similar size, both were sensitive to ether and chloroform, the stability of each was similar at the same range of temperatures, and both were shown to be RNA viruses(3,4).

Of particular interest, reciprocal induction of primary response in rabbits between HC and BVD viruses should be considered another important criterion for demonstration of heterotypic relationship. In the natural host, secondary response was not observed in calves given BVD virus followed by HC virus nor in pigs given HC virus followed by BVD virus. Certain confusion in heterotypic relationships by this criterion, therefore, may follow use of sera from natural hosts for comparative purposes. Confusion also has resulted from repeated injections of HC virus in pigs that led to cross neutralization, although homotypic titers were comparatively greater, but whether this was the result of quantitative or qualitative effects was not determined. In fact, a significant problem suggested by these studies is a determination of whether heterotypically produced antibodies are a mixture or a single antibody.

Summary. Comparison between BVD virus and HC virus in rabbits showed no cross neutralization. Inoculation of either virus followed by the other produced antibody quickly (secondary response) to both. In addition to other shared characteristics, induction of primary response is considered additional proof of heterotypic relationship.

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Endotoxin Induced Resistance to Infections and Tolerance. (28796)

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Among the various biological effects elicited by bacterial endotoxins, the most important are the ability to produce fever, to cause leukopenia, to damage tumors, to elicit the Shwartzman phenomenon and to increase resistance to a variety of infectious agents. When endotoxin is administered repeatedly, tolerance to many of its actions develops. This diminution of action on repeated administration develops with respect to the lethal effect of endotoxin, its pyrogenicity, its leukopenic action and to the occurrence of the Shwartzman phenomenon. The purpose of this present study was to ascertain whether repeated administration of endotoxin might result in a diminution of its ability to increase resistance to infections. Knowledge regarding such relationships is of importance. If it could be shown that tolerance to the endotoxin-induced increased resistance does not develop, such a finding, apart from its therapeutic implications, would be an indication that increased resistance to infections is produced in a different manner than the other actions of endotoxin to which tolerance can be produced.

Materials and methods. All experiments were carried out with Swiss-Webster CF1 male mice weighing 18 to 22 g. Endotoxin prepared from *E. coli* 026:B6, Difco Batch #449944, was used in all experiments.

To measure lethality, endotoxin was in-

TABLE I. Development of Tolerance to Lethal Effects of Endotoxin After Repeated Intraperitoneal Administration.

No. of endotoxin pretreatments	Daily pretreatment dose, mg/kg	Mean lethal dose ± S.E., mg/kg	Daily pretreatment dose, mg/kg	Mean lethal dose ± S.E., mg/kg
None	0	35 ± 9	0	30 ± 6
1	1	54 ± 20	.25	26 ± 9
4	1	72 ± 17	.25	60 ± 14
8	1	165 ± 31	.25	100 ± 15

jected intraperitoneally using 10 mice at each dose level. White cell counts were carried out with duplicate samples on groups of 10 mice with a Coulter Counter using the technique described by Brecher *et al*(1) and Richar *et al*(2). To determine the increase in resistance, mice were challenged with *Pseudomonas aeruginosa* or *Salmonella typhi*. Most experiments were carried out with *Ps. aeruginosa* which was grown on tryptose agar for 18 to 20 hours. Each mouse received intravenously approximately 2×10^7 viable organisms suspended in 0.2 ml of tryptose-gelatin-saline (0.26%, 0.1%, 0.85%). Bacterial suspensions were adjusted to this concentration by measuring their turbidity and the number of cells determined subsequently by plate counts. Deaths were observed twice daily for 4 days after infection. Attempts to produce tolerance were made by injecting endotoxin intraperitoneally in doses of 0.25 mg/kg or 1 mg/kg once daily for 4 or 8 successive days.

Results. Lethality. The development of tolerance to the lethal effects of endotoxin is illustrated in Table I. The mean lethal dose of endotoxin was about 30 mg/kg. The diminution of the toxic effect after repeated administration was such that the LD_{50} after 8 pretreatments with endotoxin (1 mg/kg per treatment) was several times larger than the LD_{50} observed in animals not previously exposed to endotoxin. Significant tolerance developed after only a single pretreatment, provided endotoxin was given in a sufficiently large dose, and tolerance increased further with the number of doses given. When small doses of endotoxin were used, several administrations of endotoxin were necessary to produce tolerance.

Leukopenia. In confirmation of results previously obtained by others, a single dose of endotoxin 1 mg/kg produced well marked leukopenia 2 hours after administration. This leukopenic effect could be eliminated by giving endotoxin 1 mg/kg for 8 successive days. The values obtained after 8 administrations were similar to those seen in control animals which received no endotoxin, showing the development of complete tolerance. Administration of endotoxin for 4 days also produced tolerance. Smaller doses of endotoxin, such as 0.25 mg/kg given once, caused leukopenia which, however, was on the borderline of statistical significance. Repetition of this small dose for 4 or 8 doses abolished this effect.

Resistance to infection. The effect of endotoxin administration on resistance to death in mice infected with *Ps. aeruginosa* is illustrated in Table III. In these experiments the number of surviving mice were counted at a time when most or all of the controls that did not receive endotoxin pretreatment were dead (bacterial control). A single injection of endotoxin (endotoxin control) significantly increased the number of survivors. Repeated injections of endotoxin produced better protection against the lethal effects of the infection at both dose levels of endotoxin

TABLE II. Development of Tolerance to Leukopenic Effect of Endotoxin 1 mg/kg After Repeated Intraperitoneal Administration.

