suggests that antibody formation does not proceed with the disease.

*Summary.* Different fractions of plasma proteins were determined by paper electrophoresis in 16 patients suffering from cholera, in 11 cases of tetanus, 10 cases of meningitis, 10 cases of small pox and 22 normal subjects. Total protein was lower than normal in all diseases studied except tetanus. Albumin was lower and fibrinogen was higher in all diseases as compared to normal values. Of the globulins, cholera cases had low $\alpha$, increased $\beta$ and normal $\gamma$-globulins. $\beta$-globulin contained $\beta_1$ and $\beta_2$ fractions not seen in normal persons. In patients suffering from small pox $\alpha$- and $\beta$-globulin fractions were normal but $\gamma$-globulin fraction which increased, contained $\gamma_1$- and $\gamma_2$-fractions. In patients suffering from meningitis and tetanus all the globulin fractions increased but in meningitis these increased values were not statistically significant. The implication of these changes has been discussed.

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## Cortisone and Mortality in Mouse Typhoid. I. Effect of Hormone Dosage and Time of Injection.* (22984)

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The effect of cortisone on progress of bacterial infections remains a controversial issue. Some studies have indicated that this hormone lowers resistance(1,2). Other experiments have demonstrated that cortisone, in small quantities, will increase natural resistance to infection(3,4). This disagreement is of interest in regard to the *Salmonella* diseases, inasmuch as cortisone has been recommended as an adjunct in treatment(5). The following data summarize a study of the effects of cortisone on mortality of 4 strains of mice genetically differentiated for resistance to *Salmonella typhimurium*.

*Materials and methods.* Four inbred mouse strains from our Laboratory were used in this study: S, Z, K, and BALB/Gw, indicated as Ba. Each strain has been inbred by over 30 generations of brother-sister matings and, as a result, has become a distinct biotype with characteristic level of resistance to mouse typhoid. The wide range in resistance of these 4 strains may be seen in Table I. All mice were 5½-6½ months old. *Bacterial inocula-*

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TABLE I. Natural Resistance of Female Mice of 4 Strains to Intraperitoneal Inoculation of $2 \times 10^5$ Typhoid Bacteria (*Salmonella typhimurium* 11C).*

| Strain | Total inoculated | Survived | % survived |
|---|---|---|---|
| S | 1232 | 1173 | 95.2 |
| K | 534 | 337 | 63.1 |
| Z | 1440 | 616 | 42.8 |
| Ba | 897 | 2 | .2 |

* These data for mice from 100 to 400 days of age have accumulated over a period of years as part of results from numerous investigations.

*tions.* Mice were infected by intraperitoneal injection of approximately 200,000 bacteria of 11C strain of *S. typhimurium.* This 11C culture has been maintained by mass transfers on nutrient agar slants and has shown consistent virulence for 26 years. Eighteen hour cultures were suspended in 0.85% saline with 0.05 peptone. Suspensions were brought to standard density by appropriate dilutions within the linear portion of the calibration curve on a Lumetron photoelectric colorimeter. Each calibration was checked by plate count. The mean bacterial count in 26 inoculations was estimated to be 1.96 ± 0.04 hundred thousand cells. *Hormones.* Progesterone (Progestone Schering)‡ and desoxycorticosterone acetate (Cortate Schering) were used as crystalline suspensions in sesame oil. Control animals received an equal volume of Plain Sesame Oil (Schering). Cortisone acetate (Cortone Merck) was used as suspension in saline. Control animals in the cortisone experiments received an equal volume of Aqueous Vehicle No. 1 (Merck). Each injection was given in single subcutaneous dose. This injection preceded typhoid inoculation by 24 hours, unless otherwise noted. In all experiments, hormone-treated animals and their litter-mate controls were confined in the same cage; identification was by pedigree numbers.

*Results.* As little as 0.25 mg of cortisone reduced resistance of a mouse to *S. typhimurium.* When this quantity was given to males of the BALB/Gw strain, their mean survival time was reduced from 5.6 ± 0.1 to 4.9 ± 0.1 days. This difference is significant ($P < 0.01$). The observations on these 148 cortisone-treated mice and their litter-mate controls are presented in Table II. In a similar experiment on 56 litter-mate pairs of mice, a single injection of 0.01 mg of cortisone had no significant effect on resistance.

