STUDIES IN MICROBIC HEREDITY

VIII. THE INFECTIVITY AND VIRULENCE OF A FILTRABLE PHASE IN THE LIFE HISTORY OF B. FUSIFORMIS AND RELATED ORGANISMS

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INTRODUCTION

A little more than seven years ago we reported the presence of a filtrable organism in the blood stream of a patient having a pyemia, due to *B. fusiformis* and related microörganisms (Mellon, 1919a). Although at that time very considerable additional work was done on the filtrability of the fusospirillary complex of organisms present in this patient it was not reported,—partly for the reason that the filtrates were cultivated with great difficulty, and partly because at that time it was not generally believed that common bacteria possessed filtrable phases.

Since then the bacteriophage has come on the scene in a way that leaves no doubt that at least certain of the enteric group have filtrable phases. Reports of filtrability of the tubercle bacillus and other organisms represent a confirmation of isolated cases, instanced not only by my own work, but by studies like that of Fontes and Almquist. Fontes (1909) reported that the tubercle bacillus was filtrable, and Almquist (1916) filtered *B. typhosus* and cultivated a minute variant typhoid bacillus which he called *B. antityphosum;* and now we can surmise that the early filtration experiments which so greatly delayed the discovery of the real cause of scarlet fever represented nothing more than a demonstration that this streptococcus had a filtrable phase in its life history.

But Miss Evans (1925) of the Hygienic Laboratory has placed

the filtrability of streptococci beyond the realm of surmise by showing that a green streptococcus and the so-called filtrable virus of encephalitis lethargica are two reversible phases of the same organism, which in addition has a third or diphtheroid phase. All of these may grow independently under proper conditions. Although the first two of these agents have been previously identified with the infection by others, each has been considered by its sponsors as the primary "causus morbi" and as a result two sharply contrasted points of view obtain.

The promise of Miss Evans' work is the reconciliation of the opposed points of view, which has been impossible heretofore on the basis of the old monomorphic conceptions of bacteria. In light of these studies our own work, done nearly ten years ago, may not seem so novel as it would had it been reported at that time. However, infection in experimental animals with the fusiform bacillus and related organisms has rarely, if ever, been accomplished. The discovery, then of an infective and filtrable phase for them definitely correlates virulence with a polymorphic life cycle, which does much to clarify the pathogenesis of diseases thought to be caused by these organisms.

SALIENT POINTS OF OUR FORMER STUDY OF B. FUSIFORMIS. THE BASIS OF THE INOCULATION EXPERIMENTS

It will be recalled that our original study of case J. B. showed that from a renal abscess there was isolated a true anaerobic Gram-negative *B. fusiformis* which was associated with a spirillary non-motile threadlike branching organism. On isolation the latter proved to be a streptothrix or, more precisely in this instance, a vibriothrix. No motile spirochetes were observed in the pus of this case. From the pus obtained by lung puncture the vibriothrix was apparently present in pure culture, and it was not until months later that multiple pleural sinuses developed, in the pus of which a typical Vincent's flora appeared. A filtrable phase of a coccus was present in the blood, which when grown out on a slant as an "adult" coccus no longer passed through a filter. Its presence in the blood in filtrable form was repeatedly demonstrated.

In addition there was described a granular aerobic diphtheroid organism which morphologically was transitional between the fusiform and the vibriothrix. It is this organism which yielded the filtrable gonidia which we pictured, and to which we alluded especially on page 518 in the statement that "Subcutaneous injection of the filtrates into animals has shown results under certain conditions." The pus from which the organisms were isolated and the organisms themselves, when injected subcutaneously into rabbits and guinea pigs, reacted negatively. This is the usual outcome with organisms of this group.

In figure 1 is shown this organism in its gonidial stage, the very numerous granules occurring chiefly in cultures six to eight hours old on fresh blood agar. (Lateral and terminal germination of gonidia at *a*, figure 1.) They were not observed when the organism was grown on other media. These granules passed a Berkefeld N candle and resisted all attempts at artificial cultivation. That is to say they never grew out as the typical bacillus that gave rise to them, although as I described, an abortive coccoid type of germination appeared to occur. Both anaerobic and aerobic technique was employed on both fluid and solid media and at different temperatures.