No. of endotoxin pretreatments	Mean leukocyte count, mm³ ± S.E. Hr after last injection 2	6	24
0 (controls)	16.1 ± 2.1	17.6 ± 2.4	17.0 ± 2.6
1	8.1 ± 1.0*	14.1 ± 1.1	20.6 ± 3.6
4	13.2 ± 1.5	11.4 ± .8	14.9 ± .9
8	17.0 ± .6	21.2 ± 2.1	16.2 ± 1.0

* Significantly different from animals receiving endotoxin 4 or 8 times and from controls.

TABLE III. Effect of Repeated Endotoxin Administration on Incidence of Death in Mice Infected with *Pseudomonas aeruginosa*.

No. of endotoxin pretreatment	Daily pretreatment dose, mg/kg	No. dead/ No. used	Daily pretreatment dose, mg/kg	No. dead/ No. used
None (bacterial control)	0	20/20	0	20/20
1 (endotoxin control)	.25	10/20	1.0	8/20
2	.25	4/20†	1.0	2/19†
4	.25	0/20*	1.0	2/20†
8	.25	8/20	1.0	1/20*

A suspension of *Pseudomonas aeruginosa* (2×10^7 viable cells) was given to each mouse 48 hr after the last endotoxin dose. Number of surviving mice was counted 46 hr after infection when all control animals had died.

* Significantly different from endotoxin control ($P < .01$).
† Significantly different from endotoxin control ($P < .05$).

employed. It appears, however, particularly when additional experiments not described here are considered, that small doses of endotoxin are somewhat less effective and produce less reproducible results than optimal doses. It must also be taken into consideration that injection of saline 48 hours prior to the challenge with microorganisms, particularly if repeated, may protect a proportion of the animals from death. This protection, however, is of a lower order than that obtained with endotoxin and never results in survival of more than 25% of the animals. It is also a fact that protection of animals from microbial infections by endotoxin cannot be reproduced with the same degree of accuracy as the inducement of leukopenia or the production of death. This may be due to slight and unavoidable differences in the virulence of the bacterial culture used for infection and other factors. However, even with all these reservations, there can be no doubt that the ability of endotoxin to increase resistance to infections is not diminished after repeated administration of endotoxin. We have observed in many experiments that repeated administration of endotoxin in non-lethal doses protects animals at least as well as, and usually better than, a single dose of endotoxin. Thus, no evidence could be obtained which would indicate the development of tolerance to the resistance-increasing action of endotoxin. Similar findings were obtained by Abernathy(3) who found when working with *Brucella melitensis* in mice that 3 weekly injections of endotoxin protected better than a single injection.

The increase of resistance to infections obtained after repeated injections of endotoxin is non-specific in character as is the increased resistance observed after single administration. This has been confirmed in experiments utilizing *Salmonella typhi* and other organisms.

Discussion. That repeated administration of endotoxin does not result in decreased resistance of animals to infection is of considerable interest, particularly in conjunction with the fact that tolerance to the other effects of endotoxin invariably develops. These observations indicate that at least two separate mechanisms of action may be responsible for the 2 types of effect. Stetson (4) expressed the view that many important effects of endotoxin, such as fever, shock and death, skin and corneal reactions, and local and generalized Shwartzman phenomena, can be reproduced by antigen-antibody interactions and that these effects may be the result of a natural hypersensitivity to endotoxin. Tolerance to all these effects is known to develop.

Our results show that the effects produced by endotoxin can be divided into 2 classes: (1) effects to which tolerance develops and (2) effects to which tolerance does not develop. The increased resistance to infections produced by endotoxin administration ap-

pears to be the only known property of endotoxin to which tolerance does not develop. All other effects lead to a diminution of response, *i.e.,* tolerance, on repeated administration of endotoxin. It then appears that endotoxin may be a mixture of at least 2 substances, one producing effects to which tolerance can be produced and the other causing an increase of resistance to infection to which tolerance does not develop. An alternative explanation, which would retain the concept of endotoxin as a single entity, would ascribe the tolerance-producing effects to a common biological action (such as release of a neurohormone) and account for the resistance increasing property by a different mechanism.

Considerable work is being carried out with the aim of producing endotoxin that would increase resistance to infections without possessing other less desirable properties, such as pyrogenicity, lethality, and ability to produce the Shwartzman phenomenon(5,6,7, 8). All these studies have attempted to modify endotoxin chemically. The results reported here suggest that endotoxin may not be a single entity and that attempts to isolate from it the resistance-increasing substance and the tolerance-producing substance may hold greater promise of success than chemical modification of the complex macromolecule.

Summary. The ability of endotoxin to increase the resistance of animals to infection is maintained or increased after repeated administration of endotoxin. This finding contrasts with the development of tolerance to the other effects of endotoxin, such as the diminution or disappearance of the lethal and granulopenic effect of endotoxin on repeated administration. These results suggest that endotoxin may be a mixture of at least two substances, one responsible for the effects to which tolerance is produced and another responsible for the increase in resistance to infections. It is also possible that these divergent effects may be mediated through two different mechanisms. The consequences following from these conclusions are briefly discussed.

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Age Changes in the Plasma Glycoproteins of the Mongolian Gerbil.* (28797)

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Certain unique characteristics of the Mongolian gerbil, *Meriones unguiculatus,* have prompted a number of investigations on the blood of this animal. For example, serum cholesterol levels in the gerbil are easily increased by dietary factors without, however, resulting in any significant atherosclerotic plaque formation(1,2,3). Normal plasma protein values covering the post-natal lifespan of the gerbil (through 28 months of age) have been reported by Ringle and Dellenback(4) using a bromophenol blue staining technique. In view of the importance of glycoprotein values in estimating physiologi-

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