Neuraminic acid was released quantitatively from α-1 glycoprotein by incubation with Vibrio L form neuraminidase. Reaction mixtures containing 1000 μg of α-1 glycoprotein yielded 104 μg of neuraminic acid when determined by the resorcinol method and 114 μg when aliquots were measured by the Warren reaction after enzyme action. These results are in close agreement with the expected value of 110 μg, since N-acetylneuraminic acid constitutes 11% of the α-1 glycoprotein. Neuraminic acid was not detected in the Vibrio L filtrate by the resorcinol method.

Discussion. The retention of various properties of the parent strain by L forms of bacteria has been noted previously. Scheibel and Assandri(12) were able to isolate L forms of *Cl. tetani* toxigenic for mice, similar to the findings of Tulasne and Lavillaureix (13) who studied a water-vibrio EZ-5. Kandler and Kandler(14) demonstrated retention of various biochemical functions by L forms of *B. proteus* and *V. cholerae,* although specific enzymatic activities were not investigated.

In the present study, the neuraminidase of *Vibrio cholerae* L form was found to be identical in all respects with the same enzyme of the parent strain. Inhibition of hemagglutination by influenza virus was indistinguishable from that produced by the parent strain and specific release of neuraminic acid from a suitable substrate was easily demonstrable. The neuraminidases of both the parent and L form exhibited the same dependence upon calcium ions for activation. Further evidence of the identical nature of the 2 enzymes is provided by the neutralization studies. Specific antiserum to neuraminidase of the parent strain suppressed the activity of the parent and L form enzymes to exactly the same degree. Absorption studies were not done. It is unlikely that they would have yielded additional information. Scheibel and Assandri(12) noted neutralization of the toxin of *Cl. tetani* L forms by tetanus antitoxin.

Summary. A stable L form of *Vibrio cholerae* (strain 4Z) has been found to produce a neuraminidase capable of inhibiting hemagglutination by influenza virus and of liberating neuraminic acid from purified α-1 glycoprotein. The enzyme activity was neutralized by antiserum to the neuraminidase of the parent *Vibrio cholerae* strain.

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Reversal of Experimental Endotoxin Shock with a Combination of Aldosterone and Metaraminol. (26752)

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A combination of hydrocortisone and metaraminol reversed endotoxin shock in the dog, which was not accomplished with either drug alone(1,2). This report is concerned with the remarkable activity of aldosterone and metaraminol under similar experimental conditions.

Materials and methods. After equilibra-

tion, anesthetized adult mongrel dogs were given a standardized dose of *E. coli* endotoxin; the systemic arterial pressure was monitored over a period of 7 hours, or until death occurred; and arterial pH determinations and hematocrit determinations were done hourly(1,2).

Ten control animals were given endotoxin alone as a test of endotoxin lethality. A second group of 10 animals each received a dose of 2 mg of synthetic d-aldosterone,* and a third group, also of 10 animals, was given 0.2 mg. The steroid was administered intravenously in a solution of 0.1 mg/cc at that time when irreversibility appeared imminent. This stage of canine endotoxin shock is characterized by progressive hypotension, hemoconcentration, acidosis and oliguria or anuria. A fourth group of 25 dogs was treated with a combination of aldosterone and metaraminol.† At the post-endotoxin stage of shock described above, 0.1 mg of aldosterone was injected into the femoral vein of the dog, followed by an infusion of metaraminol. The pressor drug was diluted in 5% dextrose and water and infused in a concentration of 0.2 mg/cc at a rate of 40-60 drops/minute. Total amounts of metaraminol varied between 0.1 and 5.0 mg. The pressor drug was given intermittently post-aldosterone in amounts sufficient to maintain systemic blood pressure between 90-100 mm Hg, or until the dogs showed hemodynamic stability. Serial serum sodium and potassium determinations were carried out in dogs receiving aldosterone alone, using the Baird flame photometer.

Results. Control animals. A dose of 0.55 mg/kg of endotoxin proved lethal in all 10 animals, death occurring in an average of 12.5 hours post-endotoxin.

Aldosterone animals. Two of 10 animals given 0.2 mg of aldosterone survived. Average survival time of the remaining 8 dogs was 8 hours. There were no significant alterations in concentrations of serum sodium and potassium for 7 hours post-endotoxin.

Two of 10 animals receiving the larger dose of 2 mg of aldosterone survived a lethal dose of endotoxin. Average post-endotoxin survival time of animals dying was 8.3 hours. There was no essential change in concentrations of serum sodium in this group; however, the animals exhibited increasing levels of potassium during the observation period. The average increase was from a control of 4.4 mEq/L to a high of 6.5 mEq/L at 4 hours post-endotoxin, in contrast to a maximum increase of 0.4 mEq/L in those animals given the lower dose of aldosterone.