Our attempts at cultivation and the incomplete results obtained led to our inoculation experiments with the hope that in the body of the animal the conditions for the complete germination of these filtrable forms might be found.[1] Injected subcutaneously into guinea pigs and rabbits the filtrates were quite negative, but intravenous injection into young rabbits resulted in their gradual loss of weight, a moderate temperature and subsequent death; but inasmuch as the cultures from the animals were negative the results could not be considered final.

We then conceived the idea of growing the gonidial filtrates

[1] It will be recalled that the central theme of these studies has grown up around the working hypothesis that bacteria elaborate special growth forms of various sorts, most of which have been collectively expressed as "involution forms" because they were supposed to be incapable of germination. The selection of suitable environments for their germination has been accomplished so often as to virtually abrogate the original meaning of this term.

in symbiosis with a saprophytic organism previous to their inoculation. The latter will be known as organism X.

EXPERIMENTAL

A. Filtrates of the diphtheroid bacillus

Experiment 1. Gonidial filtrates of the diphtheroid bacillus were grown at 37° in symbiosis with organism X, and 0.5 cc. inoculated subcutaneously into a 500-gram guinea pig. The inoculation was made immediately beneath the skin leaving an elevation of the shaved area. Six guinea pigs were so inoculated at various intervals with filtrates made from eight and twelve hour cultures showing numerous gonidia (fig. 1). The following data are characteristic for all of them.

Results. After twenty-four hours a small amount of local edema which in forty-eight hours gave way to necrosis without sloughing at this stage. By seventy-two hours marked infiltration of the surrounding tissues and frequently an early purulent exudate were noted underneath the necrosed skin.

Microscopically short fusiform bacilli, coccoid forms and perfectly typical fusiform bacilli with spiral threads were observed (figs. 2 and 3). The morphologic picture recalled that of a Vincent's angina lesion, but actual spirochetes were not demonstrated. Anaerobic Gram-negative *B. fusiformis* was isolated from the lesions, which became rapidly progressive. Usually after forty-eight hours organism X could no longer be isolated. The necrotic skin sloughed, leaving an area of thickened necrotic overhanging margins. The latter were bathed in purulent exudate, particularly beneath them and spreading into the surrounding tissues.

B. Control series

Experiment 2. All together fourteen pigs were injected with broth cultures of organism X grown twelve to fifteen hours at 37°C. Of these five received organism X in purity. The others were injected along with filtrates suspected of containing gonidia.

Results. With one exception the results were quite negative in all fourteen animals. By this I mean that although in from

twenty-four to forty-eight hours there was some local edema and perhaps necrosis present—as occurred also in the test animals at this stage—the lesions did not progress, smear and culture examinations were negative except for the injected organism, and practically all traces of the inoculation soon disappeared. In the one animal that proved the exception the small amount of pus containing granular, fusiform-like bacilli soon disappeared, and the lesion quickly healed. It seemed probable that this result was to be explained by the previous presence of skin diphtheroids that were thrown into a gonidial phase by the local inflammation resulting from organism X. But the thirteen other negative controls showed clearly that organism X alone is incapable of developing active fusiform lesions. Its morphology rendered it easily distinguishable from *B. fusiformis*.

Experiment 3. The diphtheroid culture grown on plain agar where it did not produce gonidia, at least in appreciable numbers, was emulsified in broth and grown symbiotically with organism X for from twelve to eighteen hours at 37°. Injected 1 cc. subcutaneously into three guinea pigs.

Results. Quite negative after two weeks daily observation, showing clearly that the non-filtrable stage of the organism, even in appreciable numbers and when associated with organism X, was avirulent.

Experiment 4. One cubic centimeter of gonidial filtrate *alone* was given on several occasions subcutaneously to two guinea pigs.

Results. Always negative.

Experiment 5. a. Gonidial filtrate was heated ten minutes at 55°C. It was grown as before with organism X and 0.5 cc. injected subcutaneously into a guinea pig.

b. The sterile filtrate of organism X plus the gonidial filtrate was injected subcutaneously into a guinea pig.

Results. Negative in both *a* and *b*.

Experiment 6. The other negative controls comprised three pigs that were inoculated with organism X grown with other stages of the polymorphic complex, e.g., with the vibriothrix.

