

lated by Cannon.<sup>1</sup> In the second place, however, the existence of adrenalin in the blood stream is apparently necessary for the maintenance of vasomotor tone itself. The rapid exhaustion of the available supply of adrenalin in the blood stream, obtainable in these experiments appears the factor responsible for the early breakdown of the vasomotor system. Elliott<sup>2</sup> has argued for such a function of adrenalin from evidence of a different character.

Finally the entire evidence of these studies points to a complete dependence on the functional conductivity in the brain stem of both the initiation of the vasomotor effects by the splanchnics, and the increased secretion of adrenalin through which it is maintained. In conditions of the animal when no other responses of the brain stem are being conducted, the vasomotor response also fails to appear.

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**Preliminary report on a typhoid bacteriophage.**

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I would like to report briefly on a lytic principle isolated by the d'Herelle technique from the stool of a typhoid convalescent, kindly sent to me by the Research Laboratory of the Health Department. A small particle of feces was emulsified in broth and incubated overnight. The next day about twice the volume of broth was added and the emulsion was centrifuged and filtered through a Berkefeld. The original filtrate was both inhibitory and lytic, that is, a small amount of the filtrate added to a tube of broth would, in spite of heavy inoculation with the homologous typhoid strain, prevent growth, and young turbid broth cultures became transparent on the addition of small quantities of the filtrate. The lytic principle could then be transmitted in series from both the inhibited and the dissolved cultures.

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<sup>1</sup> Cannon, W. B., 1915, "Bodily Changes in Pain, Hunger, Fear and Rage," pp. 38 and 40.

<sup>2</sup> Elliott, T. R., *Journ. Physiol.*, 1914, xvix, 38.

The lytic principle thus obtained corresponds, for the most part, to those described by d'Herelle. The action is non-specific. It acts on Shiga and Mt. Desert dysentery cultures, as well as on the homologous strain of typhoid and on other typhoid strains. It has no action on the strains of Para A and B that I have tried, and I have not undertaken any experiments to see whether the lytic principle could become acclimatized to these organisms as described by d'Herelle. It also has no action on *B. Coli communis*, or *communior*. It does not seem to dissolve or inhibit Gram positive organisms such as the pneumococcus.

It is fairly thermostable. It is not destroyed by a temperature of 70° C. for 30 minutes. It loses its activity, however, after an exposure at 75° C. for 30 minutes. The lytic principle does not maintain its activity for any length of time in sterile broth, and cannot be transmitted in series in this medium. It does not dissolve killed cultures, whether killed by heat or by the action of ether, and is not transmissible from killed cultures. The lytic principle, furthermore, does not persist and cannot be transmitted in Berkfeld filtrates of young typhoid cultures, which contain a certain amount of bacterial protein in solution. Actively growing young typhoid cultures are essential for the activity of the lytic principle.

The dissolved or inhibited cultures usually do not become absolutely sterile. If subcultures are made, it will be found that the control will give a typical confluent growth, whereas the tubes containing the lytic principle will give a small number of discrete colonies, usually occurring at the very margin of the slant. These discrete colonies are usually of two types, one the round typical typhoid colony, the other an extremely irregular jagged colony. If these two types are fished to broth, it will be found that the fishing from the round colony will cloud the broth, whereas the fishing of the irregular colony will often remain clear after 12 to 18 hours in the incubator. The lytic principle can be transmitted in series from the broth fishing of the irregular colony in the same way as from the original stool filtrate. Two types of colonies are also obtained from dissolved or inhibited Shiga and Mt. Desert cultures, one the bearer of the lytic principle, the other apparently a normal colony. The lytic action of these irregular

colonies whether they be derived from a typhoid, Shiga or Mt. Desert cultures act in the same way. The lytic principle in the experiments thus far has showed no variations due to the fact that it was carried along by typhoid or dysentery cultures. I have, therefore, worked almost exclusively with the derivatives of dissolved typhoid cultures. The stock typhoid and dysentery cultures used in these experiments have been repeatedly streaked out without obtaining the irregular type of colony which is the bearer of the lytic principle. This type of lytic colony has, as far as I know, not been described by d'Herelle, but was first reported by Bordet in connection with the lytic principle that he was able to produce in the peritoneum of guinea pigs by repeated injections of *B. Coli*.

If one of these irregular colonies, whether typhoid or dysentery, is streaked out, a certain number of typical round colonies develop which, when fished into broth, will make the broth turbid. Typical colonies have never, in my experience, given anything but typical colonies on restreaking. Usually on restreaking an irregular "lytic" colony the majority of colonies obtained will be of the lytic variety, although I have found it very difficult to gauge the proportion of typical and lytic colonies that will be obtained from any given lytic colony. If a series of lytic colonies are obtained in this way in a row, it will be found, on examining under the microscope, that there are often minute transparent masses between the lytic colonies, and have been called "appearances" by previous observers. On examining the irregular lytic colonies under the microscope it will be found that the lytic colonies owe their irregular shape to the fact that their edges have faded out into these transparent "appearances." It is impossible to predict the amount of "appearances" that will be obtained by restreaking lytic colonies, and varying degrees of transparency occur in the "appearances." Fishings of the most transparent type of "appearances" have failed in most instances to produce any growth on a variety of media. When growth did occur it proved to be colonies of the lytic type. Comparative fishings of lytic colonies and "appearances" into young turbid broth cultures of typhoid were made to see if the lytic principle was carried by the "appearances"; the former usually became

