The isolation of these organisms strengthens our already expressed belief that the respiratory flora of the Polar Eskimos is very similar to that of persons living in warmer climates without such a great degree of isolation.

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The Heat Inactivation of Bacteriophages.*

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In testing the therapeutic value of the lysed cultures of *B. diphtheriae*, it became necessary to destroy the slight amount of toxin present with bacteriophage in some of the filtrates. Since bacteriophage is comparatively resistant to heat it was thought that simple exposure to heat may destroy the toxin without destroying the phage present in the lysates. This, however, we were not able to accomplish. Exposure to heat sufficient to destroy all the toxin caused almost complete destruction of the phage. In a study of the mechanism of inactivation of phages by alcohol we concluded that the effect of alcohol was not due to the direct destruction of the active agent, but to the denaturation of the protein vehicle on which the lytic agent was adsorbed.[1] Suspecting that somewhat similar relation may exist in the inactivation of phages by heat, we attempted 2 series of experiments.

In the first we made use of our earlier finding that the addition of polyvalent cations to the medium containing phage protected it from inactivation by alcohol.[2] We found that the addition of enough $CaCl_2$, for instance, to bring its concentration between 0.002 m and 0.015 m, some degree of protection against heat inactivation may be secured. Optimum concentration of $CaCl_2$ differs with each phage, and it thus becomes necessary to find a suitable concentration for each phage and even for each batch of the same phage, a laborious and time-consuming procedure.

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[1] Bronfenbrenner, J., and Korb, C., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 177; *J. Exp. Med.*, 1926, **43**, 71.

[2] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 187.

In later experiments we used another method suggested by Spiro that glycerin, carbohydrates, urea, urethan, etc., prevent denaturation of proteins during coagulation.[3] Recently these and other substances have been used repeatedly for the protection of antibodies and of bacterial antigens against denaturation by heat. In employing a number of these "anticoagulants"[3] we succeeded in some instances in preserving a considerable degree of activity of phages during more or less prolonged exposure of the filtrates to 70°C.

In general the degree of protection varied not only among phages of different valency but among different samples of phages of similar valency. The degree of protection for each phage, in the case of polyvalent phages, is independent of the bacterial substratum used in their preparation. This relation is brought out by comparing the results secured with PC-coli and PC-Shiga phages, or Laudman-coli and Laudman-Shiga phages respectively as illustrated on Table I. In each case one volume of phage was diluted with 3 volumes of broth and 3 volumes of saturated solution of sucrose respectively. The mixtures were titrated before heating and after heating for different periods of time in sealed ampules submerged entirely under water kept at 70°C. The results represent an average of at least 5 independent experiments and are expressed in terms of the smallest fraction of a cc. exhibiting lytic activity. Essentially similar experiments were performed using dextrose, glycerine, sodium salicylate, urea and Bayer 205 in place of saccharose with varying degrees of success.

While prolonged preliminary incubation of bacteria with some of these substances (glycerine or saccharose, for instance), may render them somewhat more resistant to heat, the exposure of bacteria to heat under the conditions exactly duplicating those under

TABLE I.

	Before heating		At 70°C. for 20 min.		At 70°C. for 30 min.		At 70°C. for 45 min.	
	Broth	Sucrose	Broth	Sucrose	Broth	Sucrose	Broth	Sucrose
Laudman phage—*B. coli*	10^{-9}	10^{-9}	10^{-4}	10^{-6}	10^{-2}	10^{-5}	0	10^{-4}
" " —*B. Shiga*	10^{-9}	10^{-9}	10^{-3}	10^{-5}	10^{-1}	10^{-5}	0	10^{-4}
P. C. phage—*B. coli*	10^{-9}	10^{-9}	10^{-1}	10^{-6}	0	10^{-5}		
" " —*B. Shiga*	10^{-9}	10^{-9}	10^{-1}	10^{-5}	0	10^{-4}		
Megatherium phage	10^{-9}	10^{-9}	10^{-2}	10^{-6}	0	10^{-4}		
Friedlander phage	10^{-9}	10^{-9}	10^{-5}	10^{-8}	0	10^{-4}		
Diphtheria M 1314 phage	10^{-9}	10^{-9}	10^{-3}	10^{-5}	0	10^{-1}		
" Philippi	10^{-8}	10^{-8}	0	10^{-6}				
Staphylococcus H.	10^{-8}	10^{-8}	0	10^{-3}				
Typhosus phage	10^{-9}	10^{-9}	0	10^{-6}				

[3] Spiro, K., *Zeit. f. Physiol. Chem.*, 1900, **30**, 182; *Hofmeister's Beiträge*, 1904, **4**, 300.

which phages were exposed failed to indicate any protective effect of "anticoagulants" against destruction of bacteria by heat (70°C.). Thus these findings suggest the possibility of employing heat in the presence of saccharose as a means of isolating phages from fecal cultures and from other natural sources instead of employing filtration for the purpose of removing bacteria.

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Effects of "K" Medium on the Filterability of Bacteria.

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Kendall employed a culture medium[1] consisting presumably of whole undenatured protein, devoid of all products of digestion which he referred to as peptones. He reported the transformation of ordinary bacteria into primitive forms capable of passing filters presumably impervious to the passage of normal bacteria.[2] Kendall concluded that he was dealing with unusual forms of bacteria, not only on the basis of their filterability, their appearance under the dark-field microscope, and their irregular staining, but also because of his inability to obtain sub-cultures by transplantation of the "filterable" forms to ordinary media, whereas sub-cultures were obtained by similar transplantation to his own, so-called K medium.

We here summarize some of our experiments to acquaint ourselves with this apparently new and important phenomenon.

The appearance of viable and cultivable bacteria in the filtrates of bacterial cultures has been repeatedly recorded. It is generally understood, that the mere passage of cultivable bacteria through filters does not predicate any radical change in their original size. It has been repeatedly shown that under a variety of conditions, normally effective filters may become permeable to bacteria of ordinary and even large size.

Even the first few experiments convinced us that in the case of cultures grown on K medium, the frequency of passage and the numbers of viable units of bacterial protoplasm appearing in the filtrates at each filtration were considerably greater than in the case

[1] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32.**

[2] Kendall, A. I., *Northwestern Univ. Bull.*, 1931, **32.**