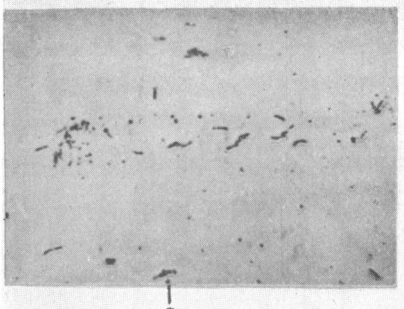

fig. 1

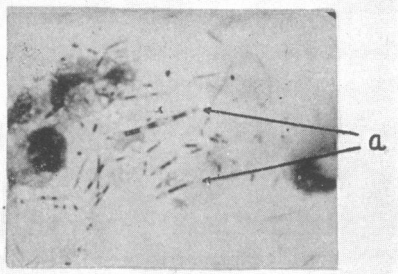

fig. 3

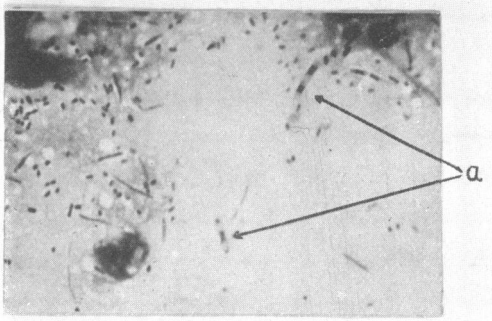

fig. 2

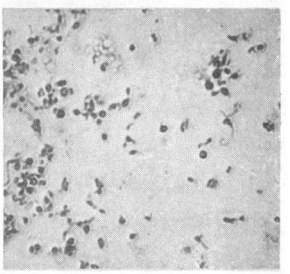

fig. 4

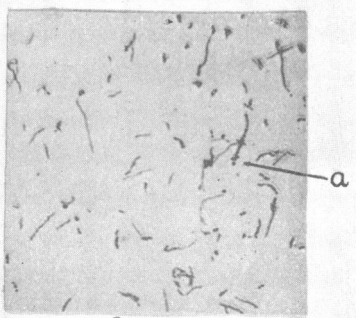

fig. 5

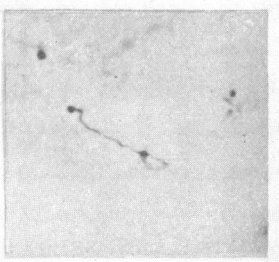

fig. 6

C. Inoculations with the filtrates of the vibriothrix

Experiment 7. Organism X and the vibriothrix were grown together on an agar slant which resulted in a marked granular transformation of the threads. These were emulsified in broth, filtered through a Berkefeld N and the filtrate incubated as usual with organism X.

Results. On February 12, 1918, 1 cc. of the culture was injected subcutaneously into a pig. In forty-eight hours a few granular bacilli and fusiforms could be detected, but on February 18, 1918 pus again appeared at the border of the lesion which contained very numerous fusiform bacilli and numerous other forms indistinguishable from *B. thetoides* (figs. 2 and 3 at *a*). After a copious discharge of mucopurulent exudate the lesion went on to complete healing by February 23, 1918. Hair grew over it and the pig appeared quite normal but its original weight of 465 grams remained stationary.

After an interval of six weeks, on April 2, 1918, the original lesion again appeared, but in place of the fusiforms which originally had dominated the morphologic picture giant coccus forms resembling the cultural "coccoids" of the bacillus appeared (fig. 4). Many of these seemed to be the origin of filamentous forms. Many of the latter were present. They often branched dichotomously and yielded lateral and terminal gonidia as seen in the pure culture of figure 5.

On April 7, 1918, the lesion was again nearly healed only to break down on April 15, 1918, being then accompanied by rapid extension into neighboring tissues. At this time 5 cc. of heart blood was withdrawn for culture, which appeared to result in the death of the animal three hours later.

Autopsy protocol. Hemorrhagic exudate was noted at the site of inoculation (left side). Extending from this point to the ribs above and the knees below the subcutaneous tissues were markedly thickened and edematous and quite adherent to the skin. The hyperplastic tissue was grayish red and very friable. A phlegmonous inflammation extended to the right side.

The abdominal viscera were matted together with a frothy

purulent exudate which almost encased the liver as a rather thin purulent looking membrane. The spleen was enlarged and friable. The lungs and kidneys were pale but mild acute purulent pleurisy without effusion was present.

