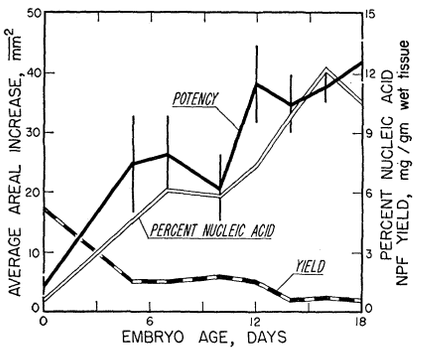

Fig. 1. Changes in yield, potency, and nucleic acid content of the nucleoprotein fraction (NPF) with age of embryonic source tissue.

crease in age of the embryo. It was further hoped that the study might disclose a source of the nucleoprotein fraction with higher yield and greater potency than 12-day embryos (*6*).

Using this streptomycin procedure, nucleoprotein fractions were prepared from nonfertile egg yolk and chick embryos that were 5, 7, 10, 12, 14, 16, and 18 days old. The egg white was devoid of streptomycin-precipitable material of appreciable quantity. The tissues were homogenized in borate with the aid of a Waring Blendor or Teflon-glass homogenizer. Streptomycin was used to precipitate the nucleoproteins from the borate extracts. Suspension of the nucleoprotein fraction in 1*M* NaCl was followed by dialysis against 1*M* NaCl and clarification in the ultracentrifuge. The clear solution was dialyzed against Gey's solution and finally was clarified in the ultracentrifuge. Dry weights and ultraviolet absorption spectra were obtained from aliquots of the clear fractions prior to culturing.

Tissue-culture assays were made using standard techniques (*7*). Fresh explants of 10- to 14-day chick embryo heart were singly cultivated in D-3.5 Carrel flasks at 37°C for a 7-day period. The basal assay medium consisted of a chicken plasma clot in which an explant (1 mm^3) was embedded, with a fluid supernatant of 20 percent horse serum and 80 percent Gey's solution. The nucleoprotein fractions were incorporated in the Gey's solution at the appropriate concentrations. Antibiotics were routinely incorporated into the media. Each test fraction was assayed in a series of six Carrel flasks, and each group of tests included a background control set of six flasks. Supernatant fluids were changed at 4 days, and cultures were traced and terminated at 7 days. Areal increases over and above the control values at 7 days were determined, and statistics (mean value and standard error) were calculated for each series of six cultures.

The average areal increase for the nucleoprotein fractions at 0.4 mg/ml in culture is shown as a function of embryonic age in Fig. 1. The points represent the averages of two extraction series. A general increase of areal outgrowth with increasing embryonic age is to be noted. The differences become significant ($P = 0.02$) when the 10-day nucleoprotein fraction is compared with the 16- or 18-day fraction.

The yields of nucleoprotein fraction per gram of wet starting tissue were calculated and plotted in Fig. 1. The steady decrease in yield with age may be partly accounted for by the difficulties of homogenization and extraction of the older tissues.

Ultraviolet absorption spectra of all the nucleoprotein fractions were similar to the spectrum of the 12-day fraction already published (*5*) with the exception of that of the egg yolk fraction. The latter had a maximum at 280 mμ, indicating a preponderance of proteins and a relatively small quantity of nucleic acids.

The content of total nucleic acids as percentage of dry weight was determined on 5 percent perchloric acid extracts (*8*) by comparing their ultraviolet absorption at 260 mμ with that of Schwartz ribonucleic acid standards. The results are shown in Fig. 1. A steady increase with age of total nucleic acids is to be noted. The nucleic acids are largely ribonucleic acid; previous analyses on the 12-day chick embryo nucleoprotein fraction disclosed that deoxyribonucleic acid accounted for less than one-tenth of the total nucleic acids (*5*).

