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.

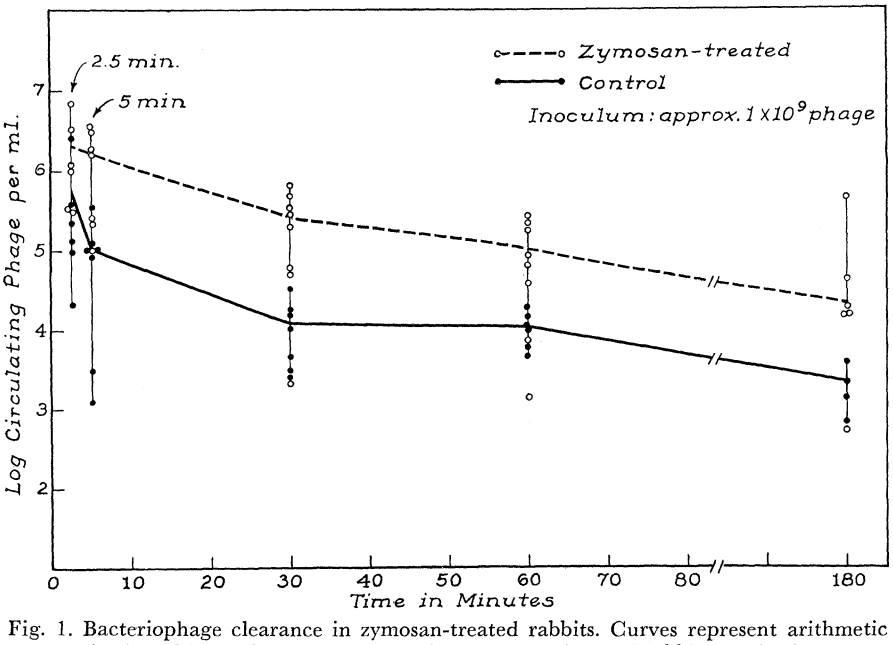

Fig. 1. Bacteriophage clearance in zymosan-treated rabbits. Curves represent arithmetic means of values derived from several experiments. Experimental rabbits received zymosan (100 mg/kg) 4 hours prior to phage inoculation.

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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28 January 1957

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

example] which promote recovery of the anticholinesterase-inhibited twitch (*1*) had no important effect on recovery of the tetanic response after poisoning with tabun (Fig. 1) or sarin. Another quaternary compound that was found (*1*) to speed recovery of the twitch response, N-benzyl atropinium chloride, had some efficacy in enhancing recovery of the tetanic response after sarin administration (Fig. 1). Figure 1 shows, however, that even with N-benzyl atropinium chloride, the tetanic response did not become reasonably normal within the observation period of 2 hours after the poisoning.

Our attention turned then to oximes, and especially to pyridine-2-aldoxime methiodide (2-PAM). This compound has been reported to be quite active *in vitro* in reactivating cholinesterase inhibited by TEPP, DFP, or sarin (*3*), in overcoming neuromuscular block induced by the same cholinesterase inhibitors in the isolated phrenic-diaphragm preparation of the rat (*4*), and in preventing mortality in mice that have been poisoned with paraoxon (*5*). Other oximes were found (*4*) to be effective *in vivo* in overcoming anticholinesterase-induced neuromuscular block in the gracilis muscle of the rat and in the tibialis of the cat.

Intravenous injection of 5 mg of 2-PAM per kilogram after intravenous administration of about 12 LD_{50} of either sarin or tabun produced comparatively rapid and complete recovery of the tetanic response (Fig. 1). This recovery

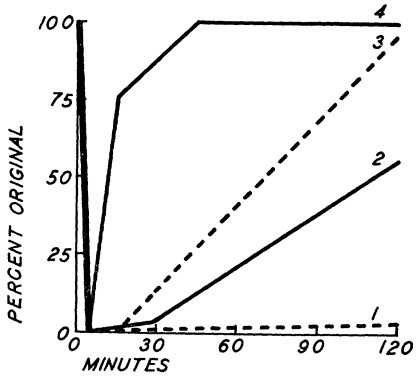

Fig. 1. Effects of various treatments on the recovery, by the gastrocnemius-soleus-tibialis anticus muscle group of the cat, of ability to maintain a tetanus for 30 seconds of indirect electric stimulation with just supramaximal square-ware stimuli. Atropine (0.5 mg/kg intravenously) was given just before the intravenous injection, at zero time, of tabun (0.8 mg/kg) (broken lines) or of sarin (0.22 mg/kg) (solid lines). Treatments, given intravenously at 5-minute intervals: (i) WIN 12306, 1 mg/kg; (ii) N-benzyl atropinium chloride, 5 mg/kg; (iii and iv) 2-PAM, 5 mg/kg.

