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## THE EFFECT OF ALDOSTERONE, CORTISOL, AND CORTICOSTERONE UPON THE SODIUM AND POTASSIUM CONTENT OF SHEEP'S PAROTID SALIVA \*

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Sodium depletion in the sheep results in a fall in parotid salivary sodium/potassium concentration ratio (Na/K) from a normal 25 to 40 (Na, 170 to 185 and K, 4 to 6 mEq per L) to as low 0.3 (Na, 40 and K, 133 mEq per L) (1, 2). Earlier work in this laboratory suggested that this reciprocal alteration in the concentration of salivary sodium and potassium was due predominantly to the simultaneous operation of 1) a fall in the salivary secretion rate and 2) an increase in the secretion of electrolyte-active adrenal steroids. If suitable allowance were made for the effects of variation in the parotid salivary secretion rate and the latency of the response, it was proposed that the salivary Na/K ratio could be used as an index of the release of electrolyte-active steroid into the circulation (2-5).

Cortisol (6), corticosterone (6), and aldosterone (7-9) have been identified in sheep adrenal venous blood. In order to obtain a basis for evaluating the contribution of each of these components of the adrenal secretion to the fall in parotid salivary Na/K ratio observed during different physiological states, the effects of these steroids upon salivary Na and K were studied in a series of experiments in normal and adrenalectomized sheep.

### METHODS

*Animals.* Eighteen crossbred Merino wethers were used in this study. All had unilateral parotid fistulae (1) and carotid loops, and 11 were bilaterally adrenalectomized (5). Of the nonadrenalectomized sheep, two

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had adrenal transplants (10, 11) and two had inflatable cuffs placed on the thoracic inferior vena cava (12).

*Diet.* The animals were allowed 0.4 kg oaten chaff and 0.4 kg lucerne chaff per day with a Na content of approximately 100 mEq per day. All animals were housed in metabolism cages, and had free access to water and sodium bicarbonate solution except during Na depletion (13). Voluntary Na intake and volumes of saliva and urine were recorded daily.

*Initial experimental conditions.* The salivary response to corticosteroids was examined in Na-replete normal and adrenalectomized animals and in adrenalectomized sheep with known Na deficit.

*Normal sodium balance.* In this instance, the animal's voluntary Na intake was recorded daily for the 3 to 4 days before the experiment (13); 3 to 4 hours before the experiment, 200 to 300 mEq NaHCO<sub>3</sub> was given into the rumen to ensure that the animal was Na-replete. In the adrenalectomized animals, the daily maintenance dose of 5 mg desoxycorticosterone and 25 mg cortisone acetate was last given 24 to 48 hours before the experiment.

*Sodium deficiency* was produced by loss of parotid saliva when the daily supplement of NaHCO<sub>3</sub> was withheld. In order to obviate endogenous aldosterone secretion, these animals were adrenalectomized. They were allowed to become adrenally insufficient as described above for Na-replete animals.

*Saliva collections.* Saliva flow in the sheep is continuous, and serial collections of saliva were made from a stainless steel tray for 2 to 3 hours before the start of steroid administration and were usually continued for 3 to 4 hours after the infusion ceased. Sodium and potassium concentrations were determined with a Beckman DU flame spectrophotometer.

### Administration of steroids

All steroids for infusion were made up aseptically in 0.9% NaCl at a concentration requiring injection at 0.2 to 0.8 ml per minute. When ethanol was required to produce solution, a minimal amount was used.

*Intravenous infusion.* A polyethylene cannula was introduced into the jugular vein at least 2 hours before infusion. A motor-driven continuous injector was used. The steroids studied<sup>1</sup> were: *dl*-aldosterone from am-

<sup>1</sup> Aldosterone = 18-formyl-11 $\beta$ ,21-dihydroxy-4-pregnene-3,20-dione, cortisol = 17-hydroxycorticosterone, corticosterone = 4-pregnene-11 $\beta$ ,21-diol-3,20-dione, and desoxycorticosterone = 21-hydroxy-4-pregnene-3,20-dione.

TABLE I  
Paper partition chromatography of compounds\*

Compound	Chromatographic systems	Marker	
Aldosterone	Cyclohexane-dioxane-water	100:100:10	Cortisol
	Benzene-methanol-water	100:50:25	
Aldosterone monacetate	Cyclohexane-benzene-methanol-water	100:100:100:40	
	Cyclohexane-dioxane-methanol-water	100:100:50:25	
Aldosterone diacetate	Cyclohexane-benzene-methanol-water	100:50:100:25	Adrenosterone 11-Keto-17 $\alpha$ -OH-progesterone
	Cyclohexane-dioxane-methanol-water	100:75:50:25	
Aldosterone 11,18-lactone-21-monoacetate	Cyclohexane-benzene-methanol-water	100:75:100:30	11-Keto-17 $\alpha$ -OH-progesterone
Cortisol	Cyclohexane-dioxane-water	100:100:25	
	Benzene-methanol-water	100:50:25	
Cortisol acetate	Cyclohexane-dioxane-methanol-water	100:100:50:25	
	Cyclohexane-benzene-methanol-water	100:100:100:40	
Corticosterone	Cyclohexane-benzene-methanol-water	100:100:100:40	
	Cyclohexane-dioxane-water	100:100:25	
Corticosterone acetate	Cyclohexane-benzene-methanol-water	100:50:100:25	
	Cyclohexane-dioxane-methanol-water	100:75:50:25	
Desoxycorticosterone	Cyclohexane-benzene-methanol-water	100:25:100:15	
	Cyclohexane-methanol-water	100:100:10	
Desoxycorticosterone acetate	Cyclohexane-benzene-methanol-water	100:25:100:15	

\* Whatman no. 1 paper was used.

poules of 1 mg free racemic steroid in saline, batches 32/744/1, 53/338/1, and 33/540/1,<sup>2</sup> *d*-aldosterone as the free alcohol,<sup>2</sup> cortisol as the hemisuccinate<sup>3</sup> and also as the free alcohol; corticosterone as the free alcohol,<sup>4</sup> and desoxycorticosterone as the free alcohol.

*Intra-arterial infusion.* *dl*-Aldosterone was also given by continuous infusion into the carotid artery supplying the parotid gland.

