17107 P. Pyrogenicity of Influenza Virus in Rabbits.*

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During an investigation of the toxicity of influenza virus in rabbits, it was noted that the intravenous injection of infected chorio-allantoic fluid was followed by fever. To study this phenomenon, animals were placed in individual stalls, 3 preliminary rectal temperatures were taken at 30-minute intervals to establish a baseline, and the virus preparation to be tested was then injected into the marginal ear-vein. Rectal temperatures were recorded every 30 minutes throughout an observation period of 6 hours after injection. All glassware and saline solutions used were uncontaminated with bacterial pyrogens. A single pool of chorio-allantoic fluid from embryos infected with the PR-8 strain, having a hemagglutinin titer of 1:1024,[1] was used.

One ml of this material consistently caused a rise in temperature beginning 1½ to 2 hours after injection, reaching a peak of 3-4°F above the baseline in the next 4 hours, and gradually falling to normal. Although doses as small as 0.025 ml produced fever, the increases in temperature were of a lower order.

Normal chorio-allantoic fluid and infected fluid from which the virus particles had been removed by centrifugation at 30,000 R.P.M. or by adsorption on chicken erythrocytes gave no fever. The virus resuspended in normal saline solution produced typical temperature elevations.

No fever followed the injection of PR-8 virus neutralized with homologous immune serum.

The febrile response in rabbits is independent of the infectivity of the virus but seems to be related to its adsorptive capacity. Heating at 56°C for 30 minutes destroyed the infectivity of PR-8 for chick embryos but a portion of the hemagglutinin was retained (1024 to 128); this material produced definite fever in rabbits. Heating at 62°C for 30 minutes destroyed the hemagglutinin titer and these preparations were then non-pyrogenic.

The injection of the Lee strain of influenza B virus produced a fever in rabbits similar to that caused by PR-8. The hemagglutinin of Lee is known to be more heat-resistant than that of PR-8.[2] When the Lee strain was heated at 62°C for 30 minutes it retained adsorptive (1024 to 256) and pyrogenic properties. Exposure to temperatures which destroyed the hemagglutinin rendered the Lee strain non-pyrogenic.

It has been shown that rabbits given a course of daily injections of a bacterial pyrogen become tolerant to the fever-producing effect of that pyrogen and those of other bacterial strains.[3] Animals made tolerant by eight daily injections of 0.1 ml typhoid vaccine responded with typical fevers when given PR-8 on the ninth day, demonstrating that no cross-tolerance existed. The heat stability of bacterial pyrogens, which are not destroyed by boiling,[4] is also in contrast to the lower temperatures at which the fever-producing principle of influenza is destroyed.

Animals which had received 1.0 ml of PR-8 virus on the first day with attendant febrile responses were completely tolerant to the effect of an equal dose on the second day, when no temperature rise occurred. Nine animals given 1.0 ml of PR-8 were re-injected in groups of three on the fourth, seventh, and eleventh days and their temperature responses recorded. As seen in Fig. 1, the responsiveness to the fever-producing effect of the virus gradually returned so that by the eleventh day,

* The opinions expressed are those of the authors alone and do not necessarily reflect those of the Navy Department or the naval service at large.

1 Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

2 Salk, J. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 134.

3 Beeson, P. B., *J. Exp. Med.*, 1947, **86**, 29.

4 Banks, H. M., *Am. J. Clin. Path.*, 1934, **4**, 260.

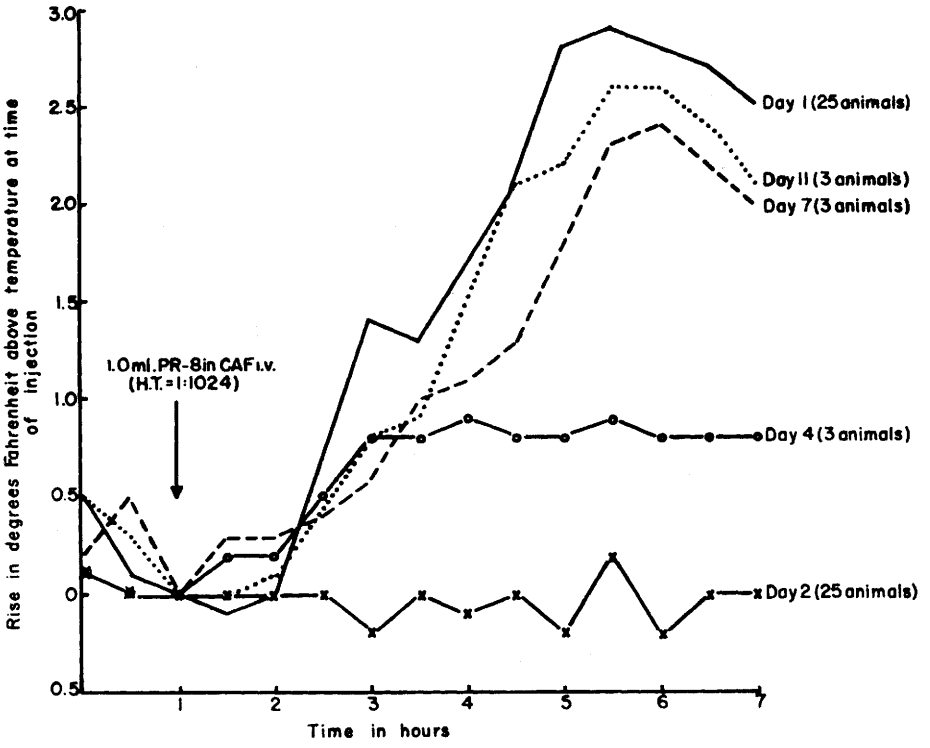

FIG. 1.

Average temperature responses of groups of rabbits to PR-8 influenza virus on day 1 and to a single injection repeated at intervals after the initial injection. Note the complete unresponsiveness on day 2 and the increase in fever through day 11.

their fevers approached first-day levels. Antibody titers (as measured by hemagglutination inhibition) of these animals showed increasing levels during this period when tolerance was being lost. Thus, there is evidence that the resistance to the pyrogenic effect of the virus is unrelated to specific antibody production, although as pointed out above, virus neutralized with immune serum before injection causes no fever.

The observation of Harris and Henle[5] that an injection of influenza virus will cause lymphopenia in rabbits was confirmed, but this effect on the lymphocytes was not seen on the second day. By the eleventh day, the lymphopenia, like the febrile response, again approached that following the first injection.

[5] Harris, S., and Henle, W., *J. Immunol.*, 1948, **58**, 9.

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