is even faster and more complete within the duration of our experiments than that produced by N-benzyl atropinium chloride.

Administration of graded doses of anticholinesterase to groups of six rabbits, followed by treatment of the same rabbits by no other procedure than intravenous injection of either 5 mg of 2-PAM per kilogram, 2 mg of atropine sulfate per kilogram, a mixture of 2 mg of atropine sulfate and 5 mg of 2-PAM per kilogram, or nothing, has yielded approximations of the LD_{50} of sarin with various drug therapies. The LD_{50} of sarin for rabbits that are given no treatment is 16 μg/kg; for rabbits that are treated with 2-PAM alone, 24 μg/kg; for those treated with atropine alone, 51 μg/kg; and for those treated with both atropine and 2-PAM, 331 μg/kg. This last figure is based on results from 13 groups of rabbits poisoned with doses of sarin ranging from 64 μg/kg to 1.2 mg/kg.

Even with the highest dose of sarin, the combined treatment with atropine and 2-PAM saved one rabbit from the two groups of animals (12 rabbits) so poisoned and treated. None of the other treatments saved any animals at doses of sarin above 130 μg/kg, although combined treatment with atropine and 2-PAM saved 30 of 66 rabbits that were given doses of 160 or more μg/kg of sarin. Chemical treatment, to be effective after large doses of anticholinesterase, must be instituted within 1 minute after sarin injection.

It is apparent that 2-PAM alone, in the dose used, is a less effective therapeutic agent in anticholinesterase poisoning than atropine, but that it and atropine together make a very effective therapeutic combination. This conclusion supplements that reached by T. A. Loomis (*6*), namely, that 2-PAM is not significantly therapeutic in animals that are poisoned by sarin and given no other treatment.

If the effectiveness of 2-PAM as an adjunct to atropine in treating anticholinesterase poisoning is attributable primarily to its ability to overcome neuromuscular block elicited by the cholinesterase inhibitors, it is easy to see why 2-PAM is of little value alone. Death in animals that are not treated with atropine or other anticholinergic tertiary amines is caused, in large part, by central respiratory failure (*7*). Because 2-PAM is a quaternary amine, it would not be expected to pass the blood-brain barrier and to affect the activity of centers within the brain. Even though the 2-PAM were capable of overcoming all the other peripheral effects of anticholinesterase compounds, in addition to restoring neuromuscular transmission, it could not benefit the poisoned subjects in the face of continued central respiratory inhibition (*8*).

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11 February 1957

Echo Virus Type 9 (New Member of Coxsackie Group Type A?) as a Cause of Epidemic Meningitis

Last summer, an extensive epidemic of aseptic meningitis occurred in many parts of Western Europe: as far as we know, in Belgium, the Netherlands, Germany, Switzerland, England, and Denmark. This disease was characterized by meningeal symptoms (sometimes accompanied by exanthem), always with a very marked pleocytosis (up to 5000 cells/mm^3) and by its highly contagious nature; often all the members of a family, children and adults, were affected.

From 399 patients with the typical syndrome, 190 virus strains were isolated from fecal specimens, 29 from spinal fluids, and 6 from throat swabs. Proof of the etiologic significance of these virus strains was obtained by direct isolation from cerebrospinal fluid and by showing a rise of fourfold or more in the titer of neutralizing and complement-fixing antibodies in all paired serums of the patients examined (to date, 112 have been examined for neutralizing antibodies and 37 for complement-fixing antibodies).

No antibodies were found in serums from patients suffering from nonparalytic poliomyelitis (13 patients), paralytic poliomyelitis (19 patients) or atypical