medium all showed increased clouding above the control tube, which had been incubated in similar fashion. Of the plain broth tubes, on the contrary, none grew; at least there was no discernible turbidity at this time. Incubation of all the cultures was again practiced at 37° C.

November 9. Noon. The K cultures of November 6 had increased very distinctly in turbidity, and the broth culture of November 6, containing 1 cc of filtrate, had developed a faint but distinct turbidity. An agglutination test performed upon this broth culture, after inoculation of sugar fermentation tubes and agar plates, was partly clumped after 2 hours, and completely clumped after 18 hours in the ice-box. Agglutination of the cultures in K medium was negative. The colonies upon agar plates were rather mucoid in appearance. Sugar fermentation reactions were typical, but rather slow in developing. Recovery of the organism in its non-filterable state after each of these three several filtrations was achieved. The identification was made in each instance from growth induced in nutrient broth, by the appearance of colonies upon agar plates (test for purity), by fermentation reactions, and by agglutination (1/500) with specific antityphoid serum.

Each of the cultures made in K medium on November 6 was inoculated into plain broth, but even after 10 days' incubation, visible growth did not occur. It is assumed therefore that this serial, prolonged cultivation of *B. typhosus* in the protein (K) medium has resulted in an acclimatization of the organism in its filterable state to the protein medium, with a concomitant loss of accommodation to the simpler (peptone, meat extractives) medium. It has, in other words, become "proteophilic" instead of "peptophilic." It is surmised that a principal function of this filtration through the Berkefeld "N" and "W" filters is to strain out, and hold back, the non-filterable forms of the organism, passing those forms which not only are indeed filterable but also which have become more and more acclimatized to the protein environment. The final result of these three serial filtrations is apparently to establish culturally this filterable, protein-cultiviable form of the organism, which is reluctant to grow in ordinary media. To

answer the obvious inquiry at this point: recovery of the bacillary form was eventually achieved through inoculation of this filterable state of *B. typhosus* upon plain, nutrient agar, after incubation under partially anaerobic conditions for several days. The details will appear later. For the present, the point at issue is the premise that *B. typhosus* is, or may be, filterable if it is cultivated for several transfers in a "protein" (K) medium. Also, that it may be recovered in the non-filterable (peptophilic) form as above indicated.[7]

The subsequent history of this thrice filtered culture of *B. typhosus* and its maintenance in the filterable state is as follows: the tube of K medium inoculated November 6, containing one drop of filtrate of that date, was used. Transfers were made to fresh K medium November 9 and November 12, respectively, with incubation at 37° C. between times. The next transfer was made in California, November 16, and daily thereafter through November 22. With the exception of November 13, 14 and 15, during which time the culture was in transit, incubation of the subcultures was practiced at 37° C. From November 22 to November 28, the organism was again in transit, but reinoculation and reincubation were resumed on this date. On January 4 the strain is still alive. It still grows in K medium, but not at all in nutrient broth. It has retained its proteophilic properties, and apparently has not regained its original peptophilic state, as manifested by lack of visible growth in nutrient broth, even after several days' incubation. Thus, so it appears, a viable organism in the filterable state, incapable of visible growth in plain, nutrient broth, has been obtained by direct descent in culture through serial growth in an artificial, sterilizable medium (K medium) from an authentic, typical, plain nutrient broth culture of *B. typhosus*. It is left to the microbic pragmatists and dialecticians to debate whether or not this viable, cultivable, filterable organism thus obtained fulfils any, some or all of the criteria of a "filterable virus."

