fected cortisone-treated mice in a warm environment, may be the result of the action of cortisone in decreasing capillary permeability (6). Also, it has been shown that cortisone blocks body temperature reducing effect of histamine(7). Weir(8) concluded that at moderate environmental temperatures (20°C) fever was not characteristic of mouse typhoid. He reported that rectal temperatures of infected mice were highly correlated with room temperature. Other studies have also shown that the mouse has little defense against increased environmental temperature(9,10). Hence, it is possible that the effect of temperature on toxicity of cortisone may be demonstrable only in the mouse.

Summary. Mice infected with *S. typhimurium* were maintained for 3 weeks either in a warm environmental temperature of 29° to 36°C or a moderate temperature 21° to 25°C. At the warmer temperature, subcutaneous injections of cortisone were more effective in reducing survival. Room temperature had no significant effect upon survival of those infected mice which received the Aqueous Vehicle of cortisone.

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Received January 4, 1957. P.S.E.B.M., 1957, v94.

Cortisone and Mortality in Mouse Typhoid. III. Effect of Natural and Acquired Immunity.* (22986)

SARANE THOMPSON BOWEN,† JOHN W. GOWEN, AND OSCAR E. TAUBER
Departments of Genetics and Zoology, Iowa State College, Ames.

Cortisone may either increase or decrease resistance of infected animals, depending upon severity of the infection(1). Conversely, survival of animals infected by the same experimental inoculum may be either increased or decreased by cortisone treatment, depending upon hormone dosage(2). Most studies of hormone-infection relationship rely upon quantitative manipulation of either the hormone or the infectious inoculum. In the present investigation, a third experimental design was possible, by using host animals of varying genetic constitution. Seven strains of mice which differed in natural resistance to *Salmonella typhimurium* were given the same amount of cortisone and were inoculated with the same number of bacteria. It was then possible to establish the effect of cortisone on innate resistance to disease. The effect of cortisone on acquired resistance was determined by comparing mortality of normal and previously immunized mice during simultaneous treatment with cortisone and infection with *S. typhimurium.*

Materials and methods. All mice were between 5 and 7 months old. Seven inbred mouse strains were used: S, RI, Z, K, E, LGw, designated L, and BALB/Gw, designated Ba. These strains were chosen because of the wide range in their innate resistance to mouse typhoid. Inoculations of $2X10^5$ living bacteria of the 11C culture of *S. typhimurium* have established the following survival values: S, 95%; RI, 78%; K, 63%; Z, 43%; E,

* This work supported in part by grant to Dr. John W. Gowen from Rockefeller Foundation. Journal Paper No. 3091 of the Iowa Agr. Exp. Station, Ames, Project Nos. 1180 and 1187.

† Present address: San Francisco State College, Calif.

