STUDIES ON THE BACTERIOPHAGE OF D'HERELLE.

I. Is the Lytic Principle Volatile?

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The lack of adequate direct evidence as to the nature of the active principle causing transmissible lysis is responsible for the existence of numerous hypotheses attempting to explain the mechanism of the bacteriophage phenomenon.

The earliest mention of the phenomenon is found in a paper by Twort.[1] He observed spontaneous transmissible lysis of micrococci isolated from vaccine virus regarding which he says: "it might almost be considered as an acute infectious disease of micrococci." However, it was d'Hérelle[2] who made the first systematic studies and elaborated this conception. He ascribed the lytic function to a living, filterable, ultramicroscopic microorganism parasitizing and rapidly multiplying at the expense of actively growing bacteria. As against this view, Bordet and Ciuca as well as Kabéshima early interposed a conception that the lysis is due to autolytic changes in bacteria themselves. Thus, according to Bordet and Ciuca[3] transmissible lysis is the result of hereditary vitiation of bacterial metabolism resulting in autolysis of cells so affected. These authors have shown that this supposed vitiation may first be caused to appear in normally growing bacteria by such extraneous influences as the action of leucocytes upon normal bacteria when the latter are introduced into the peritoneal cavity of normal guinea pigs. Using such agents as distilled water or protoplasmic poisons,[4] other investigators have induced similar changes in bacterial cultures. According to Kabéshima[5] the progressive autolysis is due to the activation of a normal proferment existing in bacterial cells. The work of Borchardt,[6] Kuttner,[7] and Hadley[8] strengthens this

[1] Twort, F. W., *Lancet*, 1915, ii, 1241.

[2] d'Hérelle, F., *Compt. rend. Acad.*, 1917, clxv, 373.

[3] Bordet, J., and Ciuca, M., *Compt. rend. Soc. biol.*, 1920, lxxxiii, 1293, 1296.

[4] Otto, R., and Winkler, W. F., *Deutsch. med. Woch.*, 1922, xlviii, 383.

[5] Kabéshima, T., *Compt. rend. Soc. biol.*, 1920, lxxxiii, 219.

[6] Borchardt, W., *Z. Immunitätsforsch., Orig.*, 1923, xxxvii, 1.

[7] Kuttner, A. G., *J. Bact.*, 1923, viii, 49.

[8] Hadley, P., *J. Infect. Dis.*, 1924, xxxiv, 260.

view—particularly that of Hadley, who pointed out the close relationship between the various enzymotic activities of bacterial cells and the process of transmissible lysis.

Bail[9] ascribes the progressive lysis to the effect of transmissible, degenerative changes in the bacterial chromosomes, and Doerr[10] believes it due to the extrusion from bacterial cells of a "growth hormone." In addition to the conditions already mentioned, which are capable of inducing the appearance of transmissible lysis, Doerr[10] stresses the accumulation of metabolic products in the environment. Other authors [11-13] record the influence of the factor of aging. Once the change appears, its products induce similar processes in gradually increasing numbers of susceptible bacteria, thus giving the impression of growth ascribed to bacteriophage by the vitalistic hypothesis of d'Hérelle.

Whatever the nature and the cause of the initial appearance of the active lytic principle in a culture, it has been assumed by the various authors cited that lysis of bacteria is essential and in fact precedes any increase in concentration of the active principle in the medium. However, Otto and Munter[14] have pointed out that this is not necessarily the case, since the observations of Doerr and Berger[15] as well as those of Meuli[16] indicate that the lytic titer of a culture may increase without any coincident lysis of susceptible bacteria. These authors suggest that the lytic principle may be of the nature of a receptor which, though usually bound to the cell, may under certain conditions be set free into the environment, while the cell, depending upon circumstances, may or may not undergo lysis.

All the attempts to explain the intimate mechanism of the phenomenon of transmissible lysis involve hypotheses for which direct evidence is lacking. Recently, Olsen and Yasaki[17] seem to have offered for the first time evidence which, if confirmed, would speak incontestably against the animate nature of the active principle of lysis.

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[9] Bail, O., *Wien. klin. Woch.*, 1922, xxxv, 765.