SUMMARY

This detailed protocol has been recited in considerable length because it contains what appears to be unequivocal evidence that a strain (Rawlin) of *B. typhosus* has been filtered not once, but actually thrice serially through Berkefeld filters, the first two filtrations through Berkefelds of N porosity, the third filtration through a Berkefeld of the W porosity, making three filtrations in all in a period of four days. These filtrations were performed on November 3, 5 and 6, respectively. Between filtrations, and prior to each of them, growth was elicited in K medium. The organisms developing after the first, the second and the third filtrations (November 3, 5 and 6) were recovered successively in the non-filterable state in nutrient broth and tested for their identity and purity by sugar reactions, by agglutination with specific typhoid serum and by plating upon agar. These tests were performed not only with the cultures of filtrates in plain nutrient broth, but also with subcultures made from K medium, in plain nutrient broth, with the exception of the K culture from the third filtration. This failed to grow in nutrient broth, although it grew well in successive K medium transfers.

Concerning the proportion of filterable to non-filterable forms, no definite information is available. However, inasmuch as the cultures were diluted with salt solution to 1/5 their original concentration prior to filtration, and inasmuch as 1 drop and 1/10 cc, respectively, of the diluted filtrates gave growths that were subsequently identified as *B. typhosus,* by fermentation reactions, by agglutination and by plating, it may be assumed that at least 1/50 cc of the unfiltered cultures in K medium contained viable typhoid microbes in the filterable state.

COMMENT

Imperfect filters: The old familiar contention that the filters which were used may have leaked, must be resurrected at this point. The theoretical possibility of leaky filters must always be admitted, and, except for two significant facts, it should be freely admitted in these experiments. Overlooking for the moment the fact that a filterable organism, incapable of growing in nutrient broth, was obtained through filtration, as above indicated, attention is directed to the fact that the filters used in these studies had been tested previously for passage of broth cultures of *B. typhosus* with negative results; hence it is assumed that the organisms do actually become filterable under the conditions specified, although the possibility still remains that the porosity of these tested filters may have changed in the interim. Also, and this may be significant, the broth culture made from the filtrate in broth (1 cc) of November 5, likewise failed to pass through one of the "N" filters used for these experiments. It is rather generally believed that Berkefeld "W" filters are too fine to pass bacteria. Attention is directed at this point to Bechhold's[8] important con-

[7] It is pertinent to inject here the rather obvious comment that the time relations met with in filtration experiments vary not only with the kind of organism, but also with the strain itself. Thus, one typhoid strain studied became "proteophilic" after one filtration; and another strain required four filtrations at two day intervals to become "proteophilic."

[8] H. Bechhold, "Porengrosse von Bakterienfiltern und Seibwirkung," *Zeit. f. Hyg. u. Infektskr.*, 112, 3 Heft, 1931.

tribution to relations between size of pores in filters (*cf.*, Berkefeld N and W, p. 416) and their capacity to withhold bacteria under definite conditions.

The other rejoinder to the plea of leaky filters, and, coincidentally perhaps the most suggestive new idea developed in these experiments, aside from the filterability and recovery of the non-filterable form of *B. typhosus,* is the gradual weaning of the filterable form of *B. typhosus* from its original (peptophilic) state, in which form it is non-filterable, to the filterable (proteophilic)[9] state, in which form it appears eventually to pass quite readily through even the finest Berkefeld (W) filter. In the peptophilic state, the organism grows readily in nutrient broth. In the fully established filterable state, achieved only by a sufficient number of serial filtrations to eliminate the peptophilic organisms, the microbe fails to grow in nutrient broth. It grows very well, however, in K medium.

It is, therefore, apparently a simple, rather rapid procedure to induce the filterable state by inoculation into K medium. Once the organism, freed from non-filterable tendencies by repeated filtration with inter-cultivation in protein (K) medium, has become accustomed, or better acclimatized, to the protein pabulum, it becomes increasingly difficult, at least in these experiments, to reinduce the non-filterable state.

