of the aorta and the rate of phospholipid synthesis in the arch was significantly less than that of the descending limb of the aorta. It is of interest that Werthessen *et al.*(10) reported a concentration gradient of cholesterol over the length of the calf's aorta. Lower concentrations of cholesterol were found in the arch than at more distal sections of the aorta. Although these facts suggest some fundamental metabolic differences between certain areas of the aorta, their possible relationship to the pathogenesis of atherosclerosis must await further studies of arterial metabolism.

Summary. Lipid partition and phospholipid metabolism of the aortic arch and descending limb of aorta, plasma and liver were determined in groups of rabbits on a normal diet and 40 and 70 days on a cholesterol-supplemented diet. In all groups, the descending limb of aorta had significantly lower total lipid, primarily due to decreased neutral fat concentration, compared with the aortic arch. In normal rabbits the specific activity of phospholipid in the descending aorta was significantly greater than that of the aortic arch. However, this distinction in phospholipid synthesis between the two areas of aorta was not found in the groups of rabbits on the high-cholesterol diet. The radioactivity data indicated this was due to an increased rate of formation of phospholipid in the aortic arch of rabbits fed the cholesterol-supplemented diet, particularly those rabbits on the diet for 70 days, and probably was related to the development of atheromatous lesions in the arch only of these rabbits. The results were discussed in relation to the general problem of local factors in the arterial wall influencing the localization and subsequent development of atherosclerotic lesions.

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Received October 15, 1956. P.S.E.B.M., 1957, v94.

Induced Resistance in Mice to Intravenous Toxicity of Influenza Virus.* (22859)

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Protection of intact animals from rapidly appearing damage, usually referred to as toxicity, which follows inoculation with massive doses of influenza viruses has been reported (1-5), but attention has largely been directed to toxicity following intracerebral injection in mice, to lung consolidation caused by nasal installation of certain strains into mice, and to the pyrogenic effect of intravenous injection into rabbits. Beyond the initial description of intravenous toxicity of influenza virus in mice(6), little study has been applied to this phenomenon. When it was found, during studies of factors modifying host reactions to

* Opinions expressed in this article are those of the authors and do not necessarily reflect the views of the Navy Department.

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virus infections, that mice could be protected from lethal effects of large intravenous doses of influenza virus by preceding sub-lethal injections of virus, it appeared desirable to determine in what respect this protection corresponded to that observed in the other systems and to explore further the relationships of viral species, dose, and injection intervals that would produce increased resistance.

Materials and methods. The PR-8 strain of influenza A virus, Lee strain of influenza B virus, Enders strain of mumps virus, and GB and (H) Najarian strains of Newcastle disease virus (NDV) were cultivated in the allantoic sac of embryonated eggs and used as infected, undiluted allantoic fluid or diluted in phosphate buffered saline. The number of agglutinating doses (A.D.) in these preparations was determined by the pattern technic using a final concentration of 0.5% chicken erythrocytes. The Chicago strain of feline pneumonitis and Madrid E strain of *R. prowazeki* were cultivated in the yolk sac of embryonated eggs and used as yolk sac suspensions clarified by light centrifugation and diluted in Hanks' balanced salt solution. Receptor destroying enzyme (RDE) was purified by the method of Ada and French (7) from filtrates of cultures of *Vibrio cholerae* (Inaba), Culture 35A3 of the National Institutes of Health. This preparation had a titer of 1:1024 as measured by the method of Burnet(8). Swiss white mice (Webster strain, mixed sexes) weighing 10-14 g and 4-5 weeks old were used. Pre-challenge injections were in volumes of 0.25 or 0.5 ml. For challenge injections and toxicity titrations virus suspensions were injected into a tail vein in 1.0 ml volume and only deaths occurring within 96 hours were considered due to toxic effects of virus. Using six mice per group and two-fold dilutions of virus, it was found in ten complete titrations of one virus preparation that the standard deviation of the distribution of the LD_{50} end points was 0.220 $\log_{10}$ units. It could be calculated from this that the chances were 19 out of 20 that a four-fold difference between the results of two titrations was significant.