These results show that survival rate of dogs with endotoxin shock was only slightly increased when aldosterone was used alone. The results are comparable to those obtained with hydrocortisone in previous studies(1).

Aldosterone + metaraminol animals. Since dogs appeared to tolerate 0.2 mg of aldosterone without ill effects, and since this dose did not significantly alter the concentrations of serum sodium and potassium, one-half this amount, or 0.1 mg, was infused into 25 animals during the post-endotoxin period of shock, followed by an injection of an amount of metaraminol sufficient to sustain the systemic blood pressure. Sixteen of the 25 dogs survived. Average survival time of the dogs dying was 8.6 hours. Although surviving animals exhibited oliguria and some degree of hemoconcentration, only a minimal degree of acidosis was detected, as determined by blood pH. The average total amount of metaraminol necessary to maintain the systemic pressure was 0.8 mg, the doses varying between 0.1 and 5 mg as compared with an average dose of 42 mg required to maintain the pressure of dogs when metaraminol was used alone in previous studies(1). When hydrocortisone preceded the infusion of metaraminol, 5.5 mg of the pressor drug was necessary.

The over-all results in the 55 dogs receiving 0.55 mg/kg of endotoxin are shown in Table I.

Discussion. Extensive studies on canine endotoxin shock have demonstrated that the reversal of shock offers a more severe therapeutic challenge than either pre-treatment, or simultaneous administration of agents with

* Supplied by Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.

† Supplied as Aramine Bitartrate 10% by Research Division, Merck Sharp and Dohme, Philadelphia, Pa.

TABLE I. Results in 55 Dogs Given a Lethal Dose of *E. coli* Endotoxin.

No. of dogs	Treatment	Survivors	Died
10	None	0	10
10	.2 mg aldosterone	2	8
10	2.0 mg aldosterone	2	8
25	.1 mg aldosterone + avg of .8 mg metaraminol	16	9

endotoxin. This viewpoint has also been expressed by Weil and Miller(3). In this laboratory reversal of shock has been most successful when a combination of adrenocorticosteroid and a pressor drug was used. It has been essential to infuse the steroid before injecting the pressor drug.

The rise in serum potassium observed with the larger doses of aldosterone may be related to the severe oliguria, or anuria, and acidosis that characterize canine endotoxin shock.

We have shown that either glucocorticoid or mineralocorticoid supplements the pressor action of metaraminol. The steroids have 2 favorable effects when used with the pressor drug. First, the amount of pressor agent necessary to sustain the systemic pressure is considerably less when preceded by administration of steroid, especially when aldosterone is employed. Second, although metaraminol will sustain the pressure when used alone, there is no significant increase in survival rate when compared to controls. Steroids not only augment the activity of pressor drug, but rate of survival is remarkably increased. The precise role shared by exogenous steroid is not known. The observations pertain only to endotoxin shock in the dog.

Summary. Lethal shock was established in 55 adult mongrel dogs with a standardized dose of *E. coli* endotoxin. The shock could not be reversed with aldosterone or metaraminol when either was used alone. However, dogs given 0.1 mg of aldosterone required approximately one-fiftieth the dose of metaraminol necessary to maintain systemic pressure, compared to the use of metaraminol alone. This action of aldosterone was about 7 times more pronounced than that obtained with hydrocortisone. The nature of the contributing effect of steroid to the pressor activity of metaraminol and to the increased survival rate is not known.

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Hemorrhagic Reactions at Sites of Passive Cutaneous Anaphylaxis in Guinea Pig after Intravenous Inoculation of Unrelated Immune Complex. (26753)

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A clear cut differentiation between anaphylactic and Arthus-type sensitivities has been afforded by the quantitative studies of Benacerraf and Kabat(1) in the guinea pig. The distinguishing features of transferred Arthus reactions are: (a) The lack of a latency period, the severity of the Arthus reaction being the same when antigen is injected immediately, 30 minutes or 24 hours after the intravenous sensitizing dose of antibody. (b) The requirement of greater amounts of antibody, *viz.*, 400 μg AbN intravenously for severe direct Arthus reactions, as contrasted with 30 μg, *i.e.*, 13 times less, the amount sufficient to convey systemic anaphylactic sensitization. In the case of passive reversed Arthus and of the local anaphylactic reaction known as "Passive Cu-