*Other steroid hormones.* Injections of 2 other physiologically active steroids had no effect upon typhoid resistance. Varying quantities of progesterone and desoxycorticosterone acetate were injected 24 hours before typhoid inoculation. Each hormone dose was given to 10 mice and their mortality was then compared with that of their 10 litter-mate controls. In doses of 0.1 mg and 1.0 mg, these 2 steroids did not significantly prolong survival of male mice of the BALB/Gw strain. In quantities of 1, 5, and 10 mg, neither hormone lowered typhoid resistance of male Z mice, a strain possessing intermediate resistance to typhoid infection. This indicates that massive doses of some steroid hormones do not lower resistance unless, like cortisone, they have a specific action in mouse typhoid.

*Large doses of cortisone.* In the following experiments the doses of cortisone ranged from 1 to 5 mg. Previous investigations had established that single doses to 5 mg were not toxic to normal mice(6,7). In our study, a 5 mg dose of cortisone had no significant effect on body weights of 6 mature male S mice when compared with their 6 litter-mate controls. Nevertheless, when smaller amounts (from 1 to 4 mg) were injected into S mice

TABLE II. Distribution of Deaths of Control and Cortisone-Treated Mice.*

| Exp. | | Day of infection 3 | 4 | 5 | 6 | 7 | 8 | 9 | Total |
|---|---|---|---|---|---|---|---|---|---|
| A | Control | 0 | 3 | 26 | 30 | 4 | 0 | 0 | 63 |
| | Treated | 0 | 12 | 34 | 16 | 1 | 0 | 0 | 63 |
| B | Control | 0 | 4 | 17 | 12 | 3 | 1 | 1 | 38 |
| | Treated | 3 | 19 | 12 | 3 | 1 | 0 | 0 | 38 |
| C | Control | 0 | 8 | 14 | 16 | 6 | 2 | 1 | 47 |
| | Treated | 1 | 17 | 15 | 12 | 2 | 0 | 0 | 47 |

* Mice were males of the Ba strain. Each treated mouse received 0.25 mg cortisone acetate one day before inoculation of *S. typhimurium.* Records for male Ba mice, tested over the same period as for females in Table I, show zero survival for 622 individuals.

‡ The authors wish to acknowledge generous supplies of hormones furnished by Merck and Co., Rahway, N. J., and by Schering Corp., Bloomfield, N. J.

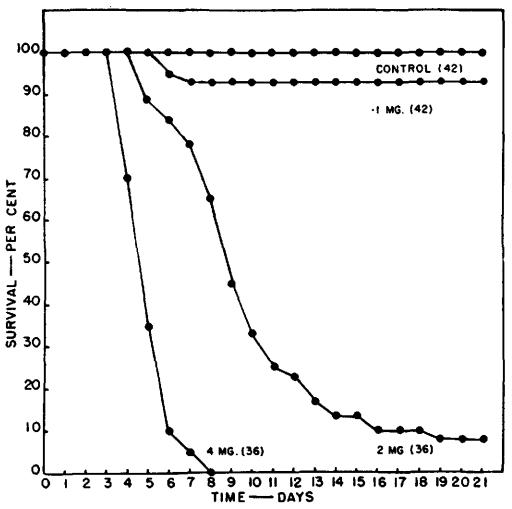


FIG. 1. Comparison of survival of control and cortisone-treated mice after inoculation with *S. typhimurium*. Figures in parentheses indicate No. of mice (S females) in each group.

which were inoculated with *S. typhimurium*, their resistance was markedly decreased. Mice of the S strain have a high resistance to typhoid. The effects of increasing doses of cortisone on mortality in mouse typhoid may be seen in Fig. 1. Twenty-one days after typhoid inoculation, none of the mice given 4 mg of cortisone had survived; 8% of those given 2 mg survived; 93% given 1 mg, and 100% receiving no cortisone had survived.

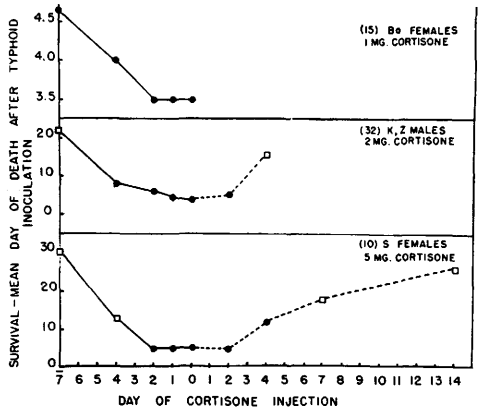


FIG. 2. Effect of single subcut. cortisone inj. given at varying times before or after inoculation with *S. typhimurium*. Experiments on 4 mouse strains (Ba, K, Z, and S) are illustrated. Numerals in parentheses indicate No. of mice used at each cortisone inj. represented by dot or square. Squares indicate that one or more mice survived beyond arbitrarily assigned day-of-death value (30th day of inj.). *Solid line*, cortisone inj. preceded typhoid inoculation. *Dotted line*, cortisone followed typhoid inoculation.