[10] Doerr, R., *Klin. Woch.*, 1922, i, 1489, 1537.

[11] Bordet, J., *Compt. rend. Soc. biol.*, 1924, xc, 96. Weinberg, M., and Aznar, P., *Compt. rend. Soc. biol.*, 1922, lxxxvi, 833; lxxxvii, 136. Lisbonne, M., and Carrère, L., *Compt. rend. Soc. biol.*, 1924, xc, 265. Otto, R., and Munter, H., *Deutsch. med. Woch.*, 1921, xlvii, 1579.

[12] Pico, C.-E., *Compt. rend. Soc. biol.*, 1922, lxxxvii, 836. Fejgin, B., and Supniewski, J., *Compt. rend. Soc. biol.*, 1923, lxxxix, 1385.

[13] Gildemeister, E., and Herzberg, K., *Centr. Bakt., 1. Abt., Orig.*, 1923–24, xci, 12.

[14] Otto, R., and Munter, H., *Ergebn. Hyg., Bakt., Immunitätsforsch., u. exp. Therap.*, 1924, vi, 592.

[15] Doerr, R., *Schweiz. med. Woch.*, 1923, liii, 1009. Doerr, R., and Berger, W., *Z. Hyg. u. Infectionskrankh.*, 1923, xcvii, 422.

[16] Meuli, H., *Z. Hyg. u. Infectionskrankh.*, 1923, xcix, 46.

[17] Olsen, O., and Yasaki, Y., *Klin. Woch.*, 1923, ii, 1879.

Subjecting an 18 hour lysed culture of bacteria to distillation at 45–50°C. under reduced pressure, these authors claim to have collected a distillate capable of propagating the lysis in series. They state that the distillate is slightly alkaline and gives a reaction for ammonia. The active substance present in the distillate forms a fairly stable solution in broth but is easily redistilled from its watery solution even at low temperatures. They conclude, therefore, that the so called bacteriophage is a volatile chemical substance.

In attempting to repeat the work of Olsen and Yasaki we closely followed the description of the procedure given by them.[17]

100 cc. of an 18 hour lysed culture of *B. coli* were placed in a side arm distilling flask and immersed in a water bath maintained at 45–50°C. The volatile products from this flask were received and condensed in a second similar side arm distilling flask which, in turn, was connected with a vacuum pump through a wash bottle containing 25 cc. of sterile broth, also imbedded in ice and used for the purpose of retaining any products of distillation which might have failed to condense in the second flask. At the end of distillation the contents of each flask were brought to the volume of the original lysate subjected to distillation (100 cc.) and titrated for lytic activity by the method of Appelmans.[18] With slight variations this experiment was repeated several times, with both the whole lysed cultures and the filtrates as the substratum for distillation. In a certain number of experiments a capillary air stream was allowed to flow through the system; in others the system was maintained under a negative pressure of from 30 to 90 mm. of mercury. The distillation time varied from 1 to 5 hours and in two experiments it was continued to complete dryness of the lysate. The results of all these experiments were essentially the same, varying only quantitatively. The findings given in Table I are expressed in terms of the minimal amount of fluid capable of causing lysis in 10 cc. of broth culture.[18] They represent the results of an experiment which was continued for 4 hours and yielded a distillate with a higher titer than that obtained in any other of this series of experiments.

TABLE I.

	Lysate.	Distillate.	Contents of wash bottle.	Original lysate control.
	cc.	*cc.*	*cc.*	*cc.*
Original volume	100.0	0.0	25.0	—
Volume after distillation	70.0	28.0	26.0	—
Diluted with broth to	100.0	100.0	100.0	—
Titer (by Applemans' method)	1×10^{-10}	1×10^{-5}	Not active in 1×10^{-1}.	1×10^{-10}

[18] Appelmans, R., *Compt. rend. Soc. biol.*, 1921, lxxxv, 1098.

As can be seen from the table, the distillate usually exhibited appreciable activity, whereas the contents of the wash bottle were invariably inactive in the largest amount used in titration.

It is to be noted that in spite of the exposure to prolonged heating at 45–50°C. and in spite of transfer of some of the active material to the distillate, the titer of the original lysate did not undergo sufficient change to be detected by the tenfold dilution method of Appelmans used for titration.

