the animals with hypothalamic islands could also be explained on this basis because these islands were extensively disrupted and hemorrhagic. The 17-hydroxycorticoid output in dogs in which the pituitary stalk had been sectioned acutely, interrupting the portal vessels and producing a degree of pituitary damage similar to brain removal, but leaving the brain intact, was similar to the output in isolated pituitary preparations.

If the hypothalamus or pituitary is activated by a humoral factor liberated from some portion of the body during trauma, the output of 17-hydroxycorticoids in dogs with hypothalamic islands or isolated pituitaries should be low in the absence of stress, while if the release of ACTH is autonomous, the rate should be high. It has been reported that in non-stressed rats with hypothalamic islands, the circulating corticosterone levels are low(10). However, there are no data on the output of 17-hydroxycorticoids in the absence of stress in dogs after similar operations, and a truly unstressed state after such extensive surgery would be difficult to achieve.

*Summary.* The adrenocortical response to the trauma of cannulating the adrenal vein has been tested in dogs 4 hours after removal of various parts of the brain. Compared to controls, 17-hydroxycorticoid output in adrenal venous blood is slightly decreased in dogs in which all brain tissue above the pons except the hypothalamus has been removed. Output was significantly lower, but still definitely above basal levels in dogs in which the entire brain above the pons had been removed. It was in the same range when the entire brain had been removed. Acute section of the pituitary stalk decreased the adrenocortical response to an approximately equal degree. Removal of the pituitary as well as the brain reduced output to low levels. These data do not support the hypothesis that the hindbrain secretes a factor which stimulates the pituitary to secrete ACTH. Other possible explanations of the results are discussed.

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## Influence of Aldosterone and Angiotensin II on Endotoxin Shock in the Primate.* (28172)

WESLEY W. SPINK, JAMES A. VICK, JAMES C. MELBY AND JOANNE FINSTAD
*Departments of Medicine, University of Minnesota Medical School, Minneapolis and University of Arkansas, Little Rock*

Canine endotoxin shock has been successfully reversed with aldosterone and angiotensin II(1).¶ Because endotoxin shock in the monkey corresponds more closely to the hemodynamic and metabolic alterations occurring in human patients, studies were extended to the primate. Features of endotoxin shock in the monkey are hypotension, oliguria or anuria, hyperkalemia and terminal acidosis.

The main objectives of the present investi-

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gations were 1) to determine if the course of shock after a lethal dose of endotoxin could be reversed by either aldosterone or angiotensin II, or by a combination of the two; 2) to measure the serum and urinary concentrations of Na and K in control and in treated animals.

*Materials and methods.* Studies were made on a total of 29 adult male and female Sooty Mangabey monkeys (Cynopithecoid group, *Cercocebus torquatus Atys)*† weighing between 2.6 and 12 kg. These rather strong but excitable animals, only 6 to 8 weeks out of their native Africa, tolerated a lethal dose of endotoxin so that observations were permitted over a period of several hours. The monkeys were first anesthetized with ether and then given sodium pentobarbital intravenously. A polyethylene catheter was inserted into the femoral vein and another was placed in the femoral artery. The latter catheter was attached to a Statham Strain gauge, and the pressure continuously registered with a Sanborn recorder. Electrocardiographic tracings of lead I were also made in this manner. An indwelling catheter in the bladder permitted the collection of urine during the period of observation.

Serial hematocrit and pH determinations were done on venous blood. The Baird flame photometer was used for determining plasma sodium and potassium values on venous blood and on urine.

The foregoing studies were made over a pre-endotoxin control period of 4 hours. The animals were then given intravenously a standardized lethal dose of 7.5 mg/kg of *Escherichia coli* endotoxin, prepared as described elsewhere(2). Similar quantitative post-endotoxin measurements were made until death occurred, or up to 8 hours. All monkeys received endotoxin and were divided into 6 groups as follows: I. 5 monkeys, received endotoxin alone. II. 5 monkeys, treated with 260-300γ angiotensin II. III. 3 monkeys, received 100-300γ aldosterone ("high dose"). IV. 6 monkeys, given 100-200γ aldosterone ("high dose") followed by 10-200γ angiotensin II. V. 5 monkeys, received 25γ aldosterone ("low dose"). VI. 5 monkeys, received aldosterone (25γ) followed by angiotensin II (average 10.2γ).

† Obtained from Trefflich's Bird & Animal Co., Inc., New York.