TABLE I. Protective Effect of Intravenous Injections of Active and Heated PR-8 Virus against Homologous Challenge 24 Hr Later.

Pre-challenge inj.	Challenge LD_{50} (A.D.)
Normal allantoic fluid	422
Active virus—96 A.D.	5120
56° heated virus—1280 A.D.*	1808
70° " " *	640

* A.D. before heating = 5120.

Results. Protective effect of sub-lethal doses of homologous virus. Groups of mice were given intravenous injections of homologous PR-8 virus, either as a small sub-lethal dose (¼ i.v. LD_{50}) of fully active virus or as a much larger quantity of heated virus. These mice were challenged 24 hours later with intravenous injection of fully active, toxic virus. It was found (Table I) that sub-lethal doses of active, homologous virus increased the LD_{50} at challenge by approximately 12-fold. There was not a complete refractoriness for if the challenge inoculum was large enough the mice died. Homologous virus heated at 56°C for ½ hour and without detectable infectivity but still retaining hemagglutinating capacity was not completely devoid of protective activity but was less effective than live virus. Virus heated at 70°C for ½ hr and lacking in hemagglutinating activity as well as infectivity did not produce significant protection even when administered in massive quantities. It is to be noted in Table I that the preparatory dose of heated virus was many times larger than the preparatory dose of active virus.

Heterologous virus. Other viruses of the mumps - influenza - Newcastle disease virus group serologically unrelated to the PR-8 strain of influenza A were similarly tested for their capacity to protect mice against the intravenous toxicity of this strain. Preliminary titration showed that each of two strains of Newcastle disease virus caused 50% mortality in mice on intravenous injection of approximately 1280 A.D. of active virus as did the Lee strain of influenza B. Fully active mumps virus in quantities available in undiluted allantoic fluid without concentration (640 A.D./cc) did not produce apparent deleterious effects. Intravenous injections of

TABLE II. Protective Effect of Intravenous Injections of Heterologous Virus Given 24 Hr before Intravenous PR-8 Challenge.

Pre-challenge inj.*	Challenge LD_{50} (A.D.)
Normal allantoic fluid	269
Active Lee	2560
70° heated Lee	422
Active mumps	2560
70° heated mumps	380
Active NDV-(H) Najarian strain	2560
" " GB strain	2560
70° heated NDV-GB	422

* Active viruses = 320 A.D.

320 A.D. of each of these viruses given 24 hours before challenge with PR-8 virus increased the LD_{50} by more than 9-fold (Table II) but injection of these preparations after exposure to sufficient heat to destroy detectable viral infectivity and hemagglutinin was without protective effect. Other experiments demonstrated that a similar result followed injection of the virus strains in reverse order, *i.e.*, if the Lee strain of influenza B was employed as the toxic challenge, pre-challenge injections of sub-lethal quantities of the PR-8 strain of influenza A served effectively to protect mice.

With the demonstration of the protective effect of several viruses of the mumps-NDV-influenza group experiments were designed to determine whether pre-challenge inoculation of unrelated viruses or a non-viral pyrogen would alter susceptibility to influenza toxicity. Feline pneumonitis virus in preparations of relatively high concentration, the Madrid E strain of epidemic typhus, and a typhoid vaccine were used. The feline pneumonitis virus and the typhus rickettsiae were titrated for intravenous toxicity in mice and doses of ¼-½ LD_{50} of fresh, live suspensions were used as preliminary inoculum. A commercial typhoid vaccine was diluted 1:4 in physiologic saline and 0.25 ml aliquots containing 6 x 10^7 *S. typhosa*, 1.6 x 10^7 *S. paratyphi* A, and 1.6 x 10^7 *S. paratyphi* B were injected intravenously into mice. There was no evidence of significant protection of mice by any of these three agents against challenge 24 hours later with 3 LD_{50} of PR-8 virus.