In view of the statement of Olsen and Yasaki that the active principle gives the reaction for ammonia, we attempted to increase the rate of its volatilization by rendering the lysate alkaline with NaOH

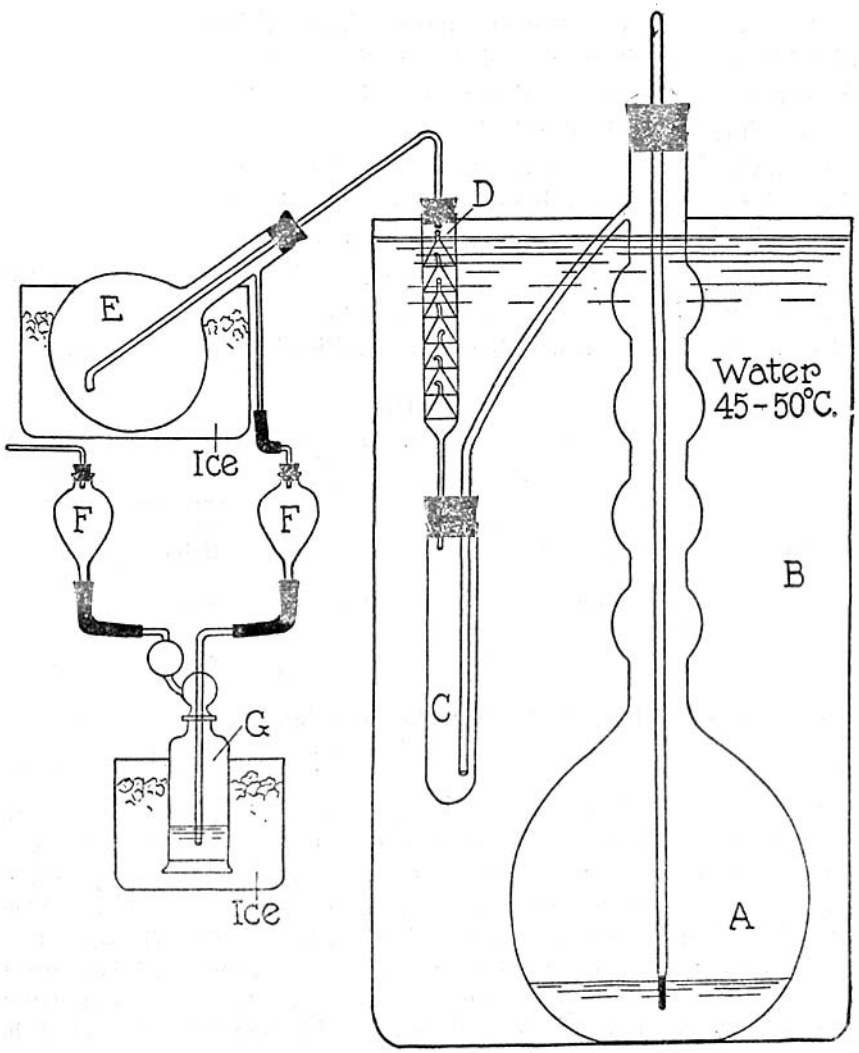
TABLE II.

	Lysate.	Trap washings.	Contents of condensing flask.	Contents of wash bottle.	Original lysate control.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
Original volume.....	100.0	0.0	0.0	25.0	—
Volume after distillation.....	4.5	0.0	95.0	25.0	—
Diluted with broth to...	100.0	100.0	100.0	100.0	—
Titer (by Applemans' method).....	1×10^{-10}	1×10^{-5}	1×10^{-1}	Not active in 1×10^{-1}.	1×10^{-10}

prior to distillation, but failed to obtain the desired results. The titer of 1×10^{-5} cc. is the highest obtained by us in any of these experiments, except in cases in which we knew definitely that some of the original lysate had grossly contaminated the contents of the distilling flask through an accident.

