Effect of Cortisone on Treatment of Tetanus with Antitoxin. (22967)

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Studies of the effect of various materials derived from the adrenal on the activity of bacterial products have yielded variable results. With diphtheria toxin, increase in susceptibility(1,2), no effect(3), and increased resistance(4,5,6) have been noted when adrenal substances were administered. Favour(8) reported that cortisone increased the susceptibility of guinea pigs to small quantities of diphtheria toxin, but raised the level of resistance to larger doses. Cortisone was noted by Brainerd and co-workers(7) not to interfere with the neutralization of diphtheria toxin by specific antitoxin.

Although studies of cortisone in tetanus intoxication in animals have indicated little or no effect(5,9,10), it has been suggested that the steroid exerts a favorable action in human tetanus(11,12). There are no reports in the literature concerning the activity of cortisone on neutralization of tetanus toxin by antitoxin in controlled experiments. It is the purpose of this paper to present evidence which indicates that cortisone interferes with the therapeutic activity of tetanus antitoxin, and enhances the effect of toxin in unprotected animals.

Methods. Highly purified tetanus toxin (0.005 mg contained 1 M.L.D. for mice) prepared by the Biologic Laboratory of Mass. State Dept. of Health, was diluted in heart infusion broth (pH 6.6), and 1 M.L.D. injected intramuscularly over the right hind leg of mice (groups of 9) weighing 20 ± 2 g. Antitoxin, a pepsin-digested immune horse serum, was administered intraperitoneally at varying times in a dose of 0.5 unit in a volume of 0.2 ml; 0.001 unit of this antitoxin neutralized 5-20 mouse M.L.D. of the toxin used in the study. Cortisone acetate (Cortone, Sharp and Dohme) was diluted with normal saline and 2 mg injected subcutaneously 4 hours and again 20 hours after the antitoxin. Control animals (groups of 9) received toxin or steroid alone. Deaths in each group were counted daily for 10 days. The treatment schedule used is described in Table I.

Results. The results obtained in these experiments are indicated in Fig. 1. When antitoxin was given 24 hours after injection of toxin, the death rate in 10 days was 11.1%. However, when cortisone was administered 4 hours after antitoxin, the number of fatalities was increased to 55.5%. Treatment with antitoxin 48 hours after tetanus toxin led, as could be predicted, to a higher incidence of deaths (55.5%), but even at this time the steroid neutralized the antitoxic effect to some degree, 77.7% of the treated animals succumbing to the intoxication. When antitoxin was delayed for 72 hours, all of the mice died within 10 days, without relation to the use of steroid. In the control animals receiving toxin alone, the fatality rate after 5 days was smaller (55.5%) than in those given toxin plus cortisone (88.8%). At the end of 10 days, however, the difference, although still present, was smaller. The average survival time with toxin alone was 82.5 hours while with toxin and cortisone, it was 69.5 hours. That the hormone in the quantity used was not itself responsible for death is illustrated by the fact that all of the mice treated with the drug alone remained alive.

Discussion. The phenomena which have been observed when cortisone has been ad-

TABLE I. Schedule of Treatment.

	Hours						
Group No.	0	24	28	48	52	72	76
1	T	A.T.	E		E		
2	T	"					
3	T			A.T.	E		E
4	T			"			
5	T		S		S		
6	T		E		E		
7			E		E		

T, tetanus toxin; A.T., antitoxin; E, cortisone; S, .85% saline.

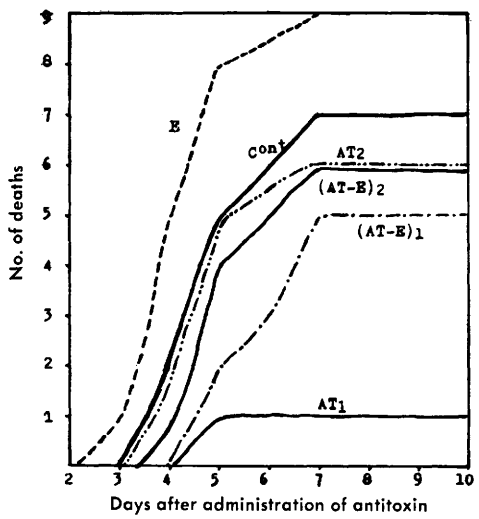

FIG. 1. Effect of cortisone on tetanus toxin and antitoxin. E, cortisone; Cont., no antitoxin or cortisone (toxin alone); AT_1, antitoxin 24 hr after toxin; $(AT\text{-}E)_1$, antitoxin and cortisone 24 hr after toxin; AT_2, antitoxin 48 hr after toxin; $(AT\text{-}E)_2$, antitoxin and cortisone 48 hr after toxin.

