this function, and not to a reduced synthesis of DNA by a given cell.

Richter and Haurowitz (13) showed that antibody synthesis to innocuous protein antigens continued unabated in rabbits for several months following either single or multiple injections. These antibodies were of the nonprecipitating type, but were detectable by the agglutination of antigen-coated red cells. This continued formation of antibody may be the result of retained antigen or antigenic fragments over prolonged periods of time as suggested by Campbell and Garvey (14). With the prolonged retention of antigen which is degraded very slowly, for example in a hibernating animal, a pseudosecondary response in animals hibernating for 1 month is not unreasonable.

Summary. Specific antibody formation to influenza A virus vaccine (strain PR_8) has been demonstrated in hibernating *Citellus tridecemlineatus*. Although only a small amount of hemagglutination-inhibiting antibody was formed on primary antigenic stimulation, it was detectable. A true anamnestic response was shown in animals given a booster dose of antigen 1 month after the initial injection when these animals were awake at room temperature for 5 days before sacrifice. Whether a true secondary response was obtained entirely in hibernation is not ascertainable from our data, although the GMT was significantly different from the primary GMT. A possible pseudosecondary response was also noted following primary antigenic stimulation when the antigen presumeably was retained in the hibernating animals.

Note added in proof: Since the submission of this paper, Jaraslow (Proc. Nat. Acad. Sci., **61**, 69, 1968) has shown the development of the secondary response in hibernating animals of the same species as we used.

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Endotoxin Induced Hypothermia and Tolerance in the Rat* (33409)

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Lipopolysaccharides extracted from gram-negative bacteria, i.e., endotoxins, are often termed pyrogens due principally to their ability to induce fever in humans, rabbits and dogs (1). In addition, tolerance to endotoxin febrility has generally been demonstrated in animals chronically pretreated with daily in-

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jections of endotoxin (2, 3). Although the mechanism of endotoxin tolerance is still unresolved, it is generally held that the reticuloendothelial system (RES) plays an important role in both host defense to endotoxemia and tolerance induction (2).

Recent studies have indicated that, while endotoxin treatment depressed the phagocytic activity of the RES in rabbits and mice (4), endotoxemia produced a hyperfunctional phagocytic state in the rat (5, 6). Since an enhanced phagocytic response may contribute to the relative resistance of the rat to the symptoms of endotoxin toxicity, the present study evaluated certain aspects of the body temperature response of the rat to endotoxin. In confirmation of previous indications (7, 8), it was found that rats respond to endotoxin with a fall in body temperature, i.e., hypothermia. In addition, induction of tolerance to endotoxin hypothermia was demonstrated and contrasted to febrile tolerance in rabbits.

Materials and Methods. Male albino rats of the Holtzman strain (250–300 g) and New Zealand rabbits (2–3 kg) were fed Purina lab chow and water *ad libitum* in animal quarters maintained at 70 ± 2°F. Endotoxins of the Boivin types prepared from *Salmonella enteritidis, Salmonella typhimurium,* and *Escherichia coli* 026:B6 were purchased from Difco Laboratories, Detroit, Mich. All endotoxins were prepared in pyrogen-free 0.9% sodium chloride solution and administered intravenously via either the dorsal vein of the penis in rats or the lateral ear vein in rabbits. Body temperature in rats and rabbits was measured in unanesthetized animals at room temperature of 70 ± 2°F via a rectal thermistor probe and an electronic recording system (Yellow Springs Instrument Co., Yellow Springs, Ohio). Data were analyzed for statistical significance at the 95% level of confidence using Student's *t* tests.

Results and Discussion. As indicated in Fig. 1, three different endotoxins injected intravenously at a dose of 0.5 mg/rat produced an abrupt but transient fall in rectal temperature. The hypothermic response was characteristically biphasic; however, the magnitude and temporal pattern of the second phase

