explanation for its potentiating action. These findings indicate that the problem of potentiation of toxicity of organic phosphate insecticides extends beyond those agents commonly used for insecticidal purposes.

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Temperature Tolerance and Kinetics of Thermal Inactivation in *E. coli communior* Phage of Various Concentrations.* (24669)

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Most studies of thermal inactivation of bacteriophages and of other viruses have indicated that the reaction is exponential over a wide temperature range and follows the first order equation $n/n_o = e^{-kt}$(1-4). This paper describes the thermal inactivation of an *E. coli communior* phage in peptone solution; under certain conditions, the kinetics of its destruction appear to deviate from the above relationship.

Materials and Methods. E. coli communior phage(5) suspended in a peptone solution (Difco Bacto-Peptone, 1%, NaCl, 0.5%, pH 7.6) was treated as follows. One ml aliquots of undiluted phage and of samples diluted 1/100 and 1/1000 in peptone solution were either dried *in vacuo* or stored as liquid preparations. For the inactivation studies, samples were heated in a water bath controlled to $\pm$ 0.5°C. The number of particles surviving 30 minutes at a given temperature was determined. In addition, estimates were made of survivors after varying times at different temperatures. The rate constant, K, was calculated from the data. The phage counts were made by the Adams technic(6).

Results. With the dried samples, a linear relationship was found between the logarithm of phage destroyed in 30 minutes and the temperature and this was independent of the initial phage concentration (Fig. 1a). On the other hand, the wet preparations behaved differently. The curves for the inactivation "rate" (logarithm of phage destroyed/30 min) plotted against temperature appeared to show a sigmoidal shape, (Fig. 1b, $1b_1$, $1b_2$). With undiluted phage (Fig. 1b) the change in inactivation rate was exponential between 50° and 75°C. Above this, there was a significant decrease in rate change/degree centigrade. In samples diluted 1/100 and 1/1000 (Fig. $1b_1$ and $1b_2$), the phage was inactivated exponentially between 55°-75°C and 58°-75° C respectively; in each case the rate change reached a plateau beyond 75°C.

The kinetics of heat inactivation in wet (undiluted) and dry conditions were studied by plotting logarithm n/n_o (the fraction of phage survivors), at any given temperature, against time, t, in seconds. In the dry state,

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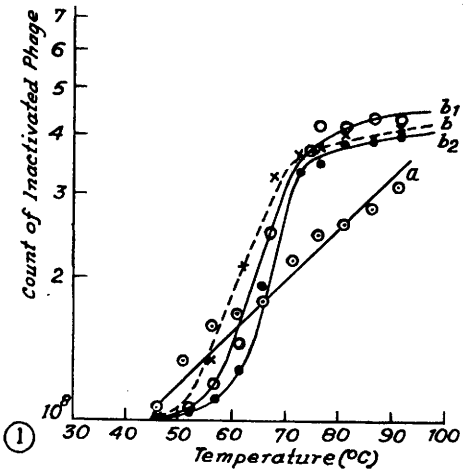
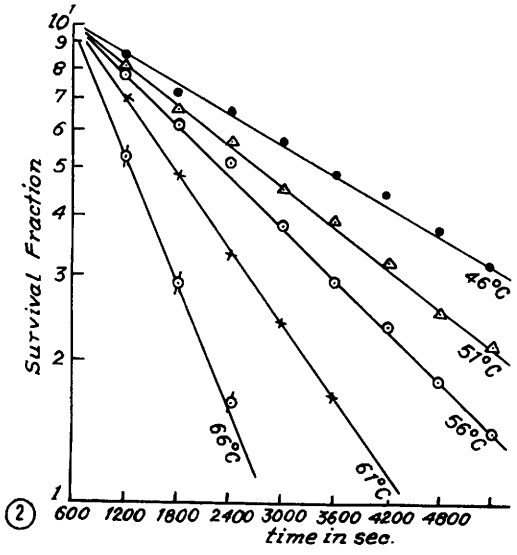
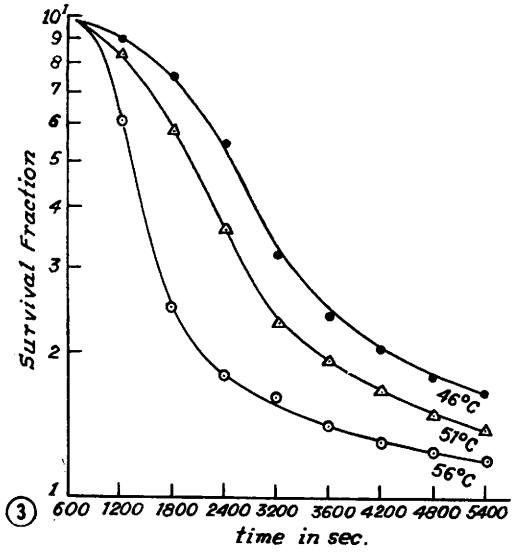


① FIG. 1. Effect of temperature on inactivation of the bacteriophage. (Each point is the mean of ten readings.)



