TABLE I. Survival of Male Skin Isografts in Pregnant and Parous Female Mice of C57Bl (Subline 1) Strain.

Group	No. of mice	No. of previous pregnancies (mean)	Time of grafting	No. and (%) of female mice accepting male skin grafts
I	16	3.6	Mid-preg.	5/16 (27.5)
II	11	3.5	9-16 days after deliv.	3/11 (27)
III	9	3.4	43-105 days after deliv.	2/9 (22)
IV	19	0	—	4/19 (21)

grafts were observed, this being approximately 8 to 12 days in all groups.

It seems, therefore, that multiparous mice of the C57Bl (Subline 1) do not show a significant increase in male isograft acceptance when compared to virgin controls. Our results seem in conflict with those recently reported by Prehn. However, this could be perhaps explained on the basis of a different genetic make up of our C57Bl (Subline 1) strain mice as compared to those of the C57 Bl/An which Prehn has used.

Summary. Experiments were undertaken to determine male skin isograft survival in multiparous female mice of the C57Bl (Subline 1) strain, as compared to virgin controls. No significant difference in male isograft acceptance was found in multiparous mice as compared to that in virgins.

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Alteration of Susceptibility of Embryonated Eggs to Newcastle Disease Virus by *Escherichia coli* and Endotoxin. (26376)

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It was recently demonstrated(1) that infection with *Escherichia coli* markedly increased resistance of chick embryos to subsequent challenge with *Vibrio cholerae*. Resistance could also be stimulated by inoculation of killed *E. coli* cells or endotoxin preparations prior to challenge. Thus, it seems apparent that the chick embryo is capable of a response to endotoxin, similar to that described in mature animals(2,3), which results in an enhanced non-specific resistance to bacterial infection. It is of obvious importance, then, to determine whether this enhanced resistance extends to virus infection as well. Newcastle disease virus was selected as the agent for test since it is stable and lethal for chick embryos in low dosage, infection can readily be proven by hemagglutination, and it has been reported on a number of occasions(4) to be inhibited by host serum factors which may play roles in natural resistance.

Materials and methods. Antibiotic-free, pullorum clean White Leghorn chick embryos were used. Eggs from a single flock were delivered at a specified age, usually 10 days, and were incubated on arrival with the air sac end up at a slight angle on racks in a humidified egg incubator at 36-37°C. Methodology was similar to the previous study(1). Embryos were challenged allantoically with 0.1 ml of virus dilution on day 13 through a small hole punched in the shell. At least 15 eggs were used per group within an experiment. Viability of embryos was determined by candling using criteria of well defined blood vessels and embryonic movement. *Escherichia coli* strain 9, serotype 0111:B4, and

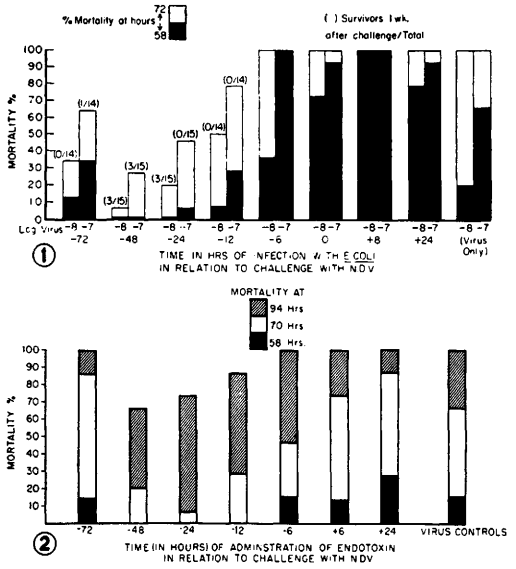

FIG. 1. Effect of *E. coli* on mortality of chick embryos challenged with N.D.V.

FIG. 2. Effect of endotoxin on mortality of chick embryos challenged with N.D.V. (10^{-5} dil'n).