transparent after several hours incubation, whereas, the latter, except in rare instances, failed to clear up. The few cases where the addition of "appearances" to the turbid broth cultures seemed to exert a dissolving action can, in my opinion, best be interpreted as lytic colonies almost completely dissolved, but which still contained a small number of living bacilli carrying the lytic principle. All attempts to find a definite structure in the "appearances" by different methods of staining have failed. Plates have been observed at frequent intervals, to see if the amount of "appearance" increased, but this was never the case. Whether the "appearances" do not increase because the lytic principle dissolves up all the susceptible bacilli present as fast as they grow, and reduces them to "appearances," and has no action on the so-called resistant bacilli which form the bulk of the lytic colony, is still to be determined. On the other hand, it might be argued that the "appearances" do not spread on further incubation because by that time the typhoid bacilli have grown too old to be susceptible to the action of the lytic principle. From my observations to date, although very incomplete, I have found nothing to indicate that these "appearances" represent living structures, they suggest much more that the same phenomenon which is indicated by the clearing of the broth when the lytic principle is acting in fluid media occurs also on solid media.

It seems possible to me that when a fluid culture is rendered transparent it means that the susceptible bacilli present in the originally turbid emulsion are reduced to the state of "appearances." A chemical analysis of the end product of the dissolved culture compared with a similar analysis of the "appearances" if they can ever be obtained in sufficient quantity, ought to prove this point. I have experiments of this nature planned at the present time.

The potency of this lytic principle has not increased from one generation to another as indicated by d'Herelle in certain instances but has remained the same over a period of three months. The original stool filtrate, dissolved culture derivatives, derivatives of broth fishing of lytic colonies all appear to be about equally active. I have carried out experiments to see whether the potency of the lytic principle depended to any extent on the amount of

protein dissolved, and have, therefore, compared the lytic action of an inhibited culture with a freshly dissolved culture. In the former case the amount of bacterial protein inoculated was very small compared to the amount of bacterial protein in the turbid culture. After 4 hours when the turbid culture had become transparent and the inhibited culture was clear, whereas, the control for the inhibition experiment already showed definite growth, both tubes, the dissolved and inhibited culture, were filtered and the lytic action of each determined in a series of dilutions. There was practically no difference in the activity of the two tubes in the interval that the tubes were observed. I intend to repeat this experiment, using a greater range of dilutions and observing at more frequent intervals. However, the amount of bacterial protein dissolved does not seem to influence the activity of the lytic principle very strikingly.

This lytic principle is very active in a dilution of 1-10, dissolving a turbid culture in from 2 to 4 hours. In a dilution of 1-100 the culture is usually dissolved in 12 hours. But in most cases the balance between the lytic action and the overgrowth by the resistant bacilli is temporary. Sooner or later, in most instances, the resistant bacilli win out, and make the transparent culture cloudy again. I have made fishings of the resistant types and tried the action of the lytic action on these bacilli. I have found that it often takes longer for the lytic principle to dissolve a resistant type than to dissolve the stock culture, but that, eventually, it seems to clear up a culture of this sort also. The resistant culture, on transplanting, seems to lose its resisting ability, but I have not finished working on this point.

I have tried to find a temperature where the lytic principle was still active and the bacilli could no longer multiply, so that, if the tubes had once become transparent, they would remain so. I have found that the lytic principle acts more quickly at a temperature of 41°-42°, a turbid culture will become transparent in half the time required for a similar tube at 37° C. These experiments are still under way, and it is very much of a question whether it will be possible to get the lytic principle to work where there are no actively growing bacilli. Between 45° and 50° the lytic principle is not active.

The most striking single fact about these lytic principles is that they are only active when added to young growing cultures. I obtain the best results in lysis experiments where I wash up the growth of a young agar culture in broth and then add enough of this heavy emulsion to 10 c.c. of sterile broth tube to make it definitely cloudy. A large amount of unused culture fluid media favors the reaction enormously. I have tried adding drops of a heavy young typhoid emulsion to a freshly dissolved transparent culture until it is again turbid, but the lytic principle which can be demonstrated to be active on other turbid young broth cultures in a dilution of 1-100, will be unable to dissolve 0.2 or 0.3 c.c. of a young typhoid emulsion in 10 c.c. of lytic principle unless fresh nutritive material is added.

Another extremely important fact about this lytic principle (similar observations have been made by other workers), is that one single contact with the lytic principle is sufficient to divide a normal culture into two types, one the typical colony, the other atypical carrying the lytic property. This can be demonstrated both in fluid and in solid media. If the lytic principle is added in a dilution of 1-10 to a turbid culture, and the culture is shaken and plated immediately, the two types, in some instances, will be obtained. If an active filtrate is allowed to drop on a young agar growth of typhoid, the culture will be dissolved at this point, and, if the plate is incubated for another day, a few lytic colonies may develop in this area. The so-called resistant bacilli must be present in the original culture, together with the susceptible bacilli, since, in the case where the broth culture is plated immediately, the resistant bacilli have not had time to become hardened to the action of the lytic principle.

The above findings have simply been enumerated without any attempt to develop a theory. The data on the subject is still accumulating too rapidly for me to take definite sides.