Minimal protective dose of homologous and heterologous virus. Time of onset and duration of resistance. The quantity of live virus required to protect against a standard intravenous challenge of 6 LD_{50} of PR-8 virus given 24 hours later was determined by varying the pre-challenge inoculum in serial 2 or 4-fold steps. All mice were protected by 20 A.D. of homologous PR-8 virus, by 20 A.D. of mumps virus, and by 20-40 A.D. of the GB strain of NDV. Smaller doses conferred diminishing protection and the gradation from complete protection to complete susceptibility was spread over 3-5 two-fold steps.

Time required for development of the resistant state and duration of resistance after injection of small, constant quantities of live mumps virus or NDV was determined using 40 A.D. of NDV or mumps virus as the pre-challenge inoculum and 6 LD_{50} of PR-8 virus as a challenge. It was found (Table III) that by 13 hours after injection of NDV all mice survived challenge with PR-8 virus and that this refractoriness persisted unabated through the 48th hour but had waned somewhat by 72 hours. With mumps virus as the preliminary inoculum, however, resistance did not become complete for 30 hours. Considerable resistance persisted through 72 hours but was subsiding at 96 hours.

Protective effect of receptor destroying enzyme. It was found that intravenous injection of 0.5 ml of purified RDE 24 hours be-

TABLE III. Time of Onset and Duration of Resistance to Intravenous PR-8 Toxicity after Pre-Challenge Injection of NDV or Mumps Virus (40 A.D.).

	Hr to challenge*									
Pre-challenge inj.	3	6	10	13	18	24	30	48	72	96
NDV	6/8†	5/8		0/8	0/8	0/8	0/8	0/8	5/8	7/8
Mumps	7/8	7/8	6/8	4/8	4/8	1/8	0/8	2/8	2/8	6/8
Normal allantoic fluid	8/8				8/8					

* 6 LD_{50}.

† Numerators give mice dying in 96 hr after challenge; denominators, total mice in group.

TABLE IV. Protective Effect of RDE against Intravenous Toxicity of PR-8 Influenza A Virus.

Pre-challenge inj.*	Challenge Agglut. doses of virus 2560	1280	640	320	160
Saline	5/5†	6/6	4/6	1/6	0/6
Unheated RDE	2/6	0/6	0/6	0/6	0/6
Heated RDE	6/6	2/6	0/6	0/6	0/6

* 0.5 ml intrav. 24 hr before challenge.
† No. of mice dying in 96 hr/total mice in group.

fore challenge completely protected mice against as many as 2560 A.D. (8 LD_{50}) of PR-8 virus although RDE given only 30 minutes after the virus was without any protective effect. The time required for development of this refractory state was tested in a manner similar to that described for sublethal doses of viruses. Mice given RDE intravenously and challenged with 2560 A.D. of PR-8 at intervals from 15 minutes to 4 hours after RDE injection were fully susceptible to the lethal effect of PR-8, but by the next test interval, 8 hours, sufficient resistance had developed so that all mice survived challenge, as they did also at 24 hours after the protective injection. The duration of this resistance was not determined.

To determine whether the protective effect of the RDE preparation was due to its content of RDE or to other unidentified components, the protection conferred on mice by the RDE preparation was compared with that produced by an aliquot of RDE which had been boiled for 30 minutes. This heating resulted in complete destruction of the erythrocyte receptor destroying activity of the RDE preparation as measured in the usual hemagglutination-inhibition test. The results of such an experiment in which mice were given intravenous injections of active and heated RDE and challenged 24 hours later with 4 LD_{50} of PR-8 virus are presented in Table IV. It is evident that the protective effect of the RDE preparation was reduced somewhat by heating but not entirely destroyed.

Discussion. The rapidity with which resistance develops, its temporary nature, and its production by serologically heterologous virus make it quite unlikely that specific immunity based upon antibody production plays any part in the increased resistance in these experiments.