② FIG. 2. Inactivation of dry phages as a function of time at various temperatures. (Each point is the mean of ten readings.)



③ FIG. 3. Inactivation of bacteriophages in broth (undiluted) as a function of time at various temperatures. (Each point is the mean of ten readings.)

TABLE I. Rate Constants of Dry Phage Samples at Different Temperatures.

Sample	Temp., °C	Time when $n/n_0 = 37\%$ (sec.)	Rate constant $\times 10^4$ (per sec.)
Dry phage	46	4740	2.110
	51	3660	2.732
(Data are same	56	3000	3.330
in all conc. of	61	2223	4.498
dry samples)	66	1659	6.026

inactivation was exponential with time (Fig. 2), but in the wet state, the curve was not linear (Fig. 3). Rate constants for dry samples are shown in Table I. They are independent of initial phage concentration.

Discussion. From the study of thermal inactivation of this coli phage, it appears that phage in the wet state behaves quite differently from phage in the dry state. The cause of this difference is obscure. It is possible that water acts to protect phage particles against the adverse effects of heat. The more dilute the virus preparation, the higher is the temperature at which rate of inactivation begins to vary exponentially with each degree rise in temperature. The reason for the decrease in rate change above 75°C is unknown. If it is assumed that the differences in rate change at the lower temperatures depend solely on initial virus concentration, then the dry samples should be expected to show a similar behavior. Instead, the dry samples exhibit a common inactivation curve regardless of concentration. The data suggest that the effect of environment on reaction rate must be taken into consideration.

Summary. The curves for heat inactivation of an *E. coli communior* phage show that in the dried state, rate of inactivation varies exponentially with each degree rise in temperature, whereas, in the wet state a more complicated relationship exists. This is also reflected by the observation that the dried

phage is inactivated logarithmically with time over a wide temperature range whereas the wet preparations of phage are not.

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Phospholipids of Human Red Blood Cells.* (24670)

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The wide disagreement in reported values for concentrations of individual phospholipids in human red blood cells(1-5) indicates inadequacy of methods employed. Recently, it was shown that the main phospholipids in serum lipid extracts could be isolated and quantitated by chromatography on silicic acid(6,7). Concentrations of individual serum phospholipids in these studies were at variance with many previously reported values. In the present study, the same chromatographic method was used to isolate and quantitate the main phospholipids of red blood cells of fasting normal human subjects. The main components found were ethanolamine- and serine-containing phospholipid, lecithin, sphingomyelin, and lysolecithin. Concentrations of these components differed from those of several previously reported series(1-4).

Materials and methods. Blood was obtained from 3 male and 3 female apparently healthy young white adults in fasting state and collected in test tube containing heparin. It was centrifuged and plasma and buffy coat removed. Red blood cells were washed 3 times with equal volume of isotonic saline, then packed by centrifugation at 3500 rpm for 30 minutes. One volume of packed red blood cells was pipetted from bottom of tube and hemolyzed with equal volume of water (8). Extraction of this hemolysate was accomplished according to the method of Folch *et al.*(9). The hemolysate was added with shaking to 20 volumes (per volume of packed red blood cells) of chloroform-methanol (2:1, v/v), let stand 30 minutes, and filtered. The red blood cell precipitate was dried, ground to powder, and reextracted with 10 volumes (per volume of packed red blood cells) of chloroform-methanol (2:1, v/v) twice, in the same manner. The 3 filtrates were pooled and then emulsified with 0.2 its volume of 0.05 N KCl solution. The emulsion was broken by centrifugation at 2500 rpm for 15 minutes to give 2 distinct phases without fluff at the interface. The upper phase was removed and the lower phase taken to dryness in rotary vacuum evaporator at maximum temperature of 60°C. This dried extract was stored *in vacuo* at –30° C. Chromatography of the lipid extract on silicic acid columns and on silicic acid-impregnated filter paper and chromatography of acid hydrolysates of phospholipid fractions on filter paper have been described(6,7). Methods used in analyses of column fractions have also been reported(6, 7). In the present study, the standard procedure in normal subjects was to extract 5 ml of packed red blood cells and chromatograph 2 aliquots of the extract, equivalent to 2 ml of packed red blood cells each, on 2 1-g silicic acid columns, respectively.

Results. Three extractions of hemolyzed red blood cells were routinely performed,

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