#### Chemical methods

Each form of aldosterone and the free alcohols of cortisol, corticosterone, and desoxycorticosterone was subjected to paper partition chromatography to determine homogeneity (Table I). Ampoules of *dl*-aldosterone of the batches listed above were found consistently to contain about 700  $\mu$ g aldosterone. This was confirmed independently by Dr. R. E. Peterson, who found, when using the double-isotope technique of Kliman and Peterson (14), that only 70% of the 1 mg of ultraviolet-absorbing material in an ampoule (batch 33/540/1) was aldosterone.

Two more polar compounds were found in amounts of 200 and 100  $\mu$ g each, and there was a trace of a less polar component. That the major fraction was aldosterone was confirmed by formation of the diacetate, the 11,18-lactone-

21-monoacetate (14), the tritium and C<sup>14</sup> diacetates, and by bioassay experiments, which also showed that the two more polar compounds were inactive.

A single chromatography with cyclohexane-dioxane-water 100:100:10 on paper freshly washed with ethanol was done routinely on 1- to 2-mg batches of the original material. The concentration of the aldosterone after elution with ethanol was determined by ultraviolet absorption at 240  $m\mu$  ( $\Sigma = 15,800$ ) with a Beckman DK2 automatic recording spectrophotometer against a paper blank. The crystalline *d*-aldosterone was chromatographed as above to remove traces of other material. With the *dl*-aldosterone, some initial experiments were carried out after dilution of material directly from the original ampoules; doses were corrected subsequently on the basis of a content of only 70% aldosterone. Some preliminary reports from this laboratory of the relation between salivary Na/K and aldosterone (15, 16) require correction on this basis. In this paper, the doses of aldosterone are recorded as micrograms per hour of the *d*-isomer, a practice justified by demonstration of the same effect with the *d*-isomer as with equivalent doses, with respect to the *d*-antipode, of the rechromatographed racemic mixture, which in turn was compared with *dl*-aldosterone directly from the ampoule. Ipsilateral intracarotid infusion of the *l*-isomer<sup>5</sup> did not affect the salivary Na/K ratio.

<sup>5</sup> Prepared by Dr. R. E. Peterson by incubation of the racemic mixture with liver homogenate.

<sup>2</sup> Ciba, Basle, Switzerland.

<sup>3</sup> Soluble Ef-Cortelan; Glaxo Laboratories Ltd., Greenford, England.

<sup>4</sup> Upjohn, Kalamazoo, Mich.

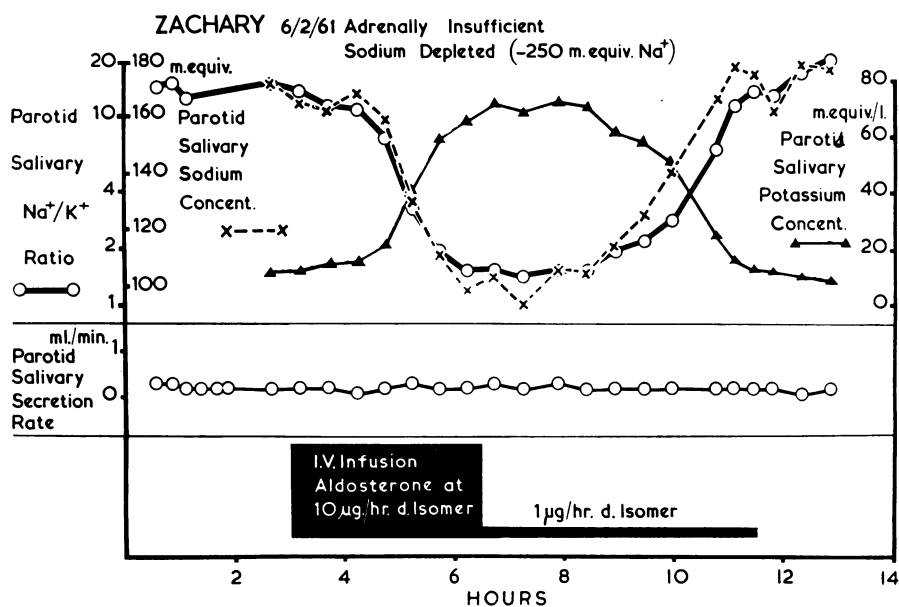


FIG. 1. ZACHARY (ADRENALLY INSUFFICIENT SHEEP WITH NA DEFICIT OF 250 MEQ). EFFECT ON PAROTID SALIVARY NA CONCENTRATION ( $\times$ --- $\times$ ), K CONCENTRATION ( $\blacktriangle$ — $\blacktriangle$ ), AND SALIVARY NA/K CONCENTRATION RATIO ( $\circ$ — $\circ$ ) OF INTRAVENOUS INFUSION OF ALDOSTERONE AT 10 AND 1  $\mu$ G PER HOUR. The parotid secretion rate is also shown.

## RESULTS

### *Effect of parotid salivary secretion rate on salivary composition during action of aldosterone*

In the normal Na-replete animal, the parotid salivary secretion rate has little or no effect on salivary Na/K ratio. In the Na-deficient animal, however, there is a linear relation between parotid secretion rate and salivary Na/K ratio which increases with the secretion rate over a limited range (3, 17). This effect is also observed in the adrenalectomized animal during infusion of electrolyte-active steroid. Large variations of parotid secretion rate due to reflex stimuli resulting from rumination or psychic influence may occur during the steroid infusion. Hence, in order to compare the effects of different rates of steroid infusion, enough saliva collections must be made at the plateau of salivary Na/K response to provide a number of samples of saliva at similar secretion rates.

### *Effect of intravenous infusion of mineralocorticoids*

**Aldosterone.** The rates of aldosterone infusion used are within the range found in adrenal venous

plasma of sheep with an adrenal transplant, i.e., 0.5 to 20  $\mu$ g per hour as determined by the double-isotope dilution derivative assay (9, 18). Eighty-one experiments were made in this series. Figure 1 shows the reciprocal nature of the effects on the Na and K concentrations of saliva when aldosterone was infused intravenously. In the Na-depleted, adrenalectomized sheep Zachary (46 kg), the Na concentration of saliva decreased and K increased reciprocally approximately 100 minutes after aldosterone infusion was begun at 10  $\mu$ g per hour. The resulting fall of salivary Na/K ratio is also shown. Reduction of the infusion rate to 1  $\mu$ g per hour caused the ratio to rise towards normal 130 minutes later, indicating that this rate of infusion was less than the threshold of parotid response in this animal. This steroid effect on ionic composition occurred without change of salivary secretion rate. The reciprocal effect on Na and K concentrations without a significant modification of the anion pattern of saliva (1) is similar to the findings in Na deficiency. In most of the subsequent figures, the effect of corticosteroid infusion on salivary Na/K ratio only is recorded.

