washings no longer gave a test for the heavy metal ion, a residue remained very similar in appearance to heat coagulated or denatured albumin and which after ignition did not give a positive test, for the heavy metal ion. The precipitation of albumin in concentrated salt solutions was not immediately complete but precipitate continued to form for a period of time which would indicate a secondary reaction or denaturing of the albumin. The precipitation with concentrated solutions of heavy metal salts is probably a salting out of the albumin followed by denaturing of the protein.

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Is the bacteriophage of d'Herelle volatile?

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Following the description of the phenomenon of transmissable lysis of bacteria by d'Herelle in 1917, an enormous amount of evidence has been brought forward in support of his observations.

At the same time, however, there has also been an ever-growing tendency to dispute the parasitic nature of the active principle responsible for the lysis as postulated by d'Herelle.

In this connection, recent findings by Olsen and Yasaki[1] appeared to offer most important evidence which we attempted to corroborate.

Subjecting 18 hour cultures of so-called "bacteriophage" to distillation at 45-50° C. under reduced pressure, Olsen and Yasaki claim to have obtained a product capable of initiating transmissible lysis of cultures of susceptible bacteria. They conclude, therefore, that the "bacteriophage" is a volatile chemical substance and not a living parasite. Following their description of the experiment, we connected three flasks in series. The first,

[1] Olsen and Yasaki, *Klin. Woch.*, 1923, xli, 1879.

a distilling flask of one liter capacity, received 100 cc. of the filtrate from an 18 hour lysed culture of the colon bacillus (having a titer of 5×10^{-10} cc.) and was placed in a water bath kept at 45° C. With this was connected a similar distilling flask containing enough sterile distilled water to submerge the opening of the side-arm of the first distilling flask. This second flask was kept cool by means of an ice bath and was connected with a vacuum pump through a wash-bottle containing 25 cc. of sterile broth, also kept under ice.

Distillation was continued for from one to four hours in the three preliminary experiments with the result that in each case the second distilling flask yielded a distillate active against susceptible culture (lytic titer of from 1×10^{-4} cc. to 1×10^{-5} cc.).

The fact, however, that the titer of the original solution remained practically unchanged after distillation suggested that the activity of the distillate was due to droplet infection rather than to volatility of the active principle. This possibility was considered also by Olsen and Yasaki, but they finally decided that they were not dealing with droplet infection, since upon the addition of trypaflavin to the filtrate, they failed to obtain evidence of the dye in the active distillate. Instead of relying upon such indirect evidence, we decided to set up the apparatus in such a way as to exclude the possibility of the transfer of droplets. Accordingly, between the first (distilling) and the second (condensing) flasks a series of inverted funnel-shaped traps was interposed at a temperature of 45° C., so that any substance volatile at this temperature would be carried into the second flask, but any liquid particles would be filtered out of the current by the repeated impact against the glass surface.* In addition to titration of the distillate as well as of the residue, the traps were washed in measured amounts of sterile broth after each distillation, and washings thus obtained were titrated for their lytic activity. All the glassware and rubber used in these experiments was thoroughly sterilized by heat before use. Five distillations were carried out for varying lengths of time—two of them until complete dryness of the original filtrate. In all cases the distillates showed no activity whatever, the trap washings contained varying amounts of active lytic principle, and the residue retained

* It has occurred to us that a long tube filled with glass wool might be used in place of funnel traps.

its original activity, practically unchanged, even when distillation was carried to dryness. Our experiments fail to indicate volatility of the lytic principle of d'Herelle at 45-50° C.

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Effect of alcohol on the so-called bacteriophage of d'Herelle.

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D'Herelle[1] has reported that by precipitating a culture of antidysentery bacteriophage with nine volumes of 96 per cent alcohol, decanting the supernatant fluid after 48 hours and re-dissolving the precipitate in saline, he obtained a substance slightly lytic for dysentery bacilli (titer 5 cc.). This lytic action, however, was not transmissible in series. D'Herelle concluded that the alcohol, while destroying the living ultra-microbe (bacteriophage), had precipitated its endo-enzyme.

Hauduroy,[2] repeating these experiments, obtained similar results by precipitating sterile bouillon with alcohol. He decided that the apparent lytic action of the precipitate obtained by d'Herelle was due to a bacteriostatic effect of the alcohol adsorbed by this precipitate. Arnold[3] was not able to obtain any active substance by precipitation with alcohol. More recently, Appelmans[4] failed to destroy completely the lytic activity of bacteriophage by exposure of bacteriophage to 50 per cent alcohol, but he does not state whether the activity of the precipitates was transmissible in series.

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1 D'Herelle, F., The Bacteriophage, English Trans., Williams & Wilkins Co., 1922, 123.

2 Hauduroy, P., Sur les Lysins du Bacteriophage d'Hérelle. *Compt. rend. Soc. Biol.*, 1922, ii, 964.

3 Arnold, L., Basteriophage Phenomena. *J. Lab. and Clin. Med.*, 1923.

4 Appelmans, R., Le Dosage du Bacteriophage. *Compt. rend. Soc. Biol.*, 1921, ii, 1098.