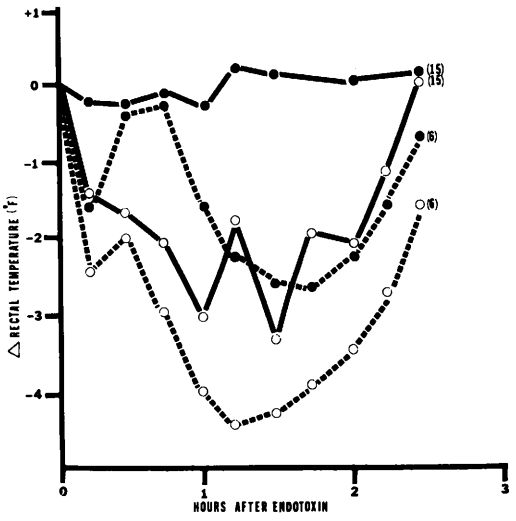

FIG. 1. Effect of three different endotoxins on change in rectal temperature in the rat. Numbers in parentheses indicate rats/group; av SE was 0.60°F; (●—), saline; (○—), *S. enteritidis*; (●- -), *E. coli*; and (○- -), *S. typhimurium.*

varied greatly among rats. It should be noted that the ambient temperature may be an important determinant of both the direction and magnitude of the rectal temperature response (4); in these studies room temperature was 70 ± 2°F.

Since it is plausible that lower endotoxin doses might elicit a different temperature response, the rectal temperature was measured after varying doses of *S. enteritidis* endotoxin. As presented in Table I, the response to doses of 0.05, 0.075, 0.1, and 0.5 mg was consistently a fall in rectal temperature.

In contrast to the hypothermia induced in rats, *S. enteritidis* endotoxin produced a typical biphasic fever in rabbits; furthermore, tolerance to febrility was produced by six daily incremental doses of endotoxin (Fig.

TABLE I. Effect of *S. enteritidis* Endotoxin Dose on Body Temperature in Male Rats.

Dose of endotoxin (mg i.v.)	No. of rats	Maximal change in rectal temperature (mean ± SE)
0.050	4	—3.5 ± 0.45
0.075	4	—4.2 ± 0.55
0.100	4	—4.8 ± 0.60
0.500	6	—4.5 ± 0.65

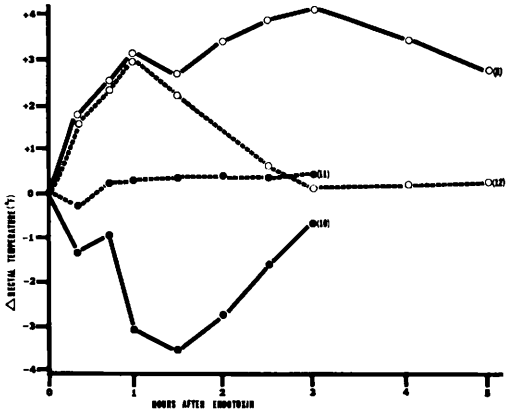

FIG. 2. Tolerance to *S. enteritidis* endotoxin induced febrility in rabbits and hypothermia in rats. Numbers in parentheses indicate rabbits or rats per group; av SE was 0.65°F. Regimens to induce tolerance were: rats - six daily injections of 0.5, 0.5, 1.0, 1.0, 2.0, and 2.0 mg; rabbits - six daily injections of 2.5, 2.5, 5.0, 5.0, 10.0, and 10.0 μg. (○—), 2.5 μg of endotoxin to control rabbits; (○- -), 2.5 μg of endotoxin to tolerant rabbits; (●—), 0.5 mg of endotoxin to control rats; and (●- -), 0.5 mg of endotoxin to tolerant rats.

2). In a similar fashion, but relating to the hypothermic effect, a tolerant state was also produced in rats by incremental pretreatment doses of endotoxin.

The data presented substantiate the ability of small mammals to respond to endotoxin with a fall, rather than a rise in body temperature (4). The apparent biphasicity of the hypothermic effect may reflect a twofold action, i.e., directly on the hypothalamus (4) and release of endogenous mediators (3, 5), similar to the mechanisms proposed for fever production. Although the precise physiology of the hypothermia is unknown, the capability to induce a tolerant state in the rat may allow analysis of the mechanism of hypothermia similar to studies performed on the mechanism of febrile tolerance in rabbits. It may be of significance that both the body temperature and RES alterations accompanying endotoxemia in rats are strikingly contrary to other animal species which are more sensitive to both lethality and other effects of endotoxin.

Summary. Endotoxins derived from *S. enteritidis, S. typhimurium,* and *E. coli* induced a fall in rectal temperature in rats maintained at 70 ± 2°F. The hypothermic response occurred over a dosage range of 0.05–0.5 mg. In correspondence to tolerance to fever induced in rabbits, incremental dose pretreatment of rats produced a tolerance to the hypothermic phenomena.

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