**Na-replete sheep.** Typical responses to intravenous infusion of *d*-aldosterone are shown for

the normal sheep Bede (35 kg) in Figure 2. The parotid response began after 70 minutes at the high dose and after 120 to 130 minutes at the threshold dose. The salivary Na/K rose 120 minutes after the infusion ceased at the high dose and 80 minutes after cessation when doses close to threshold were used. The rate of fall of salivary Na/K ratio was faster at the higher rates of infusion.

The relation between dose and response at plateau of response i.e., between rate of aldosterone infusion and salivary K concentration, is shown in Figure 3, lower panel, for a series of samples at similar secretion rates in three animals, Bede, TP 12, and TP 9. In view of the variation of salivary K concentration with secretion rate, saliva collections at secretion rates outside the range of 2 to 3 ml per minute were not employed in calculations of mean K concentrations at plateau. A

threshold of approximately 3 to 5  $\mu\text{g}$  per hour and a maximal K concentration of 35 mEq per L at aldosterone infusion of 20  $\mu\text{g}$  per hour appear to be limiting values for all Na-replete sheep tested. The general shape of the curve resembles the usual sigmoid dose-response curve, but when the basal level of K and the threshold level of aldosterone are taken into consideration, it is not possible to distinguish its shape from, for example, an exponential shape, and it almost certainly is not hyperbolic.

*Na-deficient sheep.* A typical example of these experiments on adrenalectomized sheep is shown in Figure 4 with the sheep Basil. The time of onset and decay of action is similar to that in the Na-replete sheep, but the maximal fall of Na/K is much greater, to 0.8 as against approximately 4. With the sheep Watson (55 kg), adrenalectomized and depleted of approximately 700 mEq of Na, 7

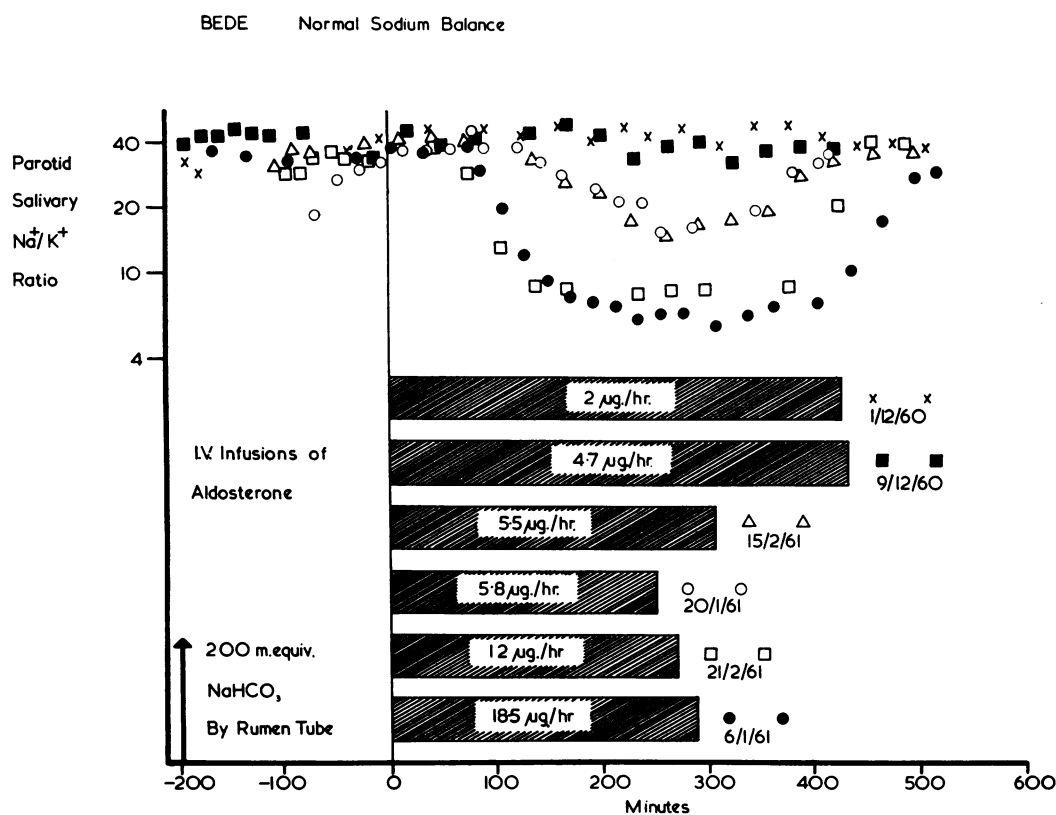


FIG. 2. BEDE (NORMAL SHEEP, NA-REPLETE). EFFECT ON PAROTID SALIVARY Na/K CONCENTRATION RATIO (ORDINATE) OF INTRAVENOUS INFUSION OF *d*-ALDOSTERONE OVER THE INTERVALS SHOWN ON THE ABSCISSA, AT THE RATES DESIGNATED. The arrow indicates oral administration of  $\text{NaHCO}_3$  200 minutes before the infusion to ensure that Na deficiency did not develop during the course of the experiment.

$\mu\text{g}$  per hour caused the salivary Na/K to fall from 21 to 1 (K concentration = 97 mEq per L). With the sheep Frazer, 14  $\mu\text{g}$  per hour reduced the salivary Na/K from 19 to 0.4. The threshold of response to aldosterone was lowered by Na deficiency. In the sheep Sigmund (Na-deficient 375 mEq), 2.8  $\mu\text{g}$  per hour caused the salivary Na/K ratio to fall to 5.8 (K concentration = 26 mEq per L). (In normal balance, 4.4  $\mu\text{g}$  per hour failed to produce any change in salivary Na/K ratio). With 10.8  $\mu\text{g}$  per hour, the ratio was 0.6 (K concentration = 96 mEq per L). With the sheep Ferdinand (Na-deficient about 450 mEq), there was a clear-cut parotid salivary response to 1  $\mu\text{g}$  per hour of iv *d*-aldosterone. With this animal, the plateau of salivary Na/K response at 4  $\mu\text{g}$  per hour was 2 and at 8  $\mu\text{g}$  per hour, 1.