At this time, an unexpected experience in the filtration of a culture of *B. coli* seems pertinent, in that it illustrates some of the pitfalls associated with the phenomena of microbic filtration. This particular strain of coli was isolated in 1909, and has been in laboratory stock ever since. The first attempt at filtration resulted in recovery of a small, yellow coccus. The yellow coccus was devoid of glucose fermenting power. Repeated platings of the original culture upon agar failed to reveal this coccus. Finally, resort was made to cultivation in K medium for 30 hours prior to plating. Then about 2 per cent. of the colonies on agar were of this yellow coccus, the remainder being typical coli. The K medium had been shown to be sterile prior to inoculation. Filtration of the purified colon bacilli, had by plating from K medium, was accomplished in good form, and the organism was filtered three times in five days with intervening growth in K medium. Between filtrations, typical *B. coli* were recovered and identified by fermentation reactions. For comparison, another stock culture of *B. coli* was examined. It failed either by direct plating, by plating after growth in K medium, or upon filtration, to reveal this yellow coccus. The possibilities enmeshed in this experience with the yellow coccus are many; space forbids more than mention of the facts as they were observed at this time.

[9] Throughout this discussion, the terms "peptophilic" and "proteophilic" are used symbolically to suggest rather definitely a chemical difference between peptone on the one hand, and the more or less unaltered protein constituents of dried intestine on the other hand.

MORPHOLOGICAL CHANGES DURING FILTRATION

Returning to the filtration of *B. typhosus,* the question very naturally arises, Are there changes discernible in the typhoid bacilli during the process of inducing the filterable state that may actually be seen under the microscope? One rather suggestive difference between the appearance of the first growth of *B. typhosus* in K medium immediately prior to filtration, and of the organism after filtration, is the relative abundance of bacillary forms, with and without granules within their substance, in the former, as shown by staining with old methylene blue, by examination with the 1/12 immersion lens directly and by dark-field illumination. This is sharply in contrast to the apparent absence of these bacillary forms, with and without granules, in the filtrate immediately after filtration. In both the unfiltered primary growth in K medium and in the filtrate of this primary K medium culture, however, there are numerous granules. Some of these granules are inherent in the medium.[10] They are bluish or greenish yellow under dark-field illumination, and usually exhibit Brownian movement. In addition, in both filtered and non-filtered K medium cultures of *B. typhosus,* there are usually small, somewhat motile granules. These are, in fresh culture in K medium, often nearly as active in their motion as typhoid bacilli themselves. It must not be assumed from this observation that the filterable forms of all bacteria will exhibit true motility, however.

In the filtered medium, these granules and not the bacillary forms, appear to be the only motile bodies. Thus far, they have not been definitely stained, even by Giemsa or silver stains, and their identification therefore depends rather upon inference than upon rigorous proof.

It is necessary to reemphasize here that repeated filtration of cultures of *B. typhosus,* with interspersed cultivation in K medium, is required to eliminate the tendency of the filterable forms to go back to the bacillary form. As acclimatization progresses, with repeated filtration for the purpose of the elimination of the non-filterable forms, a time comes, usually after two or more successive filtrations at appropriate intervals, when the filterable (proteophilic) forms no longer respond to the "peptone urge" and remain

[10] It will be found that cultures grown in broth usually contain at least some bacilli with enclosed granules, and all media containing protein ingredients will contain at least some free granules. These, in uninoculated media, are non-motile, although they usually exhibit Brownian movement.

viable, but filterable from culture to culture in K medium. Such "proteophilic" organisms, in the filterable state, are difficult indeed to reestablish in the non-filterable condition. A condition similar to this may be readily conceived of as existing in the tissues of the animal or human body, where invading bacteria, originally non-filterable, exposed for periods of time to the tissue protein in absence of peptones, may thus become proteophilic, parasitized upon the proteins of the body, as it were, and difficult to recultivate in peptone media. Emphasis is laid at this point upon the biological significance of inducing this filterable state through cultivation of bacteria in media rich in nearly unaltered protein. In this respect, the experiments recorded above differ sharply from previous procedures in which strontium salts, or very old peptone broth cultures are relied upon to induce filterability.