The results indicate that the high-molecular-weight growth factors are present at all ages and do indeed contribute to the increased potency of whole extracts of older embryos as found by Gaillard (*3*) and Fowler (*4*). However, the decrease in yield of nucleoprotein fraction with age would indicate that the 12-day embryo is the best and most productive source. The parallel increase of nucleic acid and potency of the nucleoprotein fractions may be fortuitous and may reflect only an increase in quantity of nucleic acids in the enlarging embryo since the streptomycin precipitation carries down free nucleic acids as well as nucleoproteins (*9*). Other investigations on adult tissue nucleoproteins (*10*), as well as on embryonic nucleic acids (*5, 11*), indicate that nucleic acids as such are inert in this type of tissue culture. Preliminary experiments indicate that the protein portion of the nucleoprotein fraction is active (*12*). An alternative explanation for the parallel increase of potency and nucleic acid content is possible since unpublished experiments on the stabilization of the nucleoprotein fraction suggest that an increase in nucleic acid content may promote increased protection or stabilization of the active nucleoprotein fraction during isolation procedures. The role of nucleic acids as stabilizers during isolation is being actively investigated.

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8 February 1957

Effect of Zymosan on Bacteriophage Clearance

Soon after the discovery of bacteriophage, it became evident that the initial enthusiasm over possible therapeutic application of phage was unwarranted. Among the reasons propounded for the relative lack of success were included the development of resistant bacterial strains, actual deleterious effects due to toxic cleavage products in phage lysates (*1*), failure of phage to reach the focus of infection or to lyse susceptible bacteria *in vivo*, and the inactivation of phage by host defense mechanisms (*2*).

Several investigators (*3*) have reported that human serum, purulent exudates, and other body fluids inactivate phage. The discovery by Pillemer *et al.* (*4*) of the properdin system, a natural defense mechanism effective against a variety of bacteria and animal viruses, suggested to us that this normal serum constituent may be responsible in part for the inactivation of bacterial viruses *in vivo*. Therefore, experiments were initiated to determine whether bacterial viruses are inactivated by the properdin system. While these studies were in progress, Van Vunakis and her associates (*5*) reported the *in vitro* inactivation of *Escherichia coli* phage by the properdin system.

Preliminary experiments in our laboratory revealed that approximately 60-percent inactivation resulted during incubation of staphylococcus phage (typing strain No. 53) with fresh normal rabbit serum at 37°C for 1 hour. Loss

of phage activity was insignificant in serum inactivated by heat (56°C for ½ hour) or treated with zymosan (*6, 7*) at 17°C (two times with 2 mg of zymosan per milliliter). This degree of inactivation is comparable to the results obtained by Van Vunakis *et al.* (*5*). These data suggested a series of *in vivo* experiments, herein reported, to determine the effect of zymosan on phage clearance in the blood stream of experimental animals.

Initially rats were used, but the difficulties inherent in repeated blood sampling caused us to select rabbits as more suitable experimental animals, although these animals have considerably less demonstrable properdin (*7*). The experimental animals received (usually intracardially) approximately 100 mg of zymosan per kilogram of body weight. Control animals received saline injections in lieu of zymosan. Four hours later, each animal was injected with 1×10^9 staphylococcal phage 53 via the marginal ear vein. At intervals, the animals were bled from the heart with a 21-gage needle and tuberculin syringe, and the phage content of the blood sample was immediately assayed by dilution plating on the host bacterium (PS 53).

The combined results of several series of experiments are illustrated in Figure 1. In animals pretreated with zymosan, plaque counts at any time of assay usually range from three to more than tenfold higher than their untreated counterparts. The initial blood samples, with one exception, contained considerably less phage than could be expected on the basis of blood volume dilution (approximately 100-fold, with blood volume considered as 5 percent of body weight, *8*). This observation is in accord with a previous report (*9*) of a 2500-fold reduction of phage in the blood of rats 5 minutes after injection. One must conclude, therefore, that there is an active removal of bacteriophage which is measurable 2.5 minutes after administration in both control and zymosan-treated animals. It is also apparent that pretreatment with zymosan reduces the efficiency of the mechanism(s) responsible for phage clearance.

A decreased rate of phage clearance was also observed when a sublethal dose of *E. coli* endotoxin preparation (*10*) was administered to rabbits. Similar endotoxin preparations have been demonstrated to have marked effects on the properdin system (*11*), but it must be remembered that such preparations have a variety of other effects on experimental animals.