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

TABLE I. Outcome of Endotoxin Shock in 6 Groups of Monkeys Given Lethal Dose of Endotoxin (7.5 mg/kg).

| Group and treatment | Avg survival time* (hr) | No. of survivors |
|---|---|---|
| I. Control | 13½ | 0/5 |
| II. Angiotensin II | 12 | 1/5 |
| III. "High dose" aldosterone | 5 | 0/3 |
| IV. *Idem* + angiotensin II | 8 | 2/6 |
| V. "Low dose" aldosterone | 14 | 3/5 |
| VI. *Idem* + angiotensin II | | 5/5 |

* Avg survival time based upon No. of animals that died.

*Results. Survivors in relation to treatment following lethal dose of endotoxin.* The outcome of the 29 monkeys in the 6 different groups is summarized in Table I. The significant feature is the survival of all animals given 25γ of aldosterone followed by an average of 10.2γ of angiotensin II. In trying to ascertain the optimum dose of aldosterone, 100 to 200γ of aldosterone ("high dose") was used, but when these doses were used alone or with angiotensin II the results were not encouraging. Similar results were obtained in the dogs with large doses of the steroid(3). It is not clear at this time why the larger doses of aldosterone in either species were less effective. Electrocardiographic evidence indicated the onset of ventricular arrhythmia as a possible contributing cause of death. In both dogs and in monkeys this disturbance of rhythm was seen only in those receiving endotoxin and a large dose of aldosterone. However, as much as 1000γ of aldosterone did not produce deleterious effects when given to 3 control monkeys that had not received endotoxin.

The femoral arterial pressure of a control endotoxin monkey shown in Fig. 1, demonstrates a gradual decline in the pressure associated with anuria. In Fig. 2, the pressor effect of only 5γ of angiotensin II following 25γ of aldosterone is demonstrated. Administration of the steroid in monkeys potentiated the

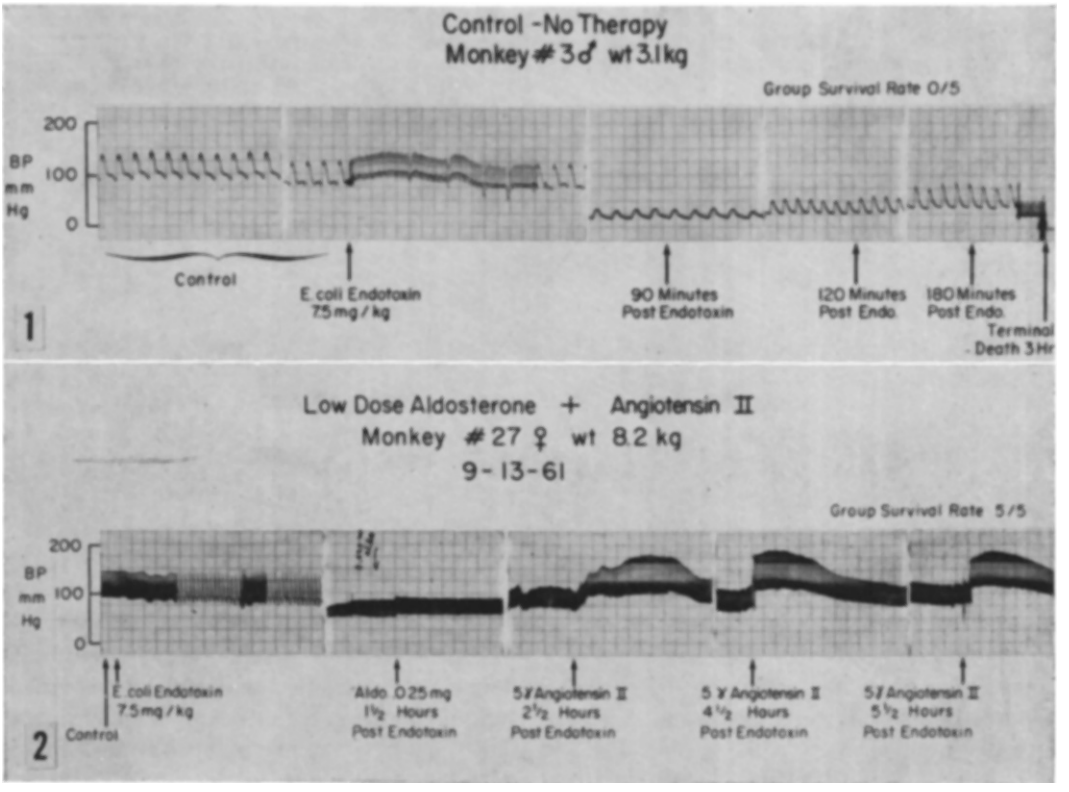


FIG. 1 and 2

pressor effect of angiotensin II, as in dogs(1). In addition, a good output of urine was maintained.