Thus far, emphasis has been laid upon actual experiments. It has been found that *B. typhosus,* under cultivation in a protein-rich, peptone-poor medium passes rather rapidly and readily to the filterable state. While it can not be dogmatically denied that leaky filters permit of this passage, the fact that five different strains of *B. typhosus* (to restrict discussion to this organism for the present) have been thus filtered and recultivated, using some 24 different filters of the Berkefeld type, each time employing the finest pored filter (W) for the final separation of non-filterable forms, would appear to be reasonable evidence of the accuracy of the assertion. The part played by filtration in these experiments is merely passive. It removes those filterable, lineal descendants of the original stock culture which still manifest a tendency toward reversion into typical, non-filterable bacilli, under the nutritional stimulus of peptone, from the filterable forms, which have developed, so to speak, proteophilic propensities.

Relation of the Filterable State to Infection

Some interesting correlative information arises from this nutritional concept of the development of filterable forms of bacteria from non-filterable forms in the body itself, especially in relation to microbic infection.

Microbic infection, generally speaking, may be considered as actually taking place when the prospective invading organism passes through the barriers which usually suffice to keep it out, and actually penetrates into the protein fastness itself. A majority of bacteria, and presumably a majority of "viruses," gain entrance to the underlying tissues through epithelia, principally those of the intestinal and the respiratory tracts. A significant chemical difference between these two tracts should be emphasized here. The intestinal mucosa is almost continually bathed, on the epithelial side, in a medium rich in protein digestive products, which pass successively from the complex peptones and albumoses to simple peptids and amino acids. The latter, according to current information, are normally absorbed from the alimentary canal through the villi, and pass to the blood stream. Hence the mucosa of the intestinal tract is in a peptone environment, using the term "peptone" symbolically merely to indicate protein in various stages of digestion. The nutritional value of this medium is reflected in the luxuriance of the intestinal flora: some thirty trillions of visible and stainable bacteria are said to be eliminated each day in the fecal mass of a normal adult enjoying a normal diet. The respiratory tract, on the contrary, is a relatively sterile tract. Protein degradation products, except at those times when purulent bacteria are at work, are apparently absent. The significance of mucus in this connection can not be answered in light of available information. Stated somewhat differently, the digestive tract is proteolytic; the respiratory tract is aproteolytic. Bacteria that enter the digestive tract find abundance of protein digestion products available for their nutrition: bacteria that enter the respiratory tract do not normally find protein digestion products available for their nutrition. It would seem to follow that bacteria within the intestinal tract are in an environment that should tend to encourage their existence in a non-filterable (peptophilic) state. Bacteria that gain entrance to the respiratory tract would appear to be in an environment that normally should tend to encourage their existence in a filterable (proteophilic) state.[11] In light of this rather striking difference between the two tracts, it is not without significance that many if indeed not most of the contagious so-called "filterable viruses" according to current information appear to enter, and to leave, the body through the respiratory rather than the intestinal path.

Microorganisms that pass from the respiratory tube actually into the tissue of the lung, whether they are initially filterable or not, become confronted with a protein-rich, peptone-poor medium, as they penetrate the epithelia of the respiratory tract. From what has been stated above, this is one condition which tends to induce and to perpetuate the filterable state. Similarly, upon passing back from lung tissue to the respiratory tube, the same condition apparently prevails unless there is pus formation. Pus, as is well known, contains products of protein digestion. It would seem to follow logically that bacteria in the filterable state should, theoretically at least, be not

[11] The pneumococcus, a frequent incitant of pneumonia, is habitually a purulent organism. Pus is said to be rich in protein degradation products.