Direct examination of the subcutaneous phlegmon and of the peritoneal exudate showed filamentous and fusiform-like organisms with numerous coccoid and gonidial forms. The picture was strikingly like that of the blood culture of the animal, which had numerous small cocci and gonidial forms with an occasional spirillary element. (Cf. figure 6 from the blood of a clinical case of gangrenous balanitis.)

Experiment 8. Blood culture of the pig of experiment 7 was incubated with organism X but was grown at room temperature. One cubic centimeter was inoculated subcutaneously into a 400-gram pig on April 17, 1918.

Results. The local induration that developed in twenty-four hours was without slough but had by April 21, 1918, developed a slough and extended into the inguinal region. The pig was obviously weak and toxic and had decreased in weight from 400 to 300 grams. Died.

Autopsy protocol. An extensive phlegmon spreading from the site of inoculation was associated with a regional and somewhat generalized lymphadenitis. The hemorrhagic lungs were encased in a purulent membrane which also enveloped the liver like a sac. Fresh adhesive pericarditis was evident. There was a generalized exudation throughout the abdominal cavity but without free fluid. Smears from various portions showed fusiform bacilli, filaments and cocci.

Pathologic summary. Discussion. There is a noteworthy similarity in the general pathology and the bacteriology of both these animals. The findings are of suggestive import in their possible relation to the origin of the clinical condition in human beings known as polyserositis. This has often been thought to be due to tuberculosis and the causative organism here is in its cultural aspects much like the tubercle bacillus except that it is not acid fast.

Especially noteworthy is the fact that we have with this

organism a fusiform-filamentous complex associated with a coccus, much as we had in the case from which the organism was isolated. In common with it, too, there was no suggestion of motile spirochetes. Such a complex, when it becomes generalized as it occasionally does in the human being, runs a course which is strikingly like that of a generalized tuberculosis, particularly in respect to the alternate remissions and exacerbations that it shows.

Whether spirochetes are present may depend on which variety of filtrable gonidium gives rise to the pathologic process. The cultural work on these two animals was not extensive enough to know with certainty whether the spirals present had actually been dissociated as a stabilized stage in the life history of the thread form, or whether they represented an ontogenetic phase in its phylogeny.[2] The same may be said of the large number of theta bacillus forms of experiment 7. This bacillus is recognized as a member of the anaerobic gangrene group, closely related to *B. fusiformis*.

Of note, too, is the difference in virulence of filtrable gonidia produced in different ways. This can best be seen by contrasting the above experiment with experiment 9 which follows. In this experiment the gonidia were derived not by symbiosis with organism X but they occurred rather spontaneously, arising laterally and terminally as illustrated in figure 5 and described in our original communication (1919a) as gonidia of the second order. Myriads of them could be seen developing from the filaments which did not break up in toto into a mass of granules as was the case when grown with organism X.

Experiment 9. Filtrate IX represented a filtrate of vibriothrix gonidia which occurred spontaneously as noted above. Immediately part of the filtrate was seeded with organism X, while a part was permitted to germinate at 37°. This it appeared to do in twenty-four hours, yielding a turbid broth containing coccoids and granular diphtheroid or fusiform looking bacilli (fig. 7 at *a*); but curiously enough all attempts to transplant

[2] For fuller discussion of these relationships involving Haeckel's Law the reader is referred to Study V of this series (see bibliography).