An approximately linear relation between aldosterone infusion rate and salivary K concentra-

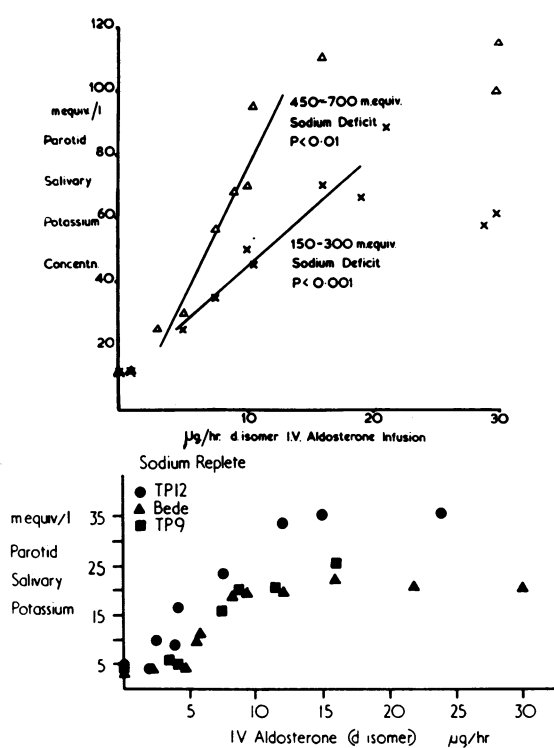


FIG. 3. EFFECT ON PAROTID SALIVARY K CONCENTRATION OF INTRAVENOUS INFUSION OF *d*-ALDOSTERONE AT THE RATES DESIGNATED ON THE ABSCISSA. Upper panel: A group of 6 adrenalectomized sheep at two different levels of Na deficit. Each point represents the mean K concentration at the plateau of parotid salivary response to a particular rate of infusion.

Lower panel: Na-replete sheep Bede, TP 9, and TP 12.

tion in the Na-depleted condition is evident in Figure 4. The nature of this relationship is shown more fully with six sheep in Figure 3, upper panel. The Na deficits shown, 150 to 300 mEq and 450 to 700 mEq, correspond to 1 and 2 days, respectively, of withholding the daily  $\text{NaHCO}_3$  supplement. The relationships are not inconsistent with a sigmoid curve. The regression lines over the range drawn in Figure 3 for two levels of Na deficiency are highly significant for linearity and the regression coefficients differ significantly ( $p < 0.01$ ). The effect of any suprathreshold dose of aldosterone was always greater with the larger Na deficit. This consistent difference is not due to falling salivary secretion rate with increasing Na deficit. Practically all data included in Figure 3 were drawn from experiments with saliva flows in the range 0.2 to 0.4 ml per minute. The points shown for aldosterone infusion rates above 15  $\mu\text{g}$  per hour give an indication of the limit of response; the large change in slope and maximum between the two groups, and the uncertainty of estimation of Na depletion (about 150 mEq) did not warrant an assessment of the relationship as hyperbolic or exponential. Comparison of the upper and lower panels of Figure 3 shows that with Na depletion, the parotid gland of the sheep becomes more sensitive to aldosterone; in the Na-depleted state, there is a reduced threshold, an increased effect upon K concentration for a given dose of aldosterone, and an increased maximal K concentration.

*Potency ratio between intravenous aldosterone and desoxycorticosterone.* In a Na-depleted sheep, a dose-response curve was obtained for the effect of intravenous infusions of desoxycorticosterone upon parotid salivary K concentration at the plateau of response. This was compared with a dose-response curve for *d*-aldosterone. The relative potency with reference to the effect on salivary K concentration at this level of Na depletion (250 to 350 mEq) and over the common linear range of response was aldosterone/desoxycorticosterone = 11.7.

#### *Effect of intravenous infusion of glucocorticoids*

In the conscious, undisturbed, Na-replete sheep with an adrenal transplant, the range of cortisol output found in 37 specimens of adrenal venous

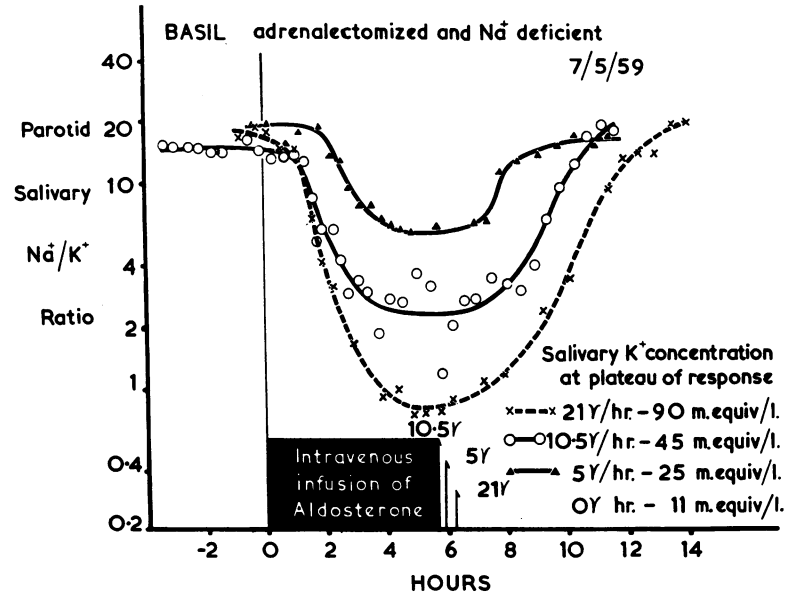


FIG. 4. BASIL (ADRENALLY INSUFFICIENT, NA-DEFICIENT SLEEP). EFFECTS ON PAROTID SALIVARY NA/K CONCENTRATION RATIO OF INTRAVENOUS INFUSION OF *d*-ALDOSTERONE AT 5, 10.5, AND 21  $\mu$ G PER HOUR.

blood was 30 to 480  $\mu$ g per hour with a mean of  $161 \pm 121$  (SD). The range of corticosterone output in 47 samples was 2 to 23  $\mu$ g per hour with a mean of  $10.0 \pm 5.9$  (SD). In several experimental conditions, including Na depletion, the aldosterone output has been found to be as high as 7 to 18  $\mu$ g per hour when the cortisol output was 50 to 200 and the corticosterone 25  $\mu$ g per hour. Under the influence of large intravenous doses of ACTH, for example, 12 IU per hour of  $\delta_1$ <sup>6</sup> and  $\alpha_b$ ,<sup>7,8</sup> the output observed is 1,600 to 3,000  $\mu$ g per hour cortisol and 100 to 200  $\mu$ g per hour corticosterone (9). Secretion rates of the same order have been recorded during a pyrogenic reaction in the conscious animal.