uncommon in non-purulent infections of the respiratory tract. Bacteria leaving the body from the intestinal tract, on the contrary, are exposed to nutritive conditions conducive to the non-filterable rather than the filterable state. Hence it is perhaps not necessary to reiterate that many, if not most, of the "filterable viruses" have been found thus far in association with the respiratory, rather than the intestinal tract. This is not to be construed as an assumption that all "filterable viruses" exist in a non-filterable as well as a filterable state: only precise experiments with each disease entity will determine just what the limits to be applied ultimately to the term "filterable virus" shall be: nor is it by any means the whole story. *B. coli,* like *B. typhosus,* seems to become filterable without much difficulty by cultivation in the proper manner in protein media, but the fact that this microbe may thus become filterable does not explain why it is not ordinarily an invader of the body. The nature of the weapons with which certain kinds of bacteria, and not others, may force entrance through the epithelia which ordinarily suffice to keep microbes out, is yet to be determined. Just what part the filterable state of bacteria may play in the vastly complex phenomena of infection and immunity remains to be revealed. And there are exceptions to this hypothesis of association between protein nutrition and the filterable state. Leprosy appears to be such a case. Leprosy bacilli are readily stained within the tissues of lepers; that is, they exist there in a presumably non-filterable state. There is little evidence that there is much, if indeed any, tissue digestion round about them. Nevertheless, exceptions to the contrary, there is after all apparently a very distinct general biological parallelism between the occurrence of non-filterable, stainable bacteria, growing in ordinary media under the nutritional stimulus of peptone (peptophilic state) and filterable, not stainable organisms growing in protein-rich, peptone-poor media under the nutritional stimulus of protein (proteophilic state). And the well-established difficulties surrounding the isolation of microbes from some of the so-called "filterable viruses" (which appear to be developing in the protein tissues of the body and are refractory to cultivation in peptone media, even enriched with tissue) have their nearly precise counterpart in this connection with the corresponding experimental difficulties encountered in cultivating the fully protein acclimatized, filterable form of *B. typhosus* in peptone media, even those enriched with blood. It would appear indeed that one rather striking feature of the experiments with filterable forms of the typhoid bacillus is this very establishment of the proteophylic state, refractory to cultivation in ordinary, or enriched peptone media. If this indeed be the case, then it might be rather confidently predicted that at least some of the infections refractory to artificial cultivation should be approachable from the use of suitable protein media. A word about K medium should be injected at this point: it is very crude. This has been emphasized again[3] and again.[4] There is no more reason, *a priori,* for expecting successful isolation of organisms from influenza, common cold and smallpox, to mention three possible sources of culture, in K medium in its present crude state, than there is in expecting successful isolation of *Tr. pallidum,* meningococcus and tubercle bacillus, using merely plain, nutrient broth. Special modifications, to meet the needs of specific organisms, both for isolation and for cultivation of specific organisms in a filterable state, must be applied to the medium, as is required of the usual laboratory media for similar reasons. This is well exemplified by the recent work of Mellon,[12] who has just reported the successful filtration of the tubercle bacillus, and its recovery in the non-filterable state, using a modified K medium.

CONCLUSIONS

(1) The intimate details of three successive filtrations of an authentic strain of *B. typhosus,* performed in four days, are recorded.

(2) The details of recovery of *B. typhosus* in the non-filterable state, and its identification by colony formation, by fermentation reactions, and by agglutination tests, are stressed.

(3) Attention is drawn to the perpetuation of *B. typhosus* in the filterable state by cultivation in a protein [K] medium.

(4) Emphasis is laid upon the biological significance of proteins in inducing the filterable state in bacteria.

(5) Certain theoretical relationships are suggested between this artificially induced filterable state of bacteria and certain microbic infections of man.

OBITUARY

MEMORIALS

THE section on medical history of the College of Physicians of Philadelphia, as reported in the *Journal* of the American Medical Association, held its stated meeting on March 14, in cooperation with the Henry Phipps Institute, the Philadelphia Health Council and Tuberculosis Committee and the Philadelphia Association of Tuberculosis Clinics to commemorate the

[12] Mellon, *Proc. Soc. Exper. Biol. and Med.,* Vol. 29, No. 2, p. 206, 1931.