Effects of Cortisone and Antibiotics on Lethal Action of Endotoxins in Mice.* (21211)

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The administration of ACTH or cortisone to humans and animals enhances the severity of many natural or experimental infections, presumably by depressing the antimicrobial defenses of the host(1-3). On the other hand, it has also been shown that ACTH or cortisone may exert an "antitoxic" action *in vivo* against the lethal effects of endotoxins derived from a wide variety of gram negative bacteria(4,5). In a few natural infections, notably typhoid fever and acute brucellosis, where the endotoxins of the infecting microorganisms are thought to play an important role in the pathogenesis of the disease, the judicious use of cortisone or ACTH in the therapeutic regimen results in a rapid suppression of "toxicity" and an improvement in the sense of well-being of the patient(6-8). However, Spink and Hall(8) have strongly cautioned against the indiscriminate use of these hormones in human brucellosis, by pointing out the undesirable side effects which may occur and also because antibiotics alone provide effective treatment in the majority of these cases. This paper summarizes experiments on the protective effects of cortisone and the combined effects of cortisone and antibiotics in animals injected with lethal amounts of endotoxins derived from a coliform bacillus.

Methods and materials. The crude endotoxin used in this study was prepared from the cells of *Escherichia intermedium* as described elsewhere(9). A single batch of endotoxin was used in all experiments. It was stored in a dry state in a desiccating jar and made up to the desired concentration with sterile water on the day used. Standardization of the endotoxin dosage on an LD_{50} basis proved to be impractical. Instead, sufficient endotoxin was injected into mice intraperitoneally in a volume of 0.5 ml to result in death of 80 to 100% of the animals within 48 hours. White Swiss mice weighing approximately 20 g and of heterogenous stock were employed. All animals were carefully observed for 48 hours after the injection of test materials and only deaths occurring within this period were recorded. Suspensions of cortisone acetate were supplied by Dr. E. Alpert of Merck and Co. Dilutions of the drug to the desired concentrations were made in sterile saline immediately before use. The cortisone was usually injected subcutaneously in a volume of 0.2 ml. The antibiotics used in this study were streptomycin calcium chloride complex, Merck, lot 2247; chloramphenicol, synthetic, Parke Davis, lot 151773, chlortetracycline, Lederle, lot 7-8557, and penicillin G, Bristol, lot G0426. The chloramphenicol was supplied by Dr. G. Rieveschel and the other antibiotics were obtained commercially. All solutions were made up fresh with sterile saline before each experiment and stored in the refrigerator until used. All antibiotics were injected subcutaneously in a volume of 0.2 ml, with the exception of chloramphenicol, which was injected in a volume of 0.5 ml.

Results. Protection by cortisone against lethal effects of endotoxin. A single 5 mg subcutaneous dose of cortisone injected one hour prior to or simultaneously with an intraperitoneal lethal dose of coliform endotoxin consistently protected from 60 to 90% of the animals from death. There was no protection, however, when the cortisone was injected one hour after the endotoxin, as shown in Table I. The route of injection of either the cortisone or the endotoxin could be varied without appreciably changing the results as long as the cortisone was given first. Thus, equally good protection was obtained when the cortisone was given subcutaneously and the endotoxin intravenously one hour later. There were no protective effects from the fluid in which the cortisone was suspended.

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TABLE I. Time Relationships of Cortisone Administration on Lethal Action of Coliform Endotoxins in Mice.

Materials injected*			Deaths		
1 hr before endotoxin	0 hr	1 hr after endotoxin	D/T†	%	P values
C	E	Sa	3/13	23	
Sa	E + C	Sa	2/13	15	>.01
Sa	E	C	10/12	83	
Sa	E	Sa	10/12	83	
C diluent only	E	Sa	5/5	100	

* C = Cortisone—5 mg inj. subcut. in a vol of .2 ml. E = Endotoxin—5 mg inj. intraper. in a vol of .5 ml. Sa = Saline, cortisone diluent—.2 ml inj. subcut.

† No. of animals dead/total No. of animals inj.