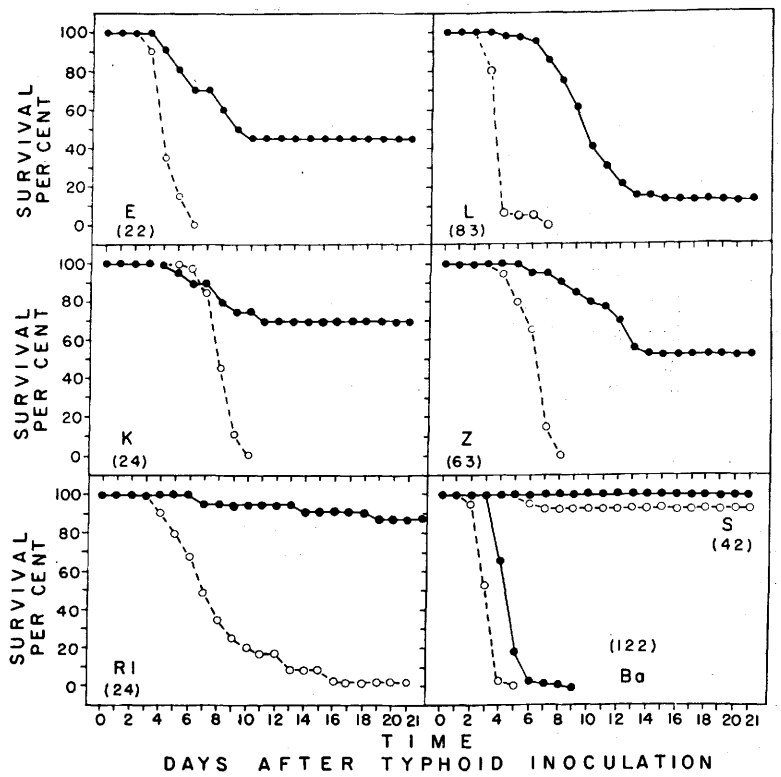

FIG. 1. Comparison of survival of control and cortisone-treated mice during mouse typhoid. The 7 mouse strains, ranked in order of decreasing resistance to *S. typhimurium*, are: S, RI, K, Z, E, L, and Ba. Numbers in parentheses indicate No. of mice in each strain which received 1 mg cortisone. An equal No. of animals was in the control group. *Solid line*, survival of controls. *Dotted line*, survival of cortisone-treated mice.

25%; L, 8%; and Ba, 0.2%. These earlier experiments utilized from 280 to 1440 female mice, 100 to 400 days old, in each strain. By testing resistance of progeny of double matings and reciprocal crosses, it has been rigorously proven that resistance to mouse typhoid of 3 of these strains (S, L, and Ba) is due to innate immunity rather than acquired immunity(4,5). The genetic basis of resistance is also demonstrated by the fact that for 15 years the breeding animals of all 7 strains have had no known contact with the pathogen, yet each strain maintains its characteristic level of resistance when tested with *S. typhimurium*(3). All mice were infected by intraperitoneal injection of $2X10^5$ living bacteria of the 11C strain of *S. typhimurium*. Treated mice were given a single subcutaneous injection of cortisone acetate (Cortone, Merck) in saline with suspending agents and benzyl alcohol as preservative. Controls were given equal volume of Aqueous Vehicle No. 1 (Merck).‡ Cortisone-treated mice and their litter-mate controls were kept in the same cage throughout the 3 week experiment. Each mouse carried its identifying pedigree number. The temperature in the isolation room ranged from 28° to 34°C.

Results. Effect of natural resistance. In the first experiment, 380 pairs of mature female mice were used. One member of each pair was given a subcutaneous injection of 1 mg cortisone, and its litter mate control was given an equal volume of Aqueous Vehicle. Twenty-four hours later, both mice were inoculated with *S. typhimurium*. In this way it was demonstrated that resistance of mice

‡ All cortisone was generously supplied by Merck and Co., Rahway, N. J.

in all 7 strains was decreased by pretreatment with cortisone. This may be seen in Fig. 1.

At the end of 3 weeks, survival of the most resistant strain, S, was reduced from 100% in controls to 93% in cortisone treated animals. In the other resistant strain (RI), survival was significantly reduced from 88% to 4% as a result of hormone administration. In 5 susceptible strains, 1 mg cortisone reduced survival to 0% within 10 days after inoculation of *S. typhimurium*. At the end of 3 weeks, survivals of controls of other strains were: K, 71%; Z, 52%; E, 45%; L, 12%; and Ba, 0%.

Differences in survival pattern of control and treated animals could be seen earlier in susceptible strains than in more resistant strains. Survival difference was seen on second day of infection in the Ba strain and on the third day in the E and L strains. In the 2 most resistant strains, the effect of cortisone was evident on the fourth day for the RI strain and on the sixth day for the S strain.

Effect of acquired resistance. Fifty sets of male mice from the most resistant S strain were used. Each set contained 3 litter-mates. One mouse in each trio had been immunized by intraperitoneal inoculation of $2X10^5$ living bacteria of the 11C culture of *S. typhimurium* 6 weeks previously. This immunized mouse and one of the normal mice were each given a single subcutaneous injection of 5 mg cortisone. The third member of each set was given an equal volume of Aqueous Vehicle. Twenty-four hours later, all 3 mice in each set were infected with $2X10^5$ cells of a viable 11C *S. typhimurium* culture. The results of this experiment are summarized in Fig. 2. Survival of immunized mice treated with cortisone was 98%. It is evident that this quantity of the infectious agent is not enough to kill either normal or immunized mice of the most resistant S strain. Infection has a high mortality rate in the S strain only when non-immunized animals are pretreated with cortisone. It had previously been determined that 5 mg cortisone is not toxic for uninfected mice (6). Hence, it has been demonstrated that survival of normal mice and of immunized infected mice is not significantly altered by a dose of cortisone which reduces survival of non-immunized infected mice from 100% to 0%.