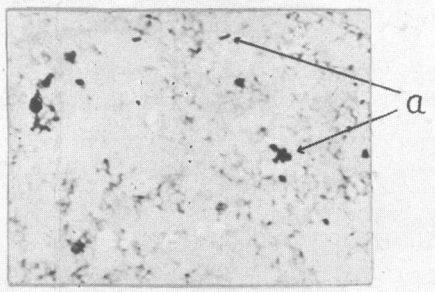

fig. 7

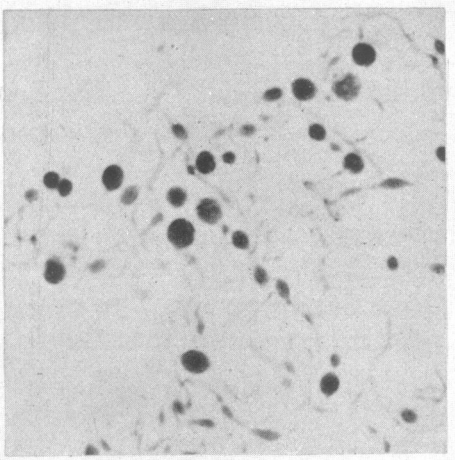

fig. 8

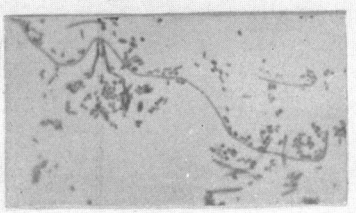

fig. 9

these forms were negative. These coccoid forms were grown with organism X as usual.

Results. The pig that received the ungerminated filtrate was quite negative in all respects after ten days' careful observation. The other one developed a rather innocuous lesion which suppurated nevertheless and gave myriads of diphtheroid and fusiform-like organisms in the pus. The lesion healed soon and gave no further trouble.

Experiment 10. The vibriothrix with giant coccoids (exospores figure 8)[3] but without visible gonidia was emulsified in broth to which was added a broth culture of organism X. Of this 0.5 cc. was injected subcutaneously into a pig.

Results. Only after a week was a slight suppuration observed and in the pus were found some long granular filaments and the solid type of *B. fusiformis.* The lesion was not progressive and healed rapidly with no further sequelae. Thus neither with adult organisms, the germinated gonidia nor the freshly filtered ones, do we get lesions that are at all comparable to those produced by gonidia of the diphtheroid or by those developed when the vibriothrix is grown symbiotically with organism X.

D. Results of filtration of clinical cases of infection with B. fusiformis and organisms other than isolated from case J. B.

Experiment 11. Diphtheroid bacillus J strain. This is a stabilized, relatively nonpleomorphic variant from the pleomorphic J strain described in our first communication on diphtheroids (1917). The organism was shown to have a nonhemolytic streptococcic phase that could be stabilized as such. It was quite granular and under certain conditions gave rise to numerous gonidial granules, as did the diphtheroid of the J. B. case.

These gonidia were emulsified in broth and filtered through a Berkefeld N. No growth was obtained under any conditions tried, but the filtrate was grown eighteen hours with organism X and injected into a 525-gram pig subcutaneously.

[3] These photographs have a magnification of 1800 diameters while the other photographs have 1200.

Results. Within three days the injection site formed an ulcer which rapidly increased in size. Its margin became thickened and indurated and underneath there was a copious purulent exudate containing myriads of fusiforms, both granular and solid types. Many cocci and coccoids were present, the bipolar appearance of the latter probably representing young *B. fusiformis.* Spiral forms were occasionally present but they were not examined for motility. Large numbers of *B. thethoides* forms occurred (figs. 2 and 3).

Cultured aerobically the fusiforms did not grow and there was no odor to the cultures. On blood agar under anaerobic conditions numerous colonies of the elevated gray colonies of fusiform bacilli of solid type, mixed with cocci, were present (fig. 9). Some of these formed threads of varying length. The culture had the characteristic odor. The threads were Gram-negative. Later a few colonies of Gram-positive diphtheroids appeared which resembled the original culture.

Although the original J strain was a pure line one it occasionally showed a moderate filamentous change, the latter being Gram-negative and originating from the ends of the Gram-positive bacilli. As such they represented an ontogenetic phase in the culture's phylogeny. In the pig the thread forms became stabilized as developmental stages of *B. fusiformis.* This is but another example of a fundamental change that we have so frequently verified in vitro.

Experiment 12. Results with a long standing B. fusiformis infection of the lungs. This case—L. C.—proved to be one of the most interesting and clear cut cases of *B. fusiformis* infection that we ever studied. It was uncomplicated by the presence of spirochetes. The patient, eighteen years of age when we first observed her, had been a sufferer from a bronchial abscess since two years of age. The lesion followed whooping cough. She had been repeatedly diagnosed as tuberculous, but the organisms were never found in her sputum although they were searched for often enough by many observers, including the Saranac group. She had periodic attacks of fever and a high polymorphonuclear leucocytosis, 20,000 to 25,000. Search of her washed sputum

showed *B. fusiformis* in purity, the colonies appearing grossly as little opaque cohesive flecks. The organisms were often in threads of moderate length.[4]

Filtrates of the sputum grown symbiotically with organism X, followed by pig inoculation, produced a small abscess containing curved mycelial and fusiform organisms, but the ulcer rapidly healed and the pig remained permanently well.