Although RDE produces a refractory state, the fact that much of the resistance inducing potency remains after heat destruction of the erythrocyte receptor destroying capacity suggests that protection by RDE is not dependent upon destruction of mucoprotein cellular receptors unless receptors on the involved tissue cells are much more susceptible to destruction by residual enzyme than are those of erythrocytes. A similar protection of mice by RDE against intracerebral toxicity has been reported(5) but little can be deduced at present as to the mechanism of this effect.

It is of interest that while homologous and heterologous influenza virus, NDV, and mumps virus readily produce a refractory state, feline pneumonitis virus, *R. prowazeki*, and typhoid vaccine had no effect on resistance to influenza virus even though the intravenous toxicity of these latter agents is similar in several respects to that of the mumps, NDV, influenza group of viruses. The refractory state thus exhibits more specificity than that of a tolerance to the endotoxin-like qualities of these agents. In the time lag prior to onset of resistance, the need for much larger quantities of heated than of unheated virus to induce resistance, the transient and incomplete nature of the refractoriness, and the limited range of effective resistance inducing agents the increased resistance to influenza toxicity appears quite similar to the phenomenon of virus interference. Some of these characteristics of the reaction also suggest that some degree of multiplication by the active pre-challenge virus may take place in the affected tissues. Limited multiplication of unadapted influenza virus in mouse brain has been demonstrated(9) and it is conceivable that similar multiplication may occur in other organs. Alteration in the susceptibility of tissue cells brought about by intracellular establishment of the pre-challenge virus would seem to be the most likely explanation of this acquired resistance. It is noteworthy, however, that Wagner(10) was unable to demonstrate an increase in resis-

tance of cells (HeLa) in culture to the cytotoxic action of influenza virus after exposure to subtoxic quantities of virus. It appears that it will be necessary to follow events in the actual cells involved in these toxicity reactions during development of host resistance in order to localize the site of altered susceptibility.

Summary. Sublethal quantities of homologous and heterologous influenza virus, mumps virus, and Newcastle disease virus injected intravenously in mice produced a state of resistance to the toxic effects of large quantities of intravenously injected PR-8 influenza A virus. Virus heated sufficiently to destroy infectivity was less effective in inducing resistance than was fully active virus; destruction of hemagglutinin caused complete loss of resistance inducing activity. Injection of sublethal doses of virus was followed by a lag period, which varied with the virus injected, before resistance became demonstrable, and resistance waned after 3-4 days. No increase in resistance to influenza toxicity was demonstrable after pre-challenge injection of feline pneumonitis virus, *R. prowazeki*, or typhoid vaccine. A preparation of receptor destroying enzyme (RDE) was effective in inducing increased resistance but its effect could not be attributed to its capacity for destruction of mucoprotein receptors since heat destruction of this activity did not completely destroy its resistance inducing effect.

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Received October 15, 1956. P.S.E.B.M., 1957, v94.

Intracellular Multiplication of *Brucella abortus* in Normal and Immune Mononuclear Phagocytes.* (22860)

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Importance of mononuclear phagocytes in pathogenesis of brucellosis has been suspected by many, but the role of these cells in resistance to brucella infection has not been investigated. The intimate relationships of mononuclear phagocytic cells and brucella can be studied *in vivo* only with great difficulty. The ease with which fairly pure cultures of guinea pig mononuclear phagocytes can be obtained and maintained *in vitro* for reasonable periods of time offered a means of studying this problem. The technics used in this work allowed the numbers of both phagocytic cells and viable brucellae to be controlled with a fair degree of accuracy. Some of the difficulties inherent in working with varying ratios of bacteria and tissue cells are thus eliminated.

Materials and methods. Tissue culture technics were similar to those described by Leighton and Hanks(1). Peritoneal exudates,

* Most of this work was done, while senior author was on sabbatical leave and Guggenheim fellow, at Department of Microbiology University of Pennsylvania School of Medicine. Aided by USPHS grant E-467 (C2).

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