It is to be noted that as much as 260 to 300γ of angiotensin II failed to stabilize the pressure or benefit renal function, when the pressor agent was used alone.

*Effect of endotoxin and treatment upon electrolytes.* Due to technical difficulties and loss of specimens complete serial plasma Na and K concentrations were carried out on only 21 of the 29 monkeys. Before injection of endotoxin the mean value of Na concentration involving 25 animals was 147.2 meq, and the K was 3.7 meq, based upon studies in 22 monkeys. Since the ion concentrations in the individual animals varied considerably after administration of endotoxin, rate of change of ion concentration per hour was computed (4).‡ These data are presented in Table II. Na concentrations for all groups studied revealed erratic variations in both the pre- and post-endotoxin periods. There is suggestive evidence that in the animals receiving the "high dose" of aldosterone, an increase in plasma Na values occurred. K concentrations were closely correlated among all of the animals during the pre-endotoxin period. It is also clear that after administration of endotoxin an elevation of K values occurred in all of the groups. However, it was not possible to draw conclusions on the influence of treatment on K concentrations because of the small number of animals used and the variation that took place between monkeys in the same group.

The data on the hourly mean urinary volumes and electrolyte excretions in 5 groups of monkeys are shown in Table III. The outstanding features are a reduction in urine output in all the groups after endotoxin; a reduction of Na excretion, except in those animals receiving the "low dose" of aldosterone; and a reduction of K excretion in all treat-

‡ We are indebted to Dr. B. W. Brown, Jr., Biostatistics Division, University of Minnesota School of Public Health, for aid in statistical analysis of the data.

TABLE II. Rate of Change in Plasma $Na^+$/hr and $K^+$/hr in 5 Groups of Monkeys Given Endotoxin and Treated with Aldosterone and Angiotensin II.

| Treatment | Animal No. | Rate of change $Na^+$/hr | | Rate of change $K^+$/hr | |
|---|---|---|---|---|---|
| | | Control period | After endotoxin | Control period | After endotoxin |
| Control | 1 | 1.02 | 2.88 | .09 | .54 |
| endotoxin | 2 | 1.68 | .96 | −.15 | .17 |
| | 3 | − .38 | 2.94 | −.05 | 3.19 |
| | 4 | .82 | −2.22 | −.15 | .78 |
| | 5 | −1.26 | .64 | −.05 | 1.72 |
| Angiotensin II | 6 | 1.83 | − .24 | .09 | .38 |
| | 7 | .32 | − .93 | −.06 | .88 |
| | 8 | − .04 | .16 | −.09 | 1.91 |
| | 9 | 4.00 | − .36 | −.54 | 5.90 |
| "High dose" | 10 | − .42 | .86 | −.10 | .26 |
| aldosterone | 14 | 1.76 | 2.16 | −.40 | 5.07 |
| | 15 | − .37 | 1.90 | .07 | .85 |
| *Idem* + | 10 | − .74 | .61 | .24 | .24 |
| angiotensin II | 11 | − .99 | 2.50 | −.03 | 3.08 |
| | 16 | .14 | 1.05 | −.16 | .39 |
| | 17 | − .90 | − .58 | .14 | .38 |
| | 18 | − .33 | 1.12 | .02 | .13 |
| "Low dose" | 21 | − .62 | − .82 | .13 | .68 |
| aldosterone | 22 | − .59 | − .72 | .13 | .43 |
| | 23 | − .58 | 1.61 | .04 | .58 |
| | 25 | .38 | — | .22 | — |

ment groups. Again, the number of animals is small and there was variation in degree of oliguria between animals within the different groups.

*Electrocardiographic changes.* Contrary to expectations, because of the observations made in canine endotoxin shock, no outstanding alterations in continuous tracings were noted. During the phase of hypotension and severe oliguria or anuria there was an elevation of the ST segment and tachycardia suggesting myocardial ischemia due to decreased coronary blood flow. Pulsus alternans was a prominent feature. Ventricular arrhythmia occurred terminally in some animals.