Three times the patient's blood was drawn into sterile broth or citrate solution. Part of it was seeded with the organism X and incubated at 37° for eighteen hours. Two of the three pigs developed the usual local lesions with *B. fusiformis* and filamentous forms. One died in two months and the other in two weeks, and at the autopsy marked serofibrinous adhesions and lung involvement were found. The same general flora was found as was present in the local abscess. The pig that was not affected received blood taken at a time when no febrile exacerbation existed.

Discussion and résumé. These results, apparently indicating the presence of a phase of *B. fusiformis* in the blood of seriously infected cases, are of interest because the fusiform organism is at best rarely isolated from the blood, even in cases of pyemia where one would suspect its presence. This may be explained by its occurrence in the filtrable form which, as we have seen, is not to be cultivated under the usual conditions. The progressive type of lesion developed from the blood culture suggests the presence of larger numbers of the gonidia there than in the sputum. From the direct examination of the sputum we could not be sure that gonidia were produced to any extent.

It is a matter of considerable interest that positive blood cultures in these cases when they occur usually yield a coccus; yet a careful examination almost always discloses the presence of filaments of whose origin from the coccus or its related coccoid forms there can be no doubt[5] (see fig. 6). It will be recalled that

[4] This patient was treated with potassium iodide and a hygienic régime. She later married and is perfectly well today.

[5] For more complete discussion and reports of blood culture findings in these cases reference may be made to our communication in 1919 (see bibliography).

this was true for our original case (J. B.) and in experiments 7 and 8 was true for our experimental animals.

The fact, too, that we have repeatedly shown in vitro that these diphtheroid organisms can dissociate stabilized coccus phases from their life cycle makes this relationship in vivo a more satisfactory explanation than the secondary infection theory. The single celled method shows clearly that the filaments as they occur in the cultures of the cocci are ontogenetic (pleomorphic) phases of the organism and not independent entities; but their presence always signifies that such a culture is *capable* of stabilizing its ontogenetic phase as a phylogenetic race or type. (See Study V.)

One of the clearest examples of the presence of these filaments as a purely pleomorphic phase of an otherwise typical coccus is shown in figure 6. This organism was repeatedly recovered from the blood of a patient with a severe gangrenous balanitis, the so-called fourth venereal disease. This condition is an example of Vincent's disease affecting the penis, and has the identical bacteriological diagnostic picture found in Vincent's angina. These cocci agglutinated the patient's serum in a 1:50 dilution, but a normal serum showed agglutination no higher than 1:10.

Experiment 13. A case of benign tumor of the large intestine (lipoma) so stretched the mucosa of the bowel as to give rise to superficial ulcerations of a very hemorrhagic character. The flora was of the fusospirillary type. The regional lymph glands were generally enlarged and presented an appearance suggestive of lymphosarcoma.

The patient developed a severe anemia, scarcely to be distinguished from pernicious anemia, with a lymphocytosis of 70 per cent. He later died with symptoms of progressive weakness and a general intoxication. The obscurity of the case and its relapsing character, combined with the uncertainty of a definite diagnosis, pointed to the probability that we dealt with a fusiform-streptothrix infection, secondary to a neoplasm that was essentially benign. It is of note that the chronic and relapsing type of the disease is exemplified in some of the experimental animals.

The lymph glands were ground up and filtered and grown with organism X, and when injected into a guinea pig yielded the same findings as gonidia from the other sources detailed—numerous fusiform and spirochete-like forms, cocci and diphtheroid forms. One of the two pigs so treated died after two weeks. There were no autopsy signs of note.

Experiment 14. This case was the filtrate from the throat of a typical ulcerous Vincent's angina. Treated as all the other filtrates were and inoculated, a similar sloughing ulcer developed with the typical flora noted before. No true spirochetes could be identified with certainty. Other cases, milder clinically, such as gingivitis associated with *B. fusiformis*, gave negative or equivocal results. We have not had adequate opportunity to apply the method to a sufficient number of cases of frank Vincent's angina to judge of its validity or to ascertain whether the infective gonidia are present only in the early stages of the disease. We have a suspicion that this is the case.