Effects of antibiotics with and without cortisone. Levaditi, Vaisman, and Reine(10) reported that in addition to its antimicrobial activity, certain sulfonamides were also able to exert an "antitoxic" action *in vivo* by protecting animals from a lethal injection of meningococcal endotoxin. These findings were confirmed by Carpenter *et al.*(11), and subsequently extended to show that penicillin (12,13), chlortetracycline, oxytetracycline, and chloramphenicol(14) also may be protective under certain conditions. A possible clue to the mechanism of this protection was noted in the bacteremia† which occurred in some animals a few hours after the injection of the endotoxin. It was postulated that the bacteremia might be due to an alteration of the intestinal mucosa by the endotoxin, and that the systemic invasion of the animal by a variety of organisms from the intestinal tract might contribute to the pathogenesis of the reaction. Experiments were therefore carried out to determine whether antibiotic therapy, by suppressing the bacteremia, would protect mice from an otherwise lethal dose of endotoxin. Of the antibiotics tested, which included chlortetracycline, penicillin, chloramphenicol, and streptomycin in a wide range of doses, only streptomycin in doses of 2 mg per mouse occasionally gave some protection, but the results were not reproducible. The possibility was considered that although streptomycin alone was not able to protect the animals consistently from the endotoxin, it might "reenforce" the protection given by cortisone and thereby increase the survival rate over that obtained with cortisone alone. However, just the contrary effects were produced. When streptomycin was injected one hour after the endotoxin and 2 hours after the cortisone, significantly higher mortality rates were obtained than with cortisone alone. The combined results from 3 experiments in which cortisone was administered in doses ranging from 1.2 to 5 mg and streptomycin in doses of 0.5 to 2 mg per mouse are shown in Table II. The results in each of the experiments were approximately of the same order, irrespective of the dosage of either agent employed. Thus, the average mortality rate in the group treated with cortisone was 11% as compared to 84% in the untreated controls. Streptomycin alone had little or no influence on the mortality rate but when it was injected into groups previously treated with cortisone, the mortality rate rose from 11% to an average of 45%. This "interference" by streptomycin with cortisone protection was demonstrable only when the antibiotic was injected after the cortisone; the simultaneous injection of both drugs one hour before the endotoxin gave results which

† The bacteremia produced in animals following injection of endotoxin was first called to our attention by Dr. A. I. Braude(15), who noted such occurrences in mice injected with coli and aerogenes endotoxins. Bacteremia occurs sporadically and varies from 2 or 3 up to 2000 bacteria/ml of blood. On the basis of preliminary work, it does not appear likely that the bacteremia plays an important role in causing death, but studies on this point are continuing.

TABLE II. Effects of Streptomycin on Cortisone Protection against Coliform Endotoxin in Mice.

Materials injected*			Deaths		
1 hr before endotoxin	0 hr	1 hr after endotoxin	D/T†	%	P values
Sa	E	Sa	42/50	84	
C	E	Sa	12/105	11	>.001
C	E	S	54/119	45	>.001
Sa	E	S	43/56	77	
C	Sa	Sa	0/15	0	
C	Sa	S	0/15	0	
Sa	Sa	S	0/15	0	

* E = Endotoxin—5 mg inj. intraper. in a vol of .5 ml. C = Cortisone—1.2 to 5 mg inj. subcut. in a vol of .2 ml. S = Streptomycin—.5 to 2 mg inj. subcut. in a vol of .2 ml. Sa = Saline—.2 ml inj. subcut.

† No. of animals dead/total No. of animals inj.

TABLE III. Effects of Penicillin on Cortisone Protection against Coliform Endotoxins in Mice.

Materials injected*			Deaths		
1 hr before endotoxin	0 hr	1 hr after endotoxin	D/T†	%	P values
Sa	E	Sa	33/34	97	
C	E	Sa	12/35	34	>.001
C	E	P	34/50	68	>.01
C + P	E	Sa	9/35	26	>.001
Sa	E	P	19/19	100	
Sa	Sa	P	0/10	0	
C	Sa	P	0/10	0	

* E = Endotoxin—5 mg inj. intraper. in a vol of .5 ml. C = Cortisone—5 mg inj. subcut. in a vol of .2 ml. P = Penicillin—10000 or 20000 units inj. subcut. in a vol of .2 ml. Sa = Saline—.2 ml inj. subcut. in a vol of .2 ml.

† No. of animals dead/total No. of animals inj.

reflected only the protective action of cortisone.