Under acute surgical conditions, secretion rates found from the left adrenal vein (right adrenal *in situ*) of six, Na-replete, anesthetized sheep were: 1.6  $\mu$ g per hour aldosterone, 52  $\mu$ g per hour corticosterone, and 1,020  $\mu$ g per hour cortisol. Blood flow averaged 7.8 ml per minute. The total adrenal output in these animals may be double this figure. In Na-deficient, surgically traumatized sheep in

which the right adrenal had been removed 1 to 7 weeks previously, the mean outputs from the cannulated left adrenal gland of 14 animals were 9.5  $\mu$ g per hour aldosterone, 106  $\mu$ g per hour corticosterone, and 1,300  $\mu$ g per hour cortisol. Mean blood flow was 9.5 ml per minute. It was considered that 5,000  $\mu$ g per hour cortisol and 350  $\mu$ g per hour corticosterone could safely be regarded as the maximal adrenal secretion rate, including acute experimentation under anesthesia.

The effects of these glucocorticoids were examined in Na-replete and Na-deficient animals under three conditions: *a*) infusion of either cortisol or corticosterone, *b*) simultaneous infusion of cortisol and corticosterone in a ratio similar to that in adrenal venous blood, and *c*) infusion of either cortisol or corticosterone concurrently with aldosterone to determine whether there is any synergistic or antagonistic effect with respect to the action of aldosterone on the parotid.

*a) Cortisol.* In two experiments, cortisol hemisuccinate was infused intravenously in Na-depleted, adrenalectomized sheep after a large "loading" injection of 18 to 20 mg given during 2 to 3 minutes. In both instances, the loading injection caused the salivary Na/K ratio to fall. The ratio was observed to rise again to normal despite a

<sup>6</sup> Dr. P. H. Bell, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

<sup>7</sup> Dr. C. H. Li.

<sup>8</sup> Armour Laboratories, Kankakee, Ill.

continuous infusion of 6 to 20 mg per hour. Cortisol was also infused into the sheep Zachary during adrenal insufficiency, first, when the animal was in normal Na balance and second, when the Na deficit was 400 to 600 mEq. In both instances, the initial infusion at 4.7 mg per hour was without effect after 4 hours, but when the infusion rate was increased to 9.0 mg per hour in the Na-replete animal, the Na/K ratio fell from 28 to 14 with a maximal K concentration of 12 mEq per L (salivary secretion rate, 1.2 ml per minute). In the Na-depleted animal, the effect was more marked, with the ratio reduced from 10 to 6 with a maximal K concentration of 25 mEq per L. Intravenous infusion of corticosterone to TP 12 when Na-replete at rates of 175 to 350  $\mu\text{g}$  per hour for 10 hours was without effect on the salivary Na/K ratio. Sigmund, when Na-replete, showed no change in Na/K ratio with an infusion at 275  $\mu\text{g}$  per hour.

b) *Simultaneous infusion of cortisol and corticosterone.* The cortisol/corticosterone ratio in adrenal venous blood of conscious, undisturbed animals is 20 to 100, and under maximal ACTH stimulation it is 10 to 20 (6). Experiments were made on three Na-replete sheep, TP 12, TP 9,

and Charon. There was no effect on salivary Na/K ratio in TP 9 (Figure 5) with infusion at 2,200  $\mu\text{g}$  per hour cortisol and 135  $\mu\text{g}$  per hour corticosterone and in TP 12 with infusion of cortisol-corticosterone at 5,000:300  $\mu\text{g}$  per hour. In TP 9 with infusion of cortisol-corticosterone at 4,400:270  $\mu\text{g}$  per hour, salivary Na/K fell slightly, with maximal salivary K concentration at 9 mEq per L (Figure 5). The effect was comparable with that caused by aldosterone infusion at 4  $\mu\text{g}$  per hour in this animal. In TP 12, cortisol-corticosterone infused at 9,000:550  $\mu\text{g}$  per hour reduced Na/K to 7.8, with a maximal K concentration of 28 mEq/L. In Charon, cortisol-corticosterone infusion at 5,500:275  $\mu\text{g}$  per hour caused salivary Na/K to fall to 11, with a maximal K concentration at 15 mEq per L. In Sigmund, bilaterally adrenalectomized and Na-deficient, infusion of cortisol-corticosterone at 750:45  $\mu\text{g}$  and 2,750:165  $\mu\text{g}$  per hour had no effect, and at 5,800:345  $\mu\text{g}$  per hour salivary Na/K fell to 6.3, with a maximal K concentration of 23 mEq per L. In this instance, plasma cortisol was measured by a modification of the double-isotope dilution derivative method of Peterson (19). With infusion at 750  $\mu\text{g}$  per hour, plasma cortisol concentration

