APPENDIX A

Remarks on a Paper Presented by Arthur Kendall, M.D., before a Meeting of the Association of American Physicians in 1932

Thomas Rivers, M.D.

Dr. Kendall's ideas and the results of his work are interesting. The notion, however, that filterable forms occur in the life cycles of bacteria is old. Furthermore, that such filterable forms represent the active agents known as viruses is also not a recent conception, because it has been expressed, from time to time during the past 8 to 10 years, by Nicolle, Kraus, Maudaroy, and others. Although most of Dr. Kendall's ideas are not new, he has, nevertheless used a new medium in attacking problems in this field. This medium is of particular interest to me because Dr. Kendall has suggested that it is suitable for the cultivation of viruses.

We have two problems with which to deal. Firstly, do bacteria have life cycles with filterable forms and has the use of K. medium added information regarding the question? Secondly, do the presumptive filterable forms of ordinary bacteria represent the etiological agents of poliomyelitis, vaccinia, yellow fever, etc., has the K. medium shed light upon the problem, and is this medium suitable for the cultivation of the viruses?

Concerning the first problem I shall say very little. The K. medium appears to be a starvation diet for nonproteolytic bacteria, and Dr. Dubos working in Dr. Avery's laboratory has shown that starvation diets regularly cause certain nonfilterable saprophytic bacteria to become granular, pleomorphic, and filterable. When these bacteria are returned to adequate diets, normal appearances and nonfilterability are resumed by them. It seems unnecessary to assume the existence of life cycles to account for such phenomena.

Rivers, Thomas M. *Tom Rivers: Reflections On a Life In Medicine and Science : an Oral History Memoir.* E-book, Cambridge, Mass.: The MIT Press, 1967, https://hdl.handle.net/2027/heb05734.0001.001.
Downloaded on behalf of 3.14.144.108

Concerning the second problem, namely, the relation of the presumptive filterable forms of bacteria to the viruses, I can speak with more assurance. No one has brought convincing evidence that any of the viruses are capable of growth in the absence of living tissue. In view of Dr. Kendall's suggestion that the K. medium is suitable for the growth of viruses, we proceed to test the importance of the suggestion in regard to the viruses of vaccinia and infectious myxomatosis of rabbits, two agents that we have for several years been successfully cultivating *in vitro* in the presence of surviving tissue suspended in Tyrode's solution or kept in a mixture of Tyrode's solution and serum. Similar amounts of the adequate medium just described, K. medium, Tyrode's solution, and meat infusion broth were inoculated with the viruses. All cultures were made in duplicate, one set of which was incubated at 30°C., and the other at 37°C. At intervals of 5 days, serial transfers of the cultures were made to fresh media and the titer of the viruses in each culture was established by animal experimentation. The experiments were repeated several times and the results are interesting. Multiplication of both viruses occurred in the medium containing bits of living tissue, while no evidence of multiplication was found in any of the other media. In fact, the active agents were not demonstrable in Tyrode's solution or in K. medium after the second transfers. We were particularly interested to find that the virus of vaccinia survived longer, 4 transfers at 30°C., in the meat infusion-peptone medium than it did in K. medium.

Dr. Kendall has also suggested that while viruses are invisible in the K. medium, they become visible when transferred to ordinary broth or to agar mixed with K. medium. We tested this point. The viruses of vaccinia and infectious myxomatosis are in the so-called invisible state when grown in the modified tissue cultures. We attempted to make them become visible by seeding, on K. medium agar, highly active material known to be free from bacterial contaminants. Sets of cultures were incubated at 30° and 37°C., respectively, for two or more weeks and then examined under the microscope for the presence of minute colonies of bacteria. Stained preparations from the surface of the agar were also examined. No evidence of organisms was found.

From the results of our work as well as from the analysis of Dr. Kendall's findings, there is no reason to suppose that the K. medium is capable of supporting the multiplication of the viruses or that its use will throw new light upon the nature of these peculiar agents.

Rivers, Thomas M. *Tom Rivers: Reflections On a Life In Medicine and Science : an Oral History Memoir.* E-book, Cambridge, Mass.: The MIT Press, 1967, https://hdl.handle.net/2027/heb05734.0001.001.
Downloaded on behalf of 3.14.144.108

APPENDIX B

Discussion of Papers on Poliomyelitis by William H. Park, M.D., and Maurice Brodie, M.D., and by John A. Kolmer, M.D. October 1935

Thomas M. Rivers, M.D.

Attempts to protect monkeys against poliomyelitis by means of inactivated virus did not arise with Dr. Brodie. Many investigators before his time made such attempts, and the results were so discouraging that the matter was dropped without pursuing it in man.

The favorable results reported by Dr. Brodie to have been obtained in monkeys admittedly depend upon his ability to titrate accurately and regularly 1 minimum completely paralyzing dose of virus. If this cannot be done, then all of his reported findings are invalid. Insofar as I know, no one has been able to obtain similar titration results, and this is not due to a lack of honest attempts on the part of other workers to do so.

At the beginning of this particular phase of his work, Dr. Brodie used in monkeys 1 or 2 doses of virus treated with 0.1 percent formalin for 12 to 16 hours at 37°C., and, according to him, favorable results were obtained. It is interesting to note that he said that just as good results were obtained with one dose of 5.0 cc. as with 2 doses of 5.0 cc. each. This does not sound reasonable unless both methods of application were without value. Indeed, that may be the case, because Dr. Schultz of California and Dr. Olitsky of New York have been able to show little, if, any, protection in monkeys vaccinated according to Dr. Brodie's method.

Recently, Dr. Brodie has been inclined to agree with others who hold that the complete inactivation of poliomyelitis virus spoils its antigenic qualities, and has been dispensing as vaccines, virus treated for 8 hours and 3 to 5 hours, respectively. He contends that the virus treated for this short

Rivers, Thomas M. *Tom Rivers: Reflections On a Life In Medicine and Science : an Oral History Memoir.* E-book, Cambridge, Mass.: The MIT Press, 1967, https://hdl.handle.net/2027/heb05734.0001.001.
Downloaded on behalf of 3.14.144.108