E. coli endotoxin, preparation #36, described previously(1) as being among several strains and endotoxin preparations which elicit the protective response against *V. cholerae* infection, were used in this study. Neither endotoxin nor *E. coli* strain 9 administered allantoically in the dosages indicated was lethal for chick embryos to any significant extent. The Newcastle disease virus (NDV) strain AMS2, was obtained from the Dept. of Virology of this Institute. For stock virus, 10-day-old eggs were inoculated with a 1:1000 dilution. The allantoic fluid was harvested aseptically at 48 hours, centrifuged and stored in vials at –20°C. This stock proved to be quite stable; over a 9 month period, 0.1 ml doses of freshly prepared 10^{-8} dilutions in saline killed approximately 50% of 13 day embryos within 3 to 4 days.

Results. Effect of E. coli on mortality of chick embryos challenged with NDV. Groups of eggs were inoculated allantoically with *E. coli* at varying intervals before and after virus challenge. The *E. coli* inocula, prepared by suspending and diluting with sterile saline the 18-hour growth from a heavily streaked meat extract agar Petri dish, ranged between 1500 and 4600 viable cells. Previous experience(1) had shown that these cells multiply to a population of approximately 10^8 per ml of egg within 24 hours. Eggs were challenged on day 13 with 0.1 ml of 10^{-6} or 10^{-7} dilutions (approximately 100 and 10 LD_{50}) of stock NDV in saline. Since deaths from the virus infection begin to occur by 48 hours post challenge in controls, the occasional eggs which succumbed by 24 hours after virus challenge were discarded. Eggs were candled again at 58 and 72 hours and results plotted in Fig. 1. *E. coli* infection had marked effects on lethality of NDV. Some evidence of protection was noted in eggs inoculated with *E. coli* 72 hours prior to NDV. It was most marked when *E. coli* cells were administered 48 hours prior to virus and the protective effect was still present at 24 and 12 hours. When the *E. coli* cells were inoculated 6 hours before virus or later, the effect was rather that of virus enhancement. Seventy-two hours after virus inoculation, all the control eggs and those inoculated with *E. coli* at –6, 0, +8, and +24 hours were dead. Mortality of embryos in the other groups increased by 72 hours in proportion to the virus inoculum and to the 58 hour mortality. Deaths continued to occur throughout the incubation period of these embryos, but at the embryonic age of 20 days there were still a few survivors some of which had pipped through their shells. The survivors were discarded at this time. Allantoic fluid of eggs dying late was found to contain hemagglutinin for chick and for human O erythrocytes which was specifically inhibited to high titer by NDV antiserum. Mortality was not affected by inoculation of saline 48 hours prior to or 6 hours after virus in groups of control eggs.

Effect of endotoxin on mortality of embryos challenged with NDV. In a similar manner, groups of eggs were inoculated allantoically at intervals with 4 μg of endotoxin (0.1 ml of a saline solution of 40 μg of endotoxin per ml) and challenged with NDV. Results (Fig. 2) were similar to those obtained in eggs infected with living *E. coli* in that there were phases of increased resistance and susceptibility. Neither effect, however, was of the magnitude observed when living *E. coli*

were used. Protection until time of hatching was not achieved in this experiment although occasional endotoxin treated eggs survived 5 days and possibly longer following virus inoculation. Living *E. coli* might be more effective since they could provide a continuing source of endotoxin. In this, and other experiments, greatest survival and also greatest mortality are obtained with correspondingly later injections of endotoxin than of viable *E. coli*. Thus, a period approximately 24 hours prior to virus appears to be the most effective time to administer endotoxin for maximal protection while −48 hours appears to be optimal in this regard when living *E. coli* are used. Similarly, enhancement of mortality is most effectively demonstrated with administration of endotoxin 24 hours after virus as opposed to +8 hours with viable *E. coli*.

Discussion. Infection of chick embryos with *E. coli* has marked effects on their susceptibility to challenge with bacterial(1) or viral pathogens. In both instances, the effects are duplicated by injection of purified endotoxin preparations at intervals which correspond to the time one would anticipate endotoxin to become available from the *E. coli* infection. Thus, it may be concluded with fair degree of certainty that the effects of *E. coli* infection may be attributed to endotoxin. In the case of NDV infection, phases of both enhanced resistance and susceptibility are observed dependent on time of administration of endotoxin or initiation of infection with *E. coli*.