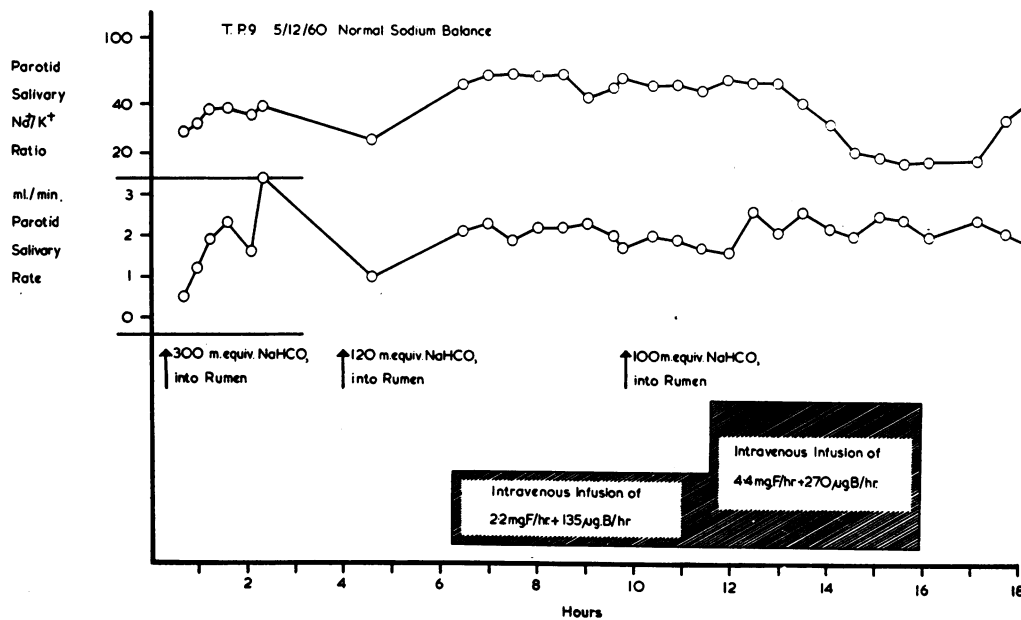


FIG. 5. TP 9 (SHEEP WITH NORMAL NA BALANCE). EFFECT OF IV INFUSION OF 2,200  $\mu\text{g}$  PER HOUR CORTISOL AND 135  $\mu\text{g}$  PER HOUR CORTICOSTERONE, AND OF 4,400  $\mu\text{g}$  PER HOUR CORTISOL AND 270  $\mu\text{g}$  PER HOUR CORTICOSTERONE UPON SALIVARY Na/K CONCENTRATION RATIO AND SALIVARY SECRETION RATE.



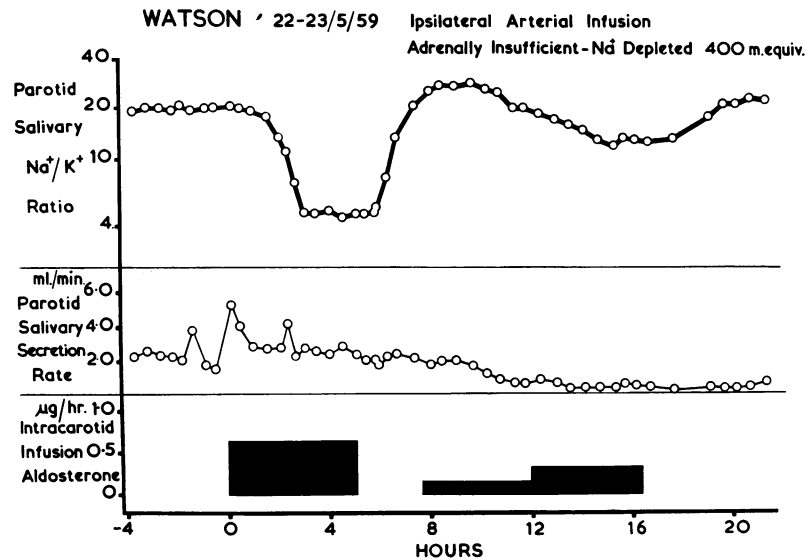


FIG. 6. WATSON (ADRENALLY INSUFFICIENT SHEEP WITH NA DEFICIT OF 400 MEQ). EFFECT ON PAROTID SALIVARY Na/K CONCENTRATION RATIO OF IPSILATERAL INTRACAROTID INFUSION OF *d*-ALDOSTERONE AT 0.7, 0.18, AND 0.35  $\mu$ G PER HOUR. Salivary secretion rate is also shown.

was 2.2  $\mu$ g per 100 ml; at 2,750  $\mu$ g per hour, 6.5  $\mu$ g per 100 ml; and at 5,800  $\mu$ g per hour, 11  $\mu$ g per 100 ml.

c) *Effect of cortisol or corticosterone infusion during aldosterone infusion.* Two experiments were made on Sigmund when Na-replete. Corticosterone was infused at 275  $\mu$ g per hour for 3½ hours without effect; thereupon aldosterone was infused at 4.3  $\mu$ g per hour for 3 hours. In this sheep when Na-replete, the threshold of response to aldosterone was 5.6  $\mu$ g per hour, and although the rate of 4.3  $\mu$ g per hour was only 1.3  $\mu$ g per hour below threshold, its combination with the high rate of corticosterone was still without effect. With an infusion of aldosterone at 8.6  $\mu$ g per hour in this same sheep, the salivary Na/K ratio fell after 4½ hours to 4.5. With inclusion of corticosterone in the infusion at 275  $\mu$ g per hour, there was no further rise or fall in ratio. In experiments on the sheep Watson when Na-replete and when 250 mEq Na-deficient, aldosterone was infused for 4 hours at 16  $\mu$ g per hour, and then cortisol was included at 4,000  $\mu$ g per hour. In neither instance did the salivary ratio change from the constant level held when aldosterone alone was being infused.

#### *Ipsilateral carotid arterial infusions*

*Aldosterone.* A representative experiment is shown in Figure 6. With Watson, adrenally insufficient and 400 mEq Na-deficient, aldosterone was infused into the carotid artery supplying the parotid gland. The initial infusion at a rate of 0.7  $\mu$ g per hour caused a fall in the salivary Na/K to 4.5 (Figure 6). The ratio was then allowed to return to normal, when infusions at 0.18  $\mu$ g per hour for 4 hours and then at 0.34  $\mu$ g per hour for 2 hours were made. This reduced the ratio to 12. When infusions ceased, the ratio returned to baseline after the usual time interval. With Zachary when Na-replete, intracarotid infusion at 2.9  $\mu$ g per hour reduced the salivary Na/K to 3.0 with maximal K concentration of 42 mEq per L. In these experiments, the earliest change of salivary Na/K began 1 hour after intracarotid infusion began.

*Infusion of steroids in blood.* The steroids for infusion were made up in blood drawn from a Na-replete, adrenally insufficient sheep. This blood was then infused into the ipsilateral carotid artery of the Na-depleted, adrenalectomized animal. An infusion of 1,000  $\mu$ g per hour of cortisol and 50  $\mu$ g per hour corticosterone in blood was without ef-

fect. The same procedure with 4  $\mu\text{g}$  per hour aldosterone added to the infusion caused the Na/K ratio to fall to 1.4 (Figure 7).

