after spaying. On the other hand 14 of 15 rabbits treated with progesterone had 1 or more normal living fetuses at time of sacrifice. None of 5 rabbits treated with 19-nortestosterone showed any protection. With 17-methyl-19-nortestosterone 1 rabbit had living fetuses and the other 4 had some further placental development although no fetuses remained. With 17-ethyl-19-nortestosterone, 9 had one or more apparently normal fetuses. Two more showed some further development after spaying but the young were resorbing at the time of sacrifice. Three additional rabbits showed no protection. With 17-propyl-19-nortestosterone, 4 of the 5 rabbits showed normal development while the fifth showed decided fetal growth, although the young died before autopsy. The higher analogs have not yet been tested.

It has been pointed out that, compared to progesterone, some of these compounds are much more effective when administered systemically rather than directly into the uterus. Preliminary data indicate that the oral activity of this series, in general, is very marked with a high oral/parenteral ratio. These findings suggest that these norsteroids may be metabolized into more active compounds. Further studies on this series are in progress.

Summary. 1. Progestational activity of a series of 17-substituted 19-nortestosterone derivatives was investigated using both intrauterine and systemic assays in rabbits. Several of the substances studied possessed a high order of progestational activity. This activity was not related to either androgenic or anabolic potency of these compounds. Of the compounds studied 17-butenyl appeared to be the most potent, possessing about 10 times and 2½ times the activity of progesterone in intrauterine and systemic assays, respectively. 2. Progesterone-like activity of these 19-nortestosterones was further demonstrated by the ability of some members of the series to maintain pregnancy in ovariectomized rabbits.

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Effects of Aldosterone and Desoxycorticosterone on Tissue Electrolytes.* (23064)

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Desoxycorticosterone (DOC) has been used in replacement therapy for adrenal insufficiency for more than 20 years. A major portion of research on the salt-retaining function of the adrenal cortex has consisted in a comparison of the effects of adrenalectomy and administration of desoxycorticosterone acetate (DCA). The recent research on aldosterone, the natural hormone whose actions DOC mimics, has revealed not only quantitative but also qualitative differences between the 2 steroids(1). Inasmuch as the major biological role of these compounds probably is to modify electrolyte transport, it seemed of interest to compare the effects of the 2 steroids on cellular ion transport systems. For this purpose, the effects of administra-

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TABLE I. Effect of DCA and Aldosterone on Total Electrolytes and Water in Plasma, Brain, and Muscle of Mice.*

	Plasma				Brain				Muscle			
	H_2O, %	Na	K	Cl	H_2O, %	Na	K	Cl	H_2O, %	Na	K	Cl
		mEq/l				mEq/kg wet tissue				mEq/kg wet tissue		
Controls	93.13 ±.25	159.0 ±3.8	3.83 ±.2	123.4 ±.9	79.76 ±.55	42.43 ±.25	105.5 ±.8	28.69 ±1.40	76.44 ±.11	23.88 ±.04	108.7 ±.3	15.84 ±.33
DCA	93.12 ±.01	163.2 ±1.8	3.37 ±.07	122.5 ±.2	80.34 ±.03	44.65 ±.14	109.2 ±.1	29.40 ±.12	76.61 ±.13	27.46 ±1.28	109.6 ±3.3	14.93 ±.12
Aldosterone	92.71 ±.34	169.8 ±5.2	3.71 ±.25	125.3 ±1.3	79.88 ±.59	44.17 ±.90	107.3 ±.8	30.00 ±.16	76.74 ±.03	26.07 ±.44	113.8 ±2.7	18.39 ±1.95

* Values given are mean ± stand. error.