Similar effects on resistance to bacterial infection in mice have been obtained by pretreatment with endotoxin(2,3) and recently reports have appeared on alteration of resistance to viral infection as well. Xerosin, an endotoxin like, heat-stable, acid-precipitable material from *Achromobacter xerosis,* has been demonstrated to suppress the neurotoxic effect of influenza virus in mice when given intracerebrally (IC) before, but not after, inoculation of virus by the same route(5). If injection of xerosin was delayed until 24 hours after virus, it was not only ineffective, but appeared to make the situation worse (enhance the neurotoxic effect). Earlier(6), this material had been tested for antiviral activity against influenza A virus in chick embryos. In tests in which xerosin was administered 1 hour before, 1 hour after, or simultaneously with virus it effected a 2-fold reduction in formation of viral hemagglutinin, but did not reduce infective titer. Possibly more striking results could have been obtained using other time intervals. Groupé *et al.*(5,7) also stated that a preparation of receptor destroying enzyme (RDE) of *Vibrio cholerae,* which was reported by Wagner(8) to protect mice against influenza neurotoxin, could be heated to 120°C and still retain protective activity. This treatment completely inactivated RDE activity of the preparation which is essentially a culture filtrate and undoubtedly contains some cholera endotoxin. This view was taken by Wagner, *et al.*(9), who reviewed the earlier literature on this subject and similarly attributed activity of xerosin to its endotoxin content.

The latter workers were able to increase resistance of mice to eastern equine encephalitis and encephalomyocarditis viruses by administration of endotoxin. Endotoxin had no effect on viral infectivity for cell cultures, nor could an antiviral substance be demonstrated in brains of mice made resistant by IC injection of endotoxin, although there was evidence of reduced virus content in brains of protected mice. Hook and Wagner(10) were also able by prior treatment with endotoxin to increase resistance of mice to neurotoxicity of influenza virus, but not to intracerebral infection with neurotropic influenza virus or lymphocytic choriomeningitis virus. Gledhill(11) found that endotoxin delayed death of mice following large doses of ectromelia virus. Similar effects were obtained with saccharated iron oxide and the author concluded that stimulation of the reticulo-endothelial system (RES) was the mechanism involved. Additional evidence for the role of RES in defense against virus infection is derived from the reduced blood clearance of NDV in thorotrast treated mice (12). The role of inflammatory cells was further implicated by the observations of Kil-

bourne(13) that survival of chick embryos infected with influenza B virus may be prolonged or shortened depending on time of injection of cortisone which was shown to suppress inflammatory response.

In our study, the protective effect is manifested primarily in a limited prolongation of survival time although occasional embryos will survive to time of hatching. Living *E. coli* seem to be more effective in this regard than endotoxin. Preliminary observations based on hemagglutinin titrations and infectivity determinations of allantoic fluid from protected and control eggs indicate that the protective effect is associated with a temporary suppression of virus levels in allantoic fluid. Therefore, whatever mechanisms may be involved in the widely demonstrated endotoxin provoked alterations of resistance, they are available, at least in part, to the developing chick embryo which should be a valuable tool in further study of these phenomena.

Summary. Phases of enhanced resistance and susceptibility to Newcastle disease virus were induced in chick embryos by infection with *E. coli*. These effects were duplicated by treatment of embryos with *E. coli* endotoxin at appropriate times.

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Effects of Various Amino Acids Upon Glucose Uptake of Rat Epididymal Adipose Tissue.* (26377)

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Insulin-like activity, as measured by glucose uptake of the rat diaphragm, has been stated to be increased by addition of the amino acids, hydroxyproline and lysine, in low concentrations to the incubation medium (1). Insulin-like or anti-insulin effects of various amino acids were measured in a bio-assay system utilizing glucose uptake by Wistar rat epididymal adipose tissue(2). This bio-assay method has been shown to be sensitive to as little as 1-10 microunits insulin/ml.

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Methods. This method of insulin bio-assay has been described and analyzed in detail previously(3). In this study, 35 to 60 mg segments of epididymal adipose tissue