ministered to animals injected with various bacterial toxins appear to be influenced to a great degree by time relationships of administration of drug and exotoxin(10). The relative quantities of toxin and hormone are also of importance in determining the results(4, 5,7,8). The experiments described in this paper were designed to simulate to some degree the situations which might arise in clinical practice. For this reason, antitoxin alone and this agent plus cortisone were given 24, 48 or 72 hours after first exposure to tetanus toxin. The quantity of antibody used was very large (0.5 unit neutralized between 2500 and 10,000 M.L.D. of toxin). Despite the greatly excessive dose of antibody, the depressing effect of steroid on protection was still marked when antitoxin was administered at a time when in animals, not receiving cortisone, a distinct therapeutic effect was produced. With relatively late injection of antitoxin, little or no difference was observed when cortisone was exhibited because the immune material itself was without effect. In addition, as can be seen in the graph, steroid therapy enhanced the activity of the toxin. It is quite possible that a different result might have been obtained had the animals been given antitoxin prophylactically. Brainerd(7), for example, has demonstrated that cortisone does not interfere with the neutralizing effect of diphtheria antitoxin when it is administered 4 days before toxin. This is not the situation which pertains, however, in the management of humans in whom there may be a relatively long period of delay between wounding and prophylaxis, or in whom active tetanus is already present and antitoxin is given therapeutically. The present study suggests that in the treatment of tetanus in which clinical signs have already appeared, or in instances in which a relatively long period of time has elapsed before prophylaxis, the administration of cortisone is not only without benefit but, in fact, sharply decreases the effectiveness of the antitoxin, as well as increases the activity of the toxin which is either fixed or still circulating.

Conclusions. 1. The therapeutic effectiveness of very large doses of tetanus antitoxin is markedly reduced by simultaneous administration of cortisone, when both agents are given 24 hours after injection of toxin. 2. Administration of steroid has little or no effect on the course of experimental tetanus if administration of antitoxin is delayed 48 or more hours after exposure to toxin. 3. Cortisone increases the activity of tetanus toxin. 4. Results suggest strongly that the use of cortisone in addition to antitoxin for treatment of human tetanus may produce a deleterious effect.

1. Belding, D. L., and Wyman, L. L., *Am. J. Physiol.*, 1926, v78, 50.

2. Scott, W., Bradford, W., Hartman, F., and McCoy, O., *Endocrinol.*, 1933, v17, 529.

3. Zwemer, R. L., and Jungeblut, C. W., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 1583.

4. Murray, R., and Branham, S. E., *ibid.*, 1951, v78, 750.

5. Bartolozzi, G., *Riv. Clin. Pediat.*, 1952, v50, 563.

6. Rosenbaum, P., and Obrinsky, W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 502.

7. Brainerd, H., and Scaparone, M., *Antibiot. Chemoth.*, 1953, v3, 693.

8. Favour, C. B., Atlas, L. T., Levine, M. I., and Osgood, C. K., *J. Lab. Clin. Invest.*, 1954, v3, 1.

9. Spector, S., and Mercer, R. D., *J. Inf. Dis.*, 1951, v89, 224.

10. Green, A. E., Ambrus, J. L., and Gershenfeld, L., *Antibiot. Chemoth.*, 1953, v3, 1121.

11. Christensen, M. A., Keith, H. M., Smith, L. A., Hanson, N. O., and Ralston, D. E., *Proc. Mayo Clin.*, 1952, v27, 28.

12. Lewis, R. A., Satoska, R. S., Joag, G. G., Dave, B. T., and Patel, J. C., *J.A.M.A.*, 1952, v156, 479.

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LSD-Like Effects Elicited by Reserpine in Rabbits Pretreated With Iproniazid.* (22968)

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Previous findings have led us to postulate that the sedative and parasympathomimetic effects of reserpine are mediated through serotonin, and that serotonin may be a chemical transmitter in the parasympathetic division of the central autonomic nervous system(1,2). These concepts are based in part on the findings that both serotonin and reserpine induce sedative effects in mice and potentiate by a central mechanism the action of certain hypnotics, the potentiation by either substance being antagonized by pretreatment with lysergic acid diethylamide (LSD); and that the prolonged effects of reserpine are associated with a persistent impairment of the capacity of the body to maintain serotonin in the bound form which normally protects the amine from the action of the active enzyme, monoamine oxidase. As a consequence, serotonin is liberated and rapidly metabolized, but as the enzyme in brain responsible for formation of serotonin remains unimpaired, the possibility has been considered that reserpine administration results in a persistent flow of serotonin from cells which have lost part of their capacity for storing the indole. The total level of serotonin is low, as would be expected because free serotonin is rapidly metabolized by monoamine oxidase, but the free amine might stimulate the central parasympathetic centers, thus producing the signs typical of reserpine.

The present paper describes studies which demonstrate that when reserpine is administered to rabbits pretreated with the monoamine oxidase inhibitor, iproniazid, the animals, instead of showing sedative and parasympathomimetic responses typical of reserpine, display instead the excitatory and sympathomimetic effects typical of lysergic acid diethylamide (LSD). Furthermore, the concentration of serotonin in brain, which declines rapidly after the administration of reserpine alone, now remains at its normal level.

Methods. Male albino rabbits were given various drugs intravenously and were killed by intravenous injection of air one hour after administration of the last drug. Serotonin in brain was assayed by the fluorometric procedure described by Bogdanski *et al.*(3).

Results. Five rabbits given reserpine (5 mg/kg) demonstrated typical responses to the drug including deep sedation and parasympathomimetic effects including miosis and relaxation of nictitating membrane. Five animals pretreated with 100 mg/kg of iproniazid and 2 hours later given 5 mg/kg of reserpine showed no parasympathomimetic effects, but rather displayed gross excitation, mydriasis, exophthalmus, piloerection, and other sympathomimetic effects. Animals given iproniazid alone, displayed no obvious signs.

It seemed possible that the sympathomimetic effects observed following the administration of reserpine to iproniazid-treated rabbits were caused by the drug combination rather than by serotonin which had been released by reserpine and protected by iproniazid from the action of monoamine oxidase. This possibility was investigated with another series of 5 rabbits in which the order of drug administration was reversed; that is, the ani-

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