TABLE II. Effect of DCA and Aldosterone on Extracellular and Intracellular Distribution of Electrolytes and Water in Brain and Muscle of Mice.*

	Cl space, %		Intracellular concentration						Extracell. Na / Intracell.		Intracell. K / Extracell.	
			H_2O, %		Na		K					
					mEq/kg cell water							
	Brain	Muscle	Brain	Muscle	Brain	Musc.	Brain	Muscle	Brain	Muscle	Brain	Muscle
Controls	20.59 ±1.22	11.40 ±.30	59.17 ±1.75	65.04 ±1.96	15.0 ±4.5	8.2 ±1.4	177.0 ±3.9	166.5 ±.3	12.0 ±3.9	20.4 ±3.8	45.6 ±3.6	42.8 ±2.5
DCA	21.22 ±.08	10.80 ±.09	59.12 ±.05	65.81 ±.04	15.7 ±.7	14.3 ±2.4	183.4 ±.1	166.1 ±5.1	10.7 ±.6	12.0 ±2.1	53.5 ±1.0	48.5 ±.5
Aldosterone	21.09 ±.24	12.92 ±1.16	58.79 ±.84	63.82 ±1.13	12.7 ±.2	5.49 ±3.6	181.3 ±1.3	177.5 ±7.0	13.7 ±.7	56.0 ±37.4	47.9 ±3.7	46.8 ±1.4

* Values given are mean ± stand. error.

tion of aldosterone and DCA on electrolyte composition of plasma, brain, and muscle were determined.

Methods. Thirty-six mice (Carworth Farm, CF #1 strain) weighing between 35 and 45 g were divided into 3 approximately equal groups. The groups consisted of (1) controls, (2) animals given DCA, and (3) animals given aldosterone. All groups were injected by the subcutaneous route once daily for a period of 4 days; the control group received 0.1 ml of isotonic sodium chloride solution containing "Tween 80," the DCA group received 0.5 mg of DCA suspended in the same vehicle, and the aldosterone group‡ received 20 μg of aldosterone dissolved in 0.1 ml of isotonic sodium chloride solution for 3 days and 8 μg of aldosterone on the last day. Sixteen hours after their terminal injection, the mice were anesthetized with 2.4 mg pentobarbital sodium (about 60 mg/kg), blood was drawn from the inferior vena cava into heparinized syringes and immediately centrifuged, and cerebral cortex and hind leg muscle were collected in tared bottles after blotting to remove superficial blood. Electrolyte determinations were performed on pooled samples comprising the plasma or tissue from 4 mice. The content of water, sodium, potassium, and chloride was determined in plasma, brain, and muscle samples according to methods previously described(2). Intracellular water and electrolyte concentrations were calculated by the method of Hastings and Eichelberger(3).

Results. The data obtained are summarized in Table I, which presents the analytical results for total plasma and tissue electrolytes, and Table II, which presents the calculated intracellular values. The administration of DCA resulted in an elevation of Na concentration and a lowering of K concentration in plasma, chloride concentration was unchanged. In brain, chloride space, which is assumed to measure extracellular

‡ The authors wish to express their appreciation to Dr. R. E. Knauff, Upjohn Co. for the gift of aldosterone.

volume, was unchanged. The concentration ratio of brain Na $\left(\frac{Na_e}{Na_c}\right)$ did not change significantly, whereas the concentration ratio of brain K $\left(\frac{K_c}{K_e}\right)$ was markedly elevated. In muscle, $\frac{K_c}{K_e}$ was elevated, despite a reduction of $\frac{Na_e}{Na_c}$. The reduction in the concentration ratio of muscle Na was related to a significant elevation of intracellular Na concentration.

Aldosterone elevated the plasma Na concentration markedly and lowered the plasma K concentration slightly. In brain, aldosterone did not affect the chloride space; it lowered intracellular Na concentration and slightly raised $\frac{Na_e}{Na_c}$ and $\frac{K_c}{K_e}$. In muscle, aldosterone definitely lowered intracellular Na and markedly increased $\frac{Na_e}{Na_c}$ ratio, and K accumulation occurred, although only to a moderate degree.

Discussion. The important effects of the 2 steroids on cellular electrolytes may be stated briefly. DCA elevated the K ratio without changing the Na ratio significantly in brain, whereas in muscle, the K ratio was elevated only slightly and the Na ratio was reduced. In contrast, aldosterone elevated K and Na ratios in both brain and muscle. The absence of an effect of DCA on brain Na differs from previously reported findings and may be presumed to be related to the short period of treatment and the low dose used. In sufficient dosage, DCA produces a definite decrease in intracellular Na and an increase in the Na ratio in brain(4,5).