THE BEARING OF THESE EXPERIMENTS ON INFECTION AND EPIDEMIOLOGY

The bearing of these experiments in the direction suggested by the subtitle is obviously of import, although it is not possible to do more than allude to these considerations here. It is desirable to point out, however, that the study as a whole represents an application of the broad principle of polymorphism—in its true mycological sense—to infection. These infective gonidia are undoubtedly a form of seed or spore, even though they are not heat resistant. Their capacity only becomes manifest when the conditions for their germination are realized.

But when they germinate as the fusiform bacilli, or the thread form perhaps, they have already passed into a nonvirulent phase. This explains the difficulty with which experimental animals have been infected with organisms from this and similar cases. Epidemics of this infection in the human being have been explained on the *presence* of fusiform bacilli in normal mouths, which does not, of course, explain why epidemics of this—or any other conditions for that matter—are not always with us.

In these alternating cycles of virulence that can be correlated with the botanical characters of microörganisms we have something that helps us to understand the well known cadences of epidemic virulence shown by infectious diseases.

But of first importance is the fact that this botanical correlation may simultaneously involve a change in morphologic type—that is to say, a fusiform infection may actually take origin from a diphtheroid, the latter being the aerobic stage which, when "adapted" to anaerobic conditions, is a fusiform bacillus. We know, too, that the reverse of this change takes place as I indicated on page 490 of my original diphtheroid monograph (Mellon, 1917). In this instance a virulent anaerobic fusiform type of diphtheroid became quite avirulent when it was transformed to its aerobic diphtheroid stage. This organism was referred to as an anaerobic diphtheroid but its ends were distinctly pointed and it was slender and granular. Its aerobic stage was a different morphologic type, but serologically identical with the anaerobe.

Study III of this series (1926) shows an acquisition of marked virulence by a *B. alkaligenes* after it had changed from a bacillary to a streptothrix type of organism; yet the serologic characters had not been altered to any extent. Thus it appears that the acquisition of virulence by a filtrable phase of an organism is but one application of the broad principle of polymorphism; for there is no a priori reason why a filtrable organism should be virulent and the nonfiltrable avirulent. But we so frequently associate the terms "filtrability" and "virus" with virulence that unless we keep in mind the *principle*, we are in danger of using the terms as catchwords.

The recent studies of Vaudremer (1923) indicate that from the tubercle bacillus a mycelial stage can be dissociated in more or less stabilized form. Although non-acid fast it is capable of reverting to an acid fast tubercle bacillus but rather different from the parent form. The organism originates from the normal bacillus as a filtrable non-acid fast form which grows out as a gonidial producing mycelium. He believes it has protective value in guinea pigs.

Vaudremer's results emphasize the inadequacy of interpretation that has been placed on Metchnikoff's well known observation of branching forms for the tubercle bacillus. Its possible taxonomic bearing has been the only significance attributed to it, but the stabilization of such a stage which is different biologically gives Metchnikoff's observation its really significant bearing. Moreover, the *distinctive* taxonomic position which branching gave the tubercle bacilli shrinks into relative insignificance, since present day work indicates that it is not different in this respect from most other bacteria.

This work with the tubercle bacillus parallels our own study with the fusiform bacillus, showing as it does that a *complex* of so-called distinct types really exists in each instance. The growing tendency to place the *B. fusiformis* near the streptothrices is borne out, *in a sense*, by showing experimentally that like the tubercle bacillus it has such a stage in its life history. Furthermore the genetic relations of *B. fusiformis* to other organisms of the anaerobic gangrene group is indicated, for in the lesions of the animals the theta bacillus developed abundantly—as a stabilized stage or a phase—it matters not which, since the potentialities are the same.

Clinically we frequently see cases with mouth lesions resembling a Vincent's angina, but not typical. We are usually able to isolate an organism, fusiform-*like* perhaps, or it may be the theta bacillus. The lesions we regard as a mild Vincent's and usually they run such a course. They do not tend to recur as does the latter. Great differences in respect of chronicity and relapse were noted in our experimental animals.