We have been unable to assay properdin according to the methods published by the Western Reserve group (*7*) but preliminary observations suggest that the bactericidal activity against *Shigella dysenteriae* of serum from zymosan-treated animals is less than that of control serums. Although elevated levels of properdin have been reported to follow administration of zymosan (*12*), we have been unable to demonstrate this "rebound" effect with phage clearance when rabbits were challenged with phage 4 to 5 days after zymosan treatment. However, it is possible that only a threshold level of properdin is required for maximal blood clearance of phage, and increases in properdin above this level may have no additional effect on this experimental system.

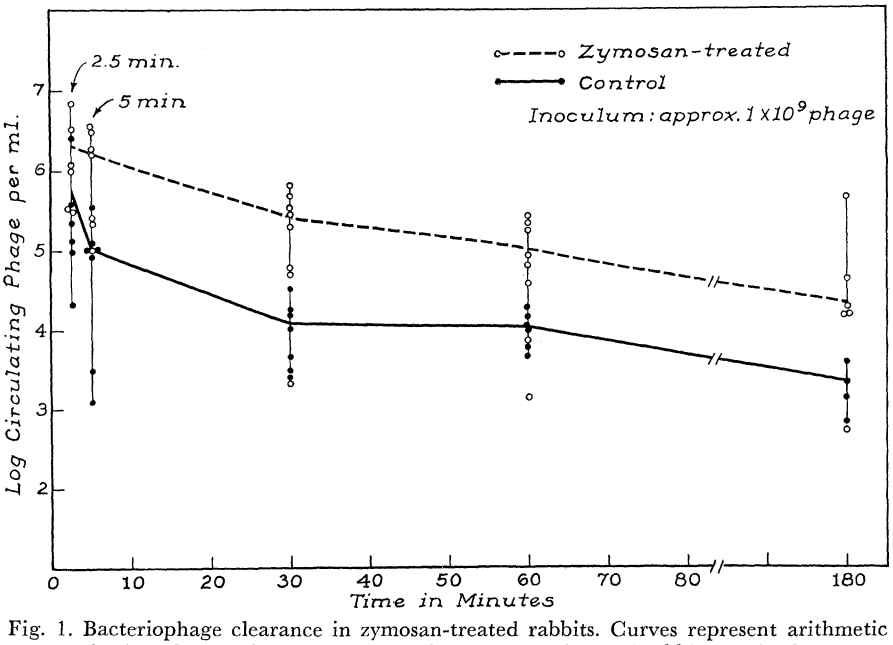

Fig. 1. Bacteriophage clearance in zymosan-treated rabbits. Curves represent arithmetic means of values derived from several experiments. Experimental rabbits received zymosan (100 mg/kg) 4 hours prior to phage inoculation.

The reported observations constitute presumptive evidence that the properdin system may be involved in the inactivation of bacteriophage *in vivo*, but additional confirmatory information is desirable.

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Pyridine-2-Aldoxime Methiodide and Poisoning by Anticholinesterases

This laboratory has reported (*1*) that certain compounds containing quaternary ammonium groups are capable of enhancing the rate at which the gastrocnemius-soleus-tibialis anticus muscle group of the cat recovers responsiveness to slow (one shock per 2 seconds) stimulation of the sciatic nerve, following weakening or abolition of this response by intravenous injection of a large dose (200 μg/kg) of the potent cholinesterase inhibitor, isopropyl methyl phosphonofluoridate (sarin). In continuation of this work, we have examined the effects of some of the same quaternary compounds on the maintenance of tension in cats poisoned by intravenous injection of about 12 LD_{50} of either sarin or ethylphosphoro dimethyl amidocyanidate (tabun), by the gastrocnemius-soleus-tibialis anticus muscle group stimulated indirectly by just supramaximal stimuli of tetanizing frequency (40 per second). The ability to maintain a tetanus with indirect electric stimulation has been shown (*2*) to be more sensitive to decrease by anticholinesterase compounds than that to produce single twitches.

Oxamide derivatives [N,N′-bis(N-dipropyl, N-2-chlorobenzyl ammonoethyl) oxamide dichloride (WIN 12306), for