The unequal partition of electrolytes across nerve and muscle membranes is consequent to active extrusion of Na from cellular fluid (6,7). Further, evidence is accumulating which indicates that this extrusion is not a simple ion pump, but that it is linked in some way to K influx. Thus, both in frog muscle and invertebrate nerve, it has been shown that Na efflux is reduced in the absence of external K(8,9). More indirect evidence for such a coupled system has been obtained in mammalian spinal motoneurons(10) and in cerebral cortex.§ Accordingly, it would be predicted that the administration of an agent that enhances ion transport would cause increases in $\frac{Na_e}{Na_c}$ and $\frac{K_c}{K_e}$ in both brain and muscle.

It is evident that aldosterone fulfills these predictions and there seems to be no difficulty in accepting the role of this hormone as a substance that promotes ion transport. In the case of DOC, although the effects on brain fit into the general hypothesis, those on muscle do not. The paradoxical finding that the administration of DCA causes a decrease in $\frac{Na_e}{Na_c}$ and a relatively minor effect on $\frac{K_c}{K_e}$ in muscle has been noted by previous authors (11,12) and is corroborated by the results presented here.

Either of 2 proposed mechanisms could be advanced to explain the effect of DOC on muscle electrolytes. It is possible that DOC simply acts differently on muscle than on brain and that its actions on muscle cause a reduction of electrolyte transport. It is also possible that the synthetic steroid is sufficiently different chemically from aldosterone that, although it mimics the hormone at some sites, it has no direct action on muscle. If this is the case, the variations in electrolyte composition which are observed after DCA administration must be secondary to its effects at other sites. Thus, the administration of DCA causes a loss of K from the body through both the kidney and the gut(12-14) and, as a result, plasma K concentration falls. Inasmuch as the membrane potential determines $\frac{K_c}{K_e}$ rather than the cellular K concentration and inasmuch as Plasma K concentration falls, K would be expected to shift from muscle cells into the extracellular fluid. The K ratio would either remain constant or be elevated slightly even though K has been lost

§ Unpublished observations.

from muscle. In association with this loss of K, muscle cells would gain Na, as demonstrated by the work of Heppel(15,16). This hypothesis, that the changes in muscle electrolytes observed after the administration of DCA result from an indirectly induced K loss and not from a direct effect of the steroid on the muscle membrane, could be tested in several ways. An indirect method would be the examination of the changes in muscle electrolytes induced by aldosterone and DCA in animals in which plasma K concentration is not allowed to fall. The observations of Ferrebee *et al.*(11) indicate that the administration of K salts prevents DCA-induced Na accumulation in muscle and may be taken as supportive evidence for the second hypothesis. A more direct test would be the measurement of muscle membrane potentials after treatment with each of the two steroids. In this connection, preliminary findings of J. W. Woodbury and Koch§ show that the resting potential of isolated frog sartorius muscle is unchanged by immersion in Ringer's solution saturated with DCA.

Thus, several independent lines of evidence suggest that DOC is devoid of direct effect on muscle and that the changes in muscle electrolyte composition which are observed are the passive result of actions of the steroid at other sites. In contrast to this, aldosterone appears to be capable of enhancing electrolyte transport at all sites which have been studied.

Summary. Plasma, brain, and muscle electrolytes were determined in mice given DCA and aldosterone. DCA increased the concentration ratio of K $\left(\frac{K_c}{K_e}\right)$ without affecting the ratio of Na $\left(\frac{Na_e}{Na_c}\right)$ in brain; it increased the concentration ratio of K moderately in muscle and decreased the concentration ratio of Na. Aldosterone increased concentration ratios of Na and K in both brain and muscle. An explanation of this difference in the effect of the two steroids on skeletal muscle electrolyte is proposed.

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