#### DISCUSSION

Intravascular infusion of aldosterone causes an increase in K concentration of the saliva of the sheep's parotid gland; there is a commensurate fall in the concentration of Na. These changes have the following characteristics. *a*) There is a threshold rate of infusion to produce any change at all. *b*) The change of salivary K and Na concentration in the Na-depleted sheep shows two phases of relation to an increasing rate of intravascular infusion of aldosterone above threshold. There is first a linear relation of high slope up to 10 to 20  $\mu\text{g}$  *d*-aldosterone per hour and then a sharp bend to low slope. *c*) Associated with increasing Na depletion of the sheep, there is a decrease in the threshold dose and an increase in the maximal K concentration under aldosterone ad-

ministration. *d*) The commencement of change shows a latent period of approximately 1 hour even with ipsilateral carotid infusion, and the decay of the effect after stopping infusion shows a similar latency. The extent of change in salivary K concentration for a given rate of aldosterone infusion is affected by the rate of salivary secretion and is smaller for higher secretion rates. Aldosterone is 12 times as potent as desoxycorticosterone on the parotid gland and over 1,000 times as potent as cortisol. There was no response to the largest doses of corticosterone given, and these were 100 times the threshold dose of aldosterone. There was no clear evidence of synergism or antagonism between any of the three steroids acting on the parotid gland. The linked, inverse changes of Na and K concentrations in the parotid saliva under the influence of aldosterone are reminiscent of the responses of muscle (20) and the kidney (21).

As Figure 3 shows, the saliva of adrenalecto-

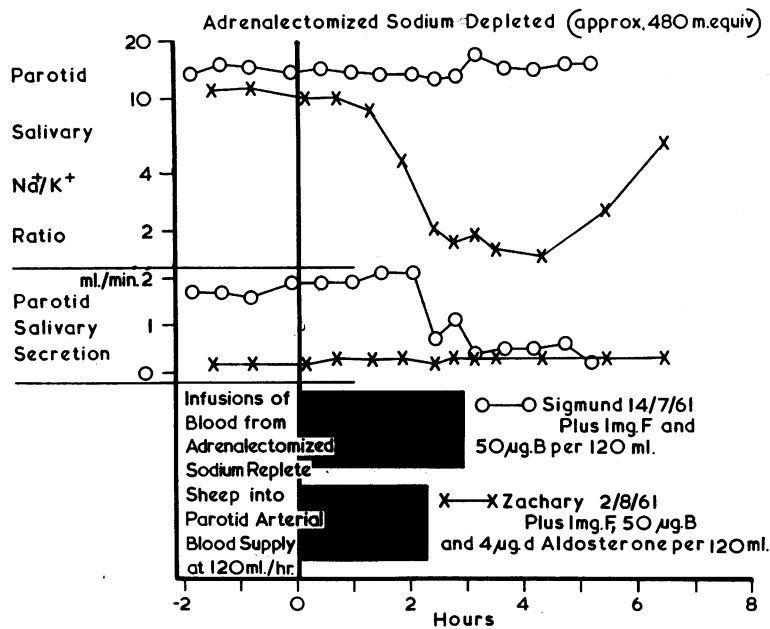


FIG. 7. EFFECT ON PAROTID SALIVARY NA/K CONCENTRATION RATIO OF IPSILATERAL INTRACAROTID INFUSION OF BLOOD FROM AN ADRENALECTOMIZED NA-REPLETE SHEEP AT 120 ML PER HOUR. In the first experiment (Sigmund, ○—○), 1,000  $\mu\text{g}$  cortisol and 50  $\mu\text{g}$  corticosterone were added to 120 ml of blood. In the second experiment (Zachary, ×—×), 1,000  $\mu\text{g}$  cortisol, 50  $\mu\text{g}$  corticosterone, and 4  $\mu\text{g}$  *d*-aldosterone were added to 120 ml. Both animals were adrenally insufficient and about 500 mEq Na-deficient. Parotid salivary secretion rate is also shown.

mized sheep without desoxycorticosterone acetate or aldosterone has a higher K concentration when the sheep are Na-depleted than when Na-replete. The maximal rise in salivary concentration in response to administered aldosterone is generally higher in Na-depleted than in the Na-replete sheep—in the cases represented, 120 mEq as against 40 mEq. The high level is also seen in the Na-depleted sheep with adrenals intact. The capacity of the parotid gland to secrete K at a high concentration is not due to long-continued action of high levels of aldosterone, since the adrenalectomized animals were given a supporting dose of desoxycorticosterone acetate until 2 days before these tests. Nor is it due to a changed concentration of electrolytes in the plasma reaching the gland, since it occurred when the Na concentration was prevented from falling by concurrent Na and water depletion (17, 22). Hypophysectomy, thyroidectomy, bilateral nephrectomy, pinealectomy associated with removal of the commissural body, or removal of the whole of the intracranial contents does not reduce the high K concentration in Na depletion. The secretory effect is not due to stimulus through the secretomotor nerve, since similar maximal K concentration occurs during Na depletion in basal saliva from parotid glands with nerves cut several weeks before. It is possible that intracellular change in ionic composition of the parotid cells modifies the secretory mechanism. A second possibility that the aldosterone effect on the gland is potentiated by aldosterone-stimulating hormone is under investigation through tests on the effect of caval constriction in the adrenalectomized animal.<sup>9</sup>

Two other features of Figure 3 are the lower threshold in the Na-depleted sheep and the increase in the slope of the relation between K and the aldosterone dose with increasing depletion. These phenomena might be contributed to by a reduced volume of distribution, a reduced rate of clearance of aldosterone from the circulation, or some change in sensitivity of the parotid gland to

aldosterone, or all of these; the maximal salivary K concentration in proportion to the degree of depletion makes likely some change in sensitivity. The latency of onset and decay of the aldosterone effect is similar to the latency for the renal effect (21, 20) and for the effect on toad's bladder (24), where aldosterone is directly applied to the epithelium affected. The latency for the salivary effect is reduced little by intra-arterial infusion of aldosterone to the parotid gland, nor is it reduced below 1 hour, when the salivary flow flushes the duct system of the gland in less than a minute. There appears to be, therefore, a substantial delay in onset of the effect, owing to slow penetration of the cells, or to a slow reaction in the cells—perhaps with a phosphatidic acid system—which precedes the effect on the ion transport, or to both.

During intravenous infusion of aldosterone, the rate of increase in peripheral concentration depends on the rate of infusion, the metabolic degradation, and the distribution of the steroid throughout the body; this rate will be lower for small doses than for large. The attainment of threshold concentration will be slower for the smaller doses and in general, the onset of salivary effect is later for smaller doses given intravenously. Similar considerations apply to the longer period for cessation of the effect with larger doses when the infusion is stopped.