Experiments of the same type as those above were carried out using penicillin, which previously had shown no influence on the mortality rate, in place of streptomycin. The results of 2 such experiments shown in Table III indicate that penicillin is equally, if not more, effective than streptomycin in interfering with the protective action of cortisone. It will be noted that doses of penicillin which are capable of interference when given after the cortisone are without effect when given at the same time as the hormone, as was also the case with streptomycin.

Discussion. A single relatively large dose of cortisone injected one hour before, or simultaneously with, a lethal dose of endotoxin derived from a coliform bacillus consistently protected the majority of animals from death. This protection was lost, however, when the cortisone was injected one hour after the endotoxin. These results are in essential agreement with those of other workers who have shown that cortisone is most effective when administered prior to the endotoxin(5,15), although occasional protection has been demonstrated when the hormone was injected after the endotoxin(4).

It has been known for many years that some sulfonamides and antibiotics are able to protect animals from a lethal dose of endotoxins(10-14), but the mechanism of this protection is unknown. A transient bacteremia, probably of intestinal origin, occurs in some animals following the injection of coliform endotoxin and it was postulated that antimicrobial agents might protect by suppression of the bacteremia. Our observations on the effects of chlortetracycline, penicillin, chloramphenicol, and streptomycin on the endotoxin mortality rate revealed occasional though irregular protection only by streptomycin. Other experiments have shown that the bacteremia varies considerably in degree from one animal to the next and probably does not contribute significantly to the death of the animal. In spite of the inconsistent protection obtained with streptomycin it was thought worthwhile to investigate the possibility that the combined administration of both cortisone and streptomycin might protect more of the animals from death than with the use of cortisone alone. However, just the opposite results were obtained. The injection of even small doses of streptomycin into animals previously treated with cortisone resulted in significantly higher death rates than in those animals given cortisone alone. This interference of streptomycin with cortisone protection can hardly by a specific reaction, since penicillin, a completely unrelated compound, shows the same interfering capacity. This effect likewise is probably unrelated to any antimicrobial action of these drugs. Both antibiotics were capable of interfering only when given after, and not simultaneously with, the cortisone. In view of the dearth of information on how cortisone may protect animals from the harmful effects of toxic substances(16), or on the precise mode of action of endotoxins *in vivo*(17) or on the pharmacologic effects of antibiotics below the toxic level(18), it is not profitable at this time to speculate on the reasons why antibiotics may interfere with cortisone protection against endotoxins. However, since such interference has been demonstrated with 2 completely unrelated agents, it is suggested that the interference is a nonspecific reaction and may simply involve an additional stress or "insult" to an animal already in a precarious balance. Studies on the effects of other agents alone and with cortisone on endotoxin mortality rates are currently in progress.

Summary. 1. Administration of cortisone

prior to or simultaneously with a lethal dose of endotoxin consistently protected the majority of mice from death. There was no protection when cortisone was administered after endotoxin. 2. A transient bacteremia, presumably of intestinal origin, occurred in some animals a few hours after injection of endotoxin, but probably did not contribute significantly to death of the animals. 3. Antibiotics were capable of suppressing bacteremia, but with the exception of streptomycin, which irregularly gave some protection, did not influence mortality rate. 4. Injection of streptomycin or penicillin into animals previously treated with cortisone resulted in significantly higher mortality rates than in animals given cortisone alone. This interference by antibiotics with cortisone protection was demonstrable only when antibiotics were given after cortisone; simultaneous administration of both cortisone and antibiotic gave results which reflected only protective effects of cortisone. 5. In view of the unrelated nature of the antibiotics capable of interfering with cortisone protection against endotoxins, it was suggested that the interference was probably of a nonspecific nature.

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Antagonism of Inotropic Responses of Frog Heart to Epinephrine and Acetylcholine.* (21212)

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In a previous study the ratio between the concentrations of epinephrine and acetylcholine present simultaneously was shown to be important in determining the amplitude of contraction of the isolated frog heart(1,2). With a mixture of appropriate concentrations of these drugs a response was obtained whose amplitude was similar to that of the heart before the addition of the mediators. This latter condition was referred to as a balanced response and was characterized by the ratio of epinephrine to acetylcholine necessary to produce it. This balanced response was readily altered by the barbiturates, which decreased the response of the heart to epinephrine.

In this investigation, using the approach

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