Since the activity of the distillate at its highest was 100,000 times weaker than that of the original filtrate and since the original titer of the lysate was not measurably affected by distillation, we were inclined to suspect droplet infection of the distillate rather than actual volatility of the lytic agent. This possibility was considered also by Olsen and Yasaki, only to be excluded on the strength of the observation that trypaflavine added to the lysate before distillation could not be detected in the distillate, which would not be the case, the authors thought, if the colored lysate were transported directly into the condensing flask.

In our attempts to exclude the possibility of droplet infection of distillate, we again repeated the distillation experiments, modifying the procedure by inserting a trap between the flask containing the



TEXT-FIG. 1.

lysate and the condensing flask. At first this trap consisted of a large glass tube (2 inches in diameter and 8 inches long) which was placed vertically, with the inlet connected with the flask containing the

lysate at the bottom and the outlet connected with the condensing flask at the upper end of the tube. This trap was maintained at 45–50°C. so as not to cause condensation of volatile products rising from the lysate. At the conclusion of the experiment this trap was washed with 100 cc. of sterile broth and the washings were titrated along with the contents of the condensing flask as before. The results of such an experiment are given in Table II.

Even the simple trap used in these experiments apparently retained the major part of the active substance which, in earlier experiments, was carried into the distillate. Since, however, some of the lytic agent was still present in the distillate, a more efficient trap was set up by interposing a series of inverted funnels between the inlet and the outlet of the trap. The complete set-up used is shown in Text-fig. 1.

TABLE III.

	Lysate.	Trap washings.	Contents of condensing flask.	Contents of wash bottle.	Original lysate.
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
Original volume.....	50.0	—	—	25.0	—
Volume after distillation.....	4.0	—	43.0	28.0	—
Diluted with broth to.....	50.0	50.0	50.0	50.0	—
Titer (by Applemans' method)....	1×10^{-10}	1×10^{-4}	Not active in 1×10^{-1}.	Not active in 1×10^{-1}.	1×10^{-10}

A Ladenburg distilling flask of 2 liter capacity (*A*) containing 50 cc. of lysate was placed in a water bath (*B*) kept at 45–50°C. and the side arm of the flask was connected with an inlet at the bottom of the trap (*C*). The latter comprised, in addition to the simple trap used previously, a large glass tube (3 by 8 inches) beyond it in which were placed seven small inverted funnels (*D*). The whole trap was kept at a temperature of from 45–50°C. The outlet at the top of the trap was connected with a condensing flask immersed in ice (*E*). The side arm of the latter was connected with a wash bottle (*G*) (imbedded in ice) containing 25 cc. of broth. Between the condensing flask and the wash bottle as well as between the wash bottle and the vacuum pump there were placed bulb traps (*F*) intended to prevent contamination between the flasks in case of the foaming of broth.

The results of one of these experiments are summed up in Table III.

This experiment was repeated ten times and, without a single exception, both the distillate and the contents of the wash bottle failed to show lytic activity.

Since the results of these experiments were first reported[19] there have come to our attention several papers[20] dealing with the same subject and similarly failing to confirm the findings of Olsen and Yasaki. However, quite recently Olsen and Yasaki have reaffirmed their original findings[21] on the basis of further experiments.

CONCLUSION.

The lytic principle concerned in the phenomenon of transmissible lysis is not volatile. The results which have been taken to indicate volatility are, in our opinion, to be attributed to the transfer to the distillate of minute droplets of the original active filtrate.

[19] The experiments described above were conducted during October and November, 1923, and a preliminary report of results was made at a meeting of the Society for Experimental Biology and Medicine (Bronfenbrenner, J., and Korb, C., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, xxi, 175, 177).

[20] Doerr, R., and Rose, G., *Schweiz. med. Woch.*, 1924, liv, 10. Meissner, G., *Centr. Bakt., 1. Abt., Orig.*, 1924, xcii, 424. Gildemeister, E., and Herzberg, K., *Klin. Woch.*, 1924, iii, 186. d'Hérelle, F., *Compt. rend. Soc. biol.*, 1924, xc, 27. Borchardt, W., *Klin. Woch.*, 1924, iii, 278. Spät, W., *Med. Klin.*, 1924, xx, 184.

[21] Olsen, O., and Yasaki, Y., *Z. Hyg. u. Infectionskrankh.*, 1924, cii, 540; *Klin. Woch.*, 1924, iii, 278.