*Discussion.* The most significant finding in this investigation is the complete reversal of severe endotoxin shock in the monkey following administration of appropriate amounts of aldosterone and angiotensin II. While each of these agents appeared to be of some benefit when used alone, optimum results were obtained with a combination of the two. Similar results occurred in canine endotoxin shock using hydrocortisone and metaraminol, aldosterone and metaraminol, and aldosterone and angiotensin II(1,5,6). In all instances the pressor action of either metaraminol or angiotensin II was markedly potentiated by administration of hydrocortisone or aldoster-

TABLE III. Hourly Mean Urinary Volume and Electrolyte Excretion in 5 Groups of Monkeys Receiving Endotoxin.

| No. of animals and treatment | Pre-endotoxin period | | | Post-endotoxin period | | |
|---|---|---|---|---|---|---|
| | Urine output (cc/hr) | $Na^+$ output (meq/hr) | $K^+$ output (meq/hr) | Urine output (cc/hr) | $Na^+$ output (meq/hr) | $K^+$ output (meq/hr) |
| 5 Endotoxin control | 17.9 | .85 ± .25 | .83 ± .02 | 4.9 | .13 ± .09 ($P = < .05$) | .40 ± .23 ($P = < .4$ ) |
| 3 Angiotensin II | 31.0 | 1.87 ± .43 | 1.85 ± .17 | 6.6 | .05 ± .00 ($P = < .05$) | .33 ± .07 ($P = < .025$) |
| 5 "High dose" aldosterone + angiotensin II | 23.7 | .83 ± .27 | 1.10 ± .20 | 7.0 | .10 ± .05 ($P = < .05$) | .32 ± .10 ($P = < .005$) |
| 5 "Small dose" aldosterone | 14.2 | .13 ± .03 | .91 ± .19 | 5.0 | .08 ± .03 ($P = < .2$ ) | .38 ± .06 ($P = < .05$) |
| 5 *Idem* + angiotensin II | 13.5 | .74 ± .038 | 1.16 ± .15 | 9.2 | .08 ± .03 ($P = < .2$ ) | .66 ± .05 ($P = < .1$) |

one. It is not clearly understood why a glucocorticoid, and particularly a mineralocorticoid, should potentiate the action of a pressor agent in a favorable manner. It has been clearly established in the dog and in man that adrenocortical insufficiency within physiologic limits is not usually present in endotoxin shock(7,8). There is accumulating evidence that normal hemodynamic function in the human may involve the interrelationship of aldosterone, angiotensin II and Na. Under the conditions of severe peripheral vascular collapse it is probable that during some periods pharmacologic amounts of hormone and a pressor agent will augment the physiologic effort of the host.

It would appear that in endotoxin shock in the dog and in the monkey an optimum amount of aldosterone must be used in conjunction with a pressor drug. Excessive amounts of aldosterone can have a deleterious effect. This may be related to the hyperkalemia and oliguria or anuria that occur. There is also an associated acidosis.

*Summary.* Severe endotoxin shock in monkeys resulted in hypotension, oliguria or anuria, hyperkalemia and death. No characteristic electrocardiographic findings were elicited. Aldosterone and angiotensin II were employed alone and in combination in attempts to reverse the shock. The most favorable results were obtained with a combination of the steroid and pressor drug. As in canine endotoxin shock, aldosterone markedly potentiated the pressor action of angiotensin II. Optimum results were correlated with the amount of aldosterone administered.

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## Antigens of *Bordetella pertussis.* III. The Protective Antigen. (28173)

J. MUNOZ AND B. M. HESTEKIN

*Department of HEW, U. S. Public Health Service, N.I.H., National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana*

The most practical prophylactic agent for immunization of children against whooping cough is still a vaccine prepared from killed whole cells of *Bordetella pertussis*(1,2). However, protection of children can be achieved with cell-free extracts of *B. pertussis*(3,4,5). All cell-free extracts so far described have been impure and the exact nature of the protective antigen is unknown. The mouse protective activity of *B. pertussis* cells is not associated with the so-called agglutinogen(6,7, 8), the hemagglutinin(9), the heat labile toxin (10,11,12), the heat stable toxin(13,14) or any substances found in the protoplasm of the cell(10,11). Since mouse protective activity of pertussis vaccines correlates well with their ability to confer immunity to children(15,16), it may be reasonable to assume that the *B. pertussis* protective antigen for children is not found in those substances lacking mouse protective activity. The mouse protective activity is found in cell walls(10,11, 17), and the active substance can be extracted from whole cells by various procedures (5,18-21). Some investigators have observed that the histamine sensitizing activity of pertussis vaccines parallels protective potency (22,23), and others have stated that the histamine sensitizing factor (HSF) is similar, if not identical, to the protective antigen, al-