Cortisol and corticosterone intravenously infused alone or together were singularly inactive in terms of the parotid salivary Na/K ratio, and only small responses were produced by infusions in the range of the maximal outputs per hour that have been recorded from the surgically traumatized, anesthetized animal, that is, outputs around 4,000  $\mu\text{g}$  per hour cortisol and 300  $\mu\text{g}$  per hour corticosterone. The changes were small compared with those produced by aldosterone under similar physiological conditions. The results of these intravenous infusions of glucocorticoids indicate a potency ratio of aldosterone/cortisol greater than 1,000 and an aldosterone/corticosterone ratio greater than 150.

Intra-arterial infusions of aldosterone, cortisol, and corticosterone would indicate that the potency ratio of aldosterone/cortisol is nearer 5,000 because infusions of cortisol at 2 mg per hour had no effect on the parotid gland, but at 4 mg per

<sup>9</sup> At the Twenty-second International Congress of Physiology held in Leiden, Netherlands, in 1962, Davis, Holman, Carpenter, Urquhart, and Higgins reported evidence for a factor causing sodium retention in caval constricted dogs that were adrenalectomized.

hour the ratio fell 2.5. The intracarotid infusion of cortisol and corticosterone in whole blood at levels 5 times the basal adrenal output was without effect. In considerations of effects of intra-arterial infusions, there are three additional factors to be allowed for: the uncertainty of mixing, the rate of binding and extent of uptake of steroid by proteins in the plasma, and recirculation.

The effect of protein binding is at present uncertain. The carotid-parotid circulation time is probably of the order of 1 second, and hence protein binding is probably incomplete by the time the steroid reaches the parotid gland. The concentration gradient of steroid from plasma to parotid gland would then be much higher than for the same apparent concentration of steroid if it were largely bound. Hence, arterial infusions of steroids with a high affinity for plasma-binding protein are likely to cause much greater effects than corresponding intravenous infusions.

The experimental results indicate that under equilibrium conditions the salivary Na/K ratio responds to changes in the rate of aldosterone infusion, and thus to the concentration of the hormone in peripheral blood. Within the physiological range of the secretion rate of the corticosteroids studied, aldosterone is the only component that affects the salivary Na/K ratio, and there is a threshold concentration for an effect, a maximal level of effect, and a delay intrinsic to the parotid gland for full development of response. There are also important influences modifying the proportional relation between aldosterone infusion rate and the salivary Na/K ratio; the sensitivity of the parotid gland to aldosterone increased in Na deficiency, and in Na deficiency the rate of parotid secretion influences the salivary Na/K.

Thus, decrease of salivary Na/K occurring during physiological experimentation on animals with intact adrenals is attributable to variation in the aldosterone component of adrenal secretion. In these circumstances, the rate of adrenal aldosterone secretion can be estimated approximately from the salivary Na/K, with the reservations mentioned above about the modifying influences. In conditions of Na equilibrium, the correspondence between salivary Na/K and adrenal aldosterone output is as close as with intravenous infusion of the steroid to an adrenally insufficient

animal with a similar Na status. For example, if a Na-deficient animal is given an intravenous infusion of 250 to 350 mEq NaCl over 40 to 50 minutes, the rise of salivary Na/K ratio that occurs has been shown to be associated with a large fall of aldosterone secretion below parotid threshold (8); but two factors, increased volume of distribution and increased hepatic blood flow, may also contribute to the fall of peripheral aldosterone level. In acute experiments under anesthesia involving extensive surgical ablation, the possibility that volume of distribution and circulatory deterioration may influence the rapidity of aldosterone degradation is clearly greater. Experiments (15) have shown, however, that adrenalectomy in the Na-deficient animal causes a rise of salivary concentration ratio towards normal within 120 to 180 minutes, and a large series of unpublished acute experiments using the double-isotope method have not shown instances of the salivary Na/K ratio remaining low when aldosterone secretion falls below the parotid threshold in a Na-deficient animal. An evident limitation of the use of the Na/K concentration ratio as an index of aldosterone secretion in a Na-deficient animal is that the dose-response curve reaches asymptote at an aldosterone infusion rate of about 12  $\mu\text{g}$  per hour, and thus a fall of the adrenal secretion rate from 20 to 12  $\mu\text{g}$  per hour would not be indicated. Practically, advantages of the use of the salivary Na/K concentration ratio are that the parotid secretion is continuous, virtually protein-free, and easily assayed by flame photometry. An experimental procedure can be monitored as it proceeds, and this information is often valuable in choosing the time of an adrenal venous blood record, since adrenal venous blood collection, is, of necessity, episodic. The same animal may be used over and over again for years as its own experimental control, and the effects on saliva composition of a given procedure can be compared with intravenous infusion of pure aldosterone, and also with adrenal venous assay in the case of sheep with autotransplanted adrenals. The parotid gland is less susceptible to hemodynamic effects than renal excretion, and whereas renal Na excretion virtually ceases with a Na deficit of 100 to 200 mEq, parotid salivary Na/K falls commensurately with Na balance to a Na deficit of 400 to 500 mEq (4).

Finally, the demonstration here of influences modifying the effect of aldosterone on the parotid electrolyte transfer mechanism raises the question of whether its action on other tissues is similarly modified. This important question in relation to target tissues is also highly pertinent to the response to stimuli of the adrenal itself. The changes of Na deficiency may modify its sensitivity to a wide variety of humoral stimuli including ACTH and, as discussed previously (9), angiotensin II.

## SUMMARY

This paper records detailed observations on the effect of administered corticosteroids, particularly aldosterone, on the Na and K content of the parotid saliva of the sheep. A dose-response relation between intravenous administration of physiological amounts of aldosterone and the salivary Na/K concentration ratio was demonstrated in Na-replete, normal and in Na-deficient, adrenalectomized sheep. It has been shown that infused glucocorticoids at levels similar to those maximally secreted by the sheep's adrenals have little or no effect on the parotid salivary Na/K concentration ratio, but infused aldosterone has the equivalent effect of endogenous aldosterone. A striking difference was shown between the sensitivity to aldosterone of the parotid gland in Na-replete and in Na-deficient sheep. Experiments on intravenous infusion of aldosterone show that the parotid gland of a 30-kg, Na-depleted sheep may respond to as little as 1 to 2  $\mu\text{g}$  per hour.

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