Since our ordinary bacteria have filtrable phases we are now in a position to ask legitimately whether some of our so-called viruses are not the stabilized stages of our visible bacteria. It appears that these filtrable stages of bacteria cannot be sharply demarcated from a virus. They may both be invisible, extremely difficult of cultivation and more resistant to certain deleterious influences than the vegetative forms of the bacteria. Even the supposedly distinctive resistance of the true viruses to 50 per cent glycerine appears to have been broken down by Miss Evans

who has cultivated the specific green streptococcus of encephalitis from these glycerinized emulsions!

So this is another point where the prevailing distinctions in biologic life may not be valid in the light of the polymorphism of the mycologist. The importance of this point of view has obvious applications, e.g., in the genetic relations of the proteus X-19 to the Ricksettia of typhus fever, a view which is finding an increasing number of proponents among those whose investigations entitles them to an opinion.

A similar situation may exist between the virus of hog cholera and *B. suipestifer*, or between a virus of epidemic virulence in guinea pigs that is constantly associated with a hog cholera-like bacillus (Petri and O'Brien, 1910); and we now know that the typhoid bacillus has a virulent filtrable phase, not ordinarily cultivable, that produces the animal picture and the serologic reactions of typhoid as faithfully as do injections of the visible organism (Friedberger, 1923).

Where a specific type of visible organism is constantly associated with a disease whose existing agent is a virus, the prevailing notion that the visible organism is an unrelated secondary invader will, I fancy, require a degree of modification that will fit more closely the increasing number of facts. A coccus consistently found in the blood stream of patients with an apparent streptothrix or *B. fusiformis* infection is in principle not different from the situations just cited.

Nor is this situation fundamentally different from that of the human disease of blastomycosis. Studies VI and VII of this series (Mellon, 1926) show beyond reasonable doubt that the polymorphism of these fungi finds expressions in vivo and in vitro by a dissociated complex of stabilized types that have proved a stumbling block for the monomorphic etiology of infectious diseases; for even the most reactionary bacteriologist cannot but acknowledge that the blastomycetes of this disease have a real life cycle. The experimental pathogenesis of blastomycosis is notoriously obscure and the probabilities are that the actual infectious phase of this fungus complex that we conventionally isolate is as unknown to us as have been the infective gonidia of *B. fusiformis*.

It may be indeed as Friedberger suggests that the initial infecting stage of all microbic diseases is an invisible uncultivable virus *phase*, and the organisms that we isolate are as often as not the avirulent stage. In this connection it is interesting to recall the classic experiment of Pettenkofer who in the cholera epidemic of 1892 drank pure cultures of the *B. cholera* cultivated from the dejecta of patients, but with negative results; while those drinking water from which no *B. cholera* bacillus could be isolated came down with the disease. Such a paradox may be linked up with the ancient view of those doubting Thomases who believed that the *disease produced the bacteria*, and not bacteria the disease!

However this may be, it is becoming increasingly difficult to dissociate entirely the saprophytes—so-called—of nature from the parasites of living things. The *thinking* epidemiologist is now admitting this point of view to his consideration as evidenced by the excellent paper of Richard Strong read before the Association for the Advancement of Science in Washington two years ago.

CONCLUSIONS

1. Evidence has been adduced that *B. fusiformis* and related organisms often associated with it in infective processes have a filtrable or gonidial phase on which their virulence primarily depends—an infective granule, so to speak.

2. This phase is usually not to be cultivated under the same conditions that enables the parent form to germinate. Some of these forms are probably invisible.

3. The conditions that are suitable for the germination of this phase, particularly in vivo, may result in its cyclic evolution to a totally different stage (bacterial type) in the infectious complex.

4. The various members of this complex, or part of them, may be avirulent in their adult phase, but quite virulent in the filtrable phase.

5. Different orders of these gonidia seem to differ greatly in virulence. Some may be of lesser virulence than the parent form of the organism.

6. The fact that common nonpathogenic organisms may have a virulent, filtrable, but uncultivable phase in their life history, suggests a revaluation in our conception of the viruses, so-called.

7. It also suggests a revaluation in our views as to what constitutes a secondary infection.

8. The latter are obviously of two kinds: *First*, those genetically related to the infective or filtrable form and helping to make up the infective complex, and *second*, chance invaders having no genetic relation to any member of the complex.

9. Such considerations have an obvious and important bearing on current views of epidemiology, infection and immunity.

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