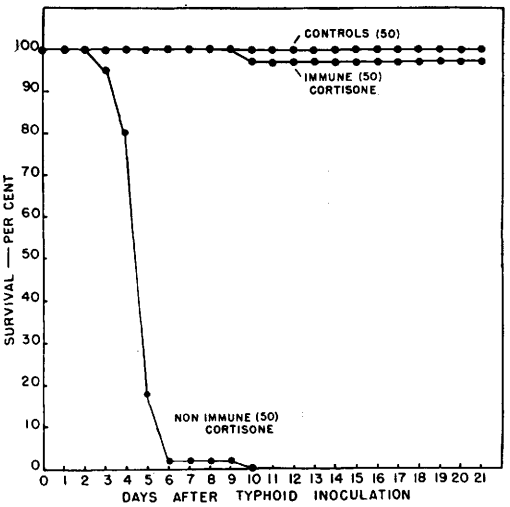

FIG. 2. Comparison of survival of normal and immunized male mice of S strain inj. with 5 mg cortisone one day before typhoid inoculation. Controls received an equal quantity of Aqueous Vehicle of cortisone. Figures in parentheses indicate No. of mice.

Discussion. Inbred strains of animals which differ in their innate resistance to a disease are an ideal material for the study of relationship between hormone dosage and severity of infection. The use of such strains obviates variation of amount of hormone or virulence of infectious agent. In studies in which dosage must be varied, there is a possibility that the effect of the hormone will be confounded with the effect of the suspending agent. This would certainly be true if the two had a synergistic action. It has been demonstrated that the cortisone vehicle reduces resistance of mice to monilial infections(1). In experiments in which the severity of the disease is controlled by gradations in the quantity of infectious agent, other problems of interpretation arise. The effect of a given quantity of hormone on a mild infection may represent an action upon bacterial invasiveness, whereas the effect of this same amount of hormone on a severe infection may represent an action upon resistance to toxins. Experiments which are based upon animals of varying natural resistance are free of these interactions.

Scherr has shown that cortisone increases resistance of mice to severe monilial infections, but decreases their resistance in mild infections(1). It is possible that a similar relationship holds true in mouse typhoid, but, if this were the case, one would expect that cortisone would be less harmful in the more susceptible strains. On the contrary, the detrimental action of cortisone showed itself earlier in the course of infection in susceptible strains.

Cortisone may have a more specific detrimental effect in mouse typhoid than in other diseases. It is well established that glucocorticoids and *Salmonella* infections both produce hyperglycemia, lymphopenia, and eosinopenia(7,8,9). Because cortisone administration simulates mouse typhoid in these respects, it might lower resistance through its action upon carbohydrate metabolism, or upon white blood cells. It is also possible that the level of cortical secretions may account for innate resistance or susceptibility of the strains. This concept has been investigated by Lurie *et al.*(10) in studies of tuberculosis in genetically resistant and susceptible rabbits.

We have shown that cortisone decreases natural immunity but not active acquired immunity to *S. typhimurium*. In studies of streptococcus infections in rabbits, Mogabgab and Thomas(11) also found that cortisone did not interfere with the mechanisms of active immunity. Payne *et al.*(12) have reported that both active and passive immunization protect cortisone-treated mice from *Pasteurella pestis* infections which are lethal for non-immunized mice.

Summary. Seven mouse strains which were selected for the wide range in their natural resistance to *S. typhimurium* were used. Typhoid resistance of every strain was decreased when 1 mg cortisone was given one day before typhoid inoculation. The difference between survival of cortisone treated mice and that of controls was evident at an earlier time in the genetically susceptible strains than in resistant strains. A single injection of 5 mg cortisone which reduced survival of non-immunized mice from 100% to 0% had no significant effect on survival of immunized mice.

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Received January 4, 1957. P.S.E.B.M., 1957, v94.