*Time of cortisone injection.* Cortisone had the greatest effect in reducing survival when administered within 2 days before or after typhoid inoculation. This may be seen in Fig. 2. In this experiment, equal numbers of mice were assigned at random to groups all of which were inoculated with *S. typhimurium* on the same day, but which received a single dose of cortisone either before or after typhoid inoculation. When cortisone was given after typhoid inoculation, there were 2 ways of calculating mean day-of-death-values. Length of survival could be measured from 1) time of typhoid inoculation, or 2) from time of cortisone injection. The former method was chosen for graphic presentation of data in Fig. 2. Because experiments on the 4 strains were done at different times, strain comparisons are not valid for this experiment. However, it is evident that each replication of this experiment, though on a different strain, is consistent: single injections of cortisone had the greatest effect in decreasing resistance when given within 2 days of typhoid inoculation.

*Discussion.* Other workers have shown that adrenal extracts have protected adrenalectomized animals from *Salmonella* endotoxins(8).§ Therefore, it is possible that the unfavorable action of cortisone on mouse typhoid resistance might be attributed to use of unphysiological doses. Robinson(3) has demonstrated that there is an optimal dose of cortisone which protects rats in *Diplococcus pneumoniae* infections, while greater amounts of hormone decrease their resistance. Nevertheless, one might expect that cortisone, when given in dosage which protects adrenalectomized mice from toxins, would increase resistance to infection. This is not the case. Delaunay and Lebrun found that 2 mg of cortisone protected adrenalectomized mice from *Salmonella* endotoxins. Yet in our study, less than 1 mg was effective in decreas-

§ Preliminary report by Delaunay and Lebrun in *Compt. Rend. Acad. Sci.*, 1951, v233, 1698.

ing resistance to infection. Because these small doses were shown to be non-toxic to the normal animal, it would seem that cortisone has some specific detrimental effect in mouse typhoid.

*Summary.* Cortisone, administered in single injections ranging from 0.25 to 5 mg, markedly decreased resistance of mice to *S. typhimurium*. Cortisone had the greatest effect on resistance when administered within 2 days before or after typhoid inoculation. Subcutaneous injections of two other steroids, progesterone and desoxycorticosterone acetate, in dosage range of 0.1 to 10 mg, had no effect on mortality in mouse typhoid.

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## Cortisone and Mortality in Mouse Typhoid. II. Effect of Environmental Temperature.* (22985)

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In an investigation of effects of cortisone‡ on mice infected with *Salmonella typhimurium,* it was observed that during the hot summer months cortisone treatment was more effective in reducing survival. To determine the effect of environmental temperature on cortisone treatment, the following experiments were completed under conditions of controlled room temperature.

*Procedure.* Two mouse strains were used: an innately resistant strain (RI), and a strain (Z) of intermediate resistance to *S. typhimurium*. Of 280 untreated female RI mice, 100 to 400 days old, 77.9% survived a single dose of 200,000 11C *S. typhimurium,* whereas only 42.8% of 1440 Z females survived the same inocula. Evidence that this disease resistance is innate and not acquired has been reviewed by Gowen(1). Hormone-treated mice, 5½ to 7 months old, were given a single subcutaneous injection of cortisone acetate (Cortone, Merck) in saline with suspending agents (polyoxyethylene sorbitan monooleate and sodium carboxymethylcellulose) and benzyl alcohol as preservative. Control animals received an equal volume of Aqueous Vehicle No. 1 (Merck). Cortisone and aqueous vehicle injections were given 24 hours before typhoid inoculation. All mice were inoculated with $2X10^5$ viable cells of 11C strain of *S. typhimurium*. Throughout each 3 week experiment, cortisone-treated mice and their litter-mate controls were confined in the same cage; identification was by pedigree numbers. This investigation was conducted during the summer and an air conditioning unit was used to attain "moderate" temperature of 21° to

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