Titrations, in quadruplicate, of type 1 poliovirus (Mahoney strain) were carried out, by the plaque-assay technic, in bottle cultures of rhesus monkey kidney cells. Cultures inoculated with various amounts of virus were exposed to light for varying periods of time, and compared with control cultures which had not been exposed to light. Periods of exposure insufficient to produce discernible changes in the cell sheets were chosen. After 48 hours of incubation in the dark, following infection, plaques were often found to be smaller and less numerous in the cultures exposed to light than in unexposed controls. On further incubation, however, these differences were minimized or obviated. Fig. 2 illustrates the effect of light on the plaque formation of type 1 poliovirus in tissue cultures.

Summary. Degenerative changes occur in agar-overlaid tissue culture cells which have been exposed to light in the presence of neutral red. Temporary modification of the ability to form poliovirus plaques may occur in such cells when exposure to light is insufficient to produce discernible changes in the cell sheet.

Addendum. After this paper had been submitted for publication a report (Klein, S. W., Goodgal, S. H., *Science,* 1959, v130, 629) appeared which contained data similar to that presented above.

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Action of Testosterone in Blocking Urinary Electrolyte Effects of Desoxycorticosterone.* (25304)

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Two distinct types of steroids were reported recently to antagonize renal electrolyte effects of mineralocorticoids. The steroidal 17-spirolactones(1) were discussed as first synthetic examples with aldosterone- and desoxycorticosterone-blocking activity in the laboratory (2-4) and in man(4,5). By virtue of electrolyte effects which were dependent upon 1) presence of mineralocorticoids and 2) dosage ratio of blocking agents/mineralocorticoids, the mechanism of action of spirolactones was ascribed to competitive inhibition. A naturally occurring steroid, progesterone, was also reported to possess a similar blocking ability(6-9), but it demonstrated desoxycorticosterone-like effects on electrolyte excretion when given alone at large doses(6). The present report summarizes ability of testosterone, another gonadal hormone, to reverse electrolyte effects of desoxycorticosterone in rats.

Method. Sprague-Dawley male rats (150 to 200 g) were adrenalectomized under ether anesthesia, then maintained on 0.85% sodium chloride solution and Purina Chow *ad lib.* Approximately 6 days postoperatively animals were injected subcutaneously with 2.5 ml isotonic saline and with 12 μg of desoxycorticosterone acetate (DCA) in 0.1 ml Mazola oil. Groups were injected additionally with 0, 2, 4, 8 and 20 mg of testosterone in 0.5 ml for study of blocking effects. Other animals received corresponding doses of testosterone without DCA. Animals were placed in metabolism cages (5 rats/cage) for collection of 4-hour sample of urine. Pooled urine from each cage was diluted with water and analyzed for total Na and K content using Beckman flame photometer. Values for each sample were used directly for computation of the Na/K ratio. DCA alone reduces the ratio of

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TABLE I. Effects of Testosterone Given Alone and in Combination with DCA on Urinary Na, K and Na/K in Adrenalectomized Rats.

Treatment/rat		Mean urinary values		
DCA, μg	Testosterone, mg	Na	K	Na/K
12	—	1.34	1.32	.91
"	2	1.57	1.27	1.24*
"	4	1.35	.98*	1.30*
"	8	1.70	1.03*	1.64*
"	20	1.84*	.97*	1.93*
—	—	2.10	1.20	1.76
—	2	1.95	1.14	1.72
—	4	1.57*	.92*	1.72
—	8	1.66	.97	1.75
—	20	1.69	.95*	1.79

* $P < .05$, relative to DCA or untreated controls; least significant difference ($P = .05$) = ± .45 for Na, ± .25 for K and ± .27 for Na/K; Na and K values = mM output/4 hr/5 rats; 4 measurements for each test response and 8 for DCA and untreated controls.

Na/K in the test by causing Na retention and K loss. Ability of testosterone to block DCA was assessed by reversal of the Na/K response. Progesterone was also tested conjointly in this study, alone and in combination with DCA, for comparative data. The pooled standard deviation (s) of various groups was 0.217 for Na/K; thus, the least significant difference ($P = 0.05$) between controls ($N = 8$) and treated ($N = 4$) groups was ± 0.27 ($t = 2.0$ at 62 degrees of freedom). The pooled s for Na and K response was 0.371 and 0.205, and thus, the least changes for significance were ± 0.45 and ± 0.25, respectively.

Results. The action of testosterone in blocking electrolyte effects of DCA is illustrated in Table I. DCA alone at the standard dose of 12 μg/rat produced a low value of 0.91 for Na/K (*cf.* Na/K = 1.76, for untreated controls). Testosterone significantly blocked DCA at a dose of 2 mg; larger doses progressively reversed the Na/K response to that found in untreated controls. In general, blocking resulted from simultaneous antagonism of Na retention and K loss. The Table also shows that testosterone alone was without effect on Na/K, with responses at 2 to 20 mg approximating closely the value obtained for untreated controls. There was at the same time a tendency for a slight but definite degree of Na and K retention at 4 and 20 mg levels.

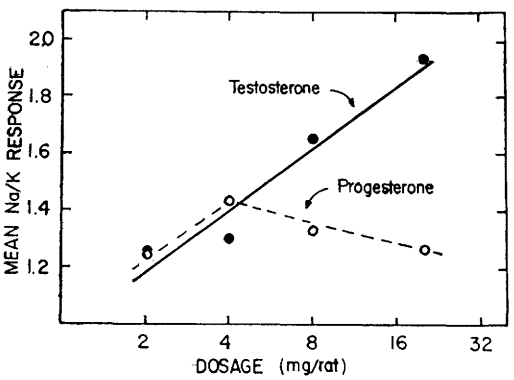

FIG. 1. Dose-response curves demonstrating action of testosterone and progesterone in blocking effects of 12 μg of DCA on urinary ratio of Na/K in adrenalectomized rats. All responses are significant ($P < .05$), relative to DCA controls.

Fig. 1 demonstrates the dose-response curves with testosterone and progesterone in terms of Na/K effect of DCA. The 2 steroids were approximately equipotent in reversing the Na/K response at 2 and 4 mg doses. An increasing dose-response curve was obtained with testosterone (slope = 0.72/10-fold range of log doses). With progesterone, the strongest reversal was achieved with the 4 mg dose; increasing doses produced, as a contrast, smaller degrees of blocking effects. The Na/K responses following treatment with the 2 steroids were significantly different at 8 and 20 mg dosage levels ($P<0.05$). Reduction of blocking potency with progesterone appears to have resulted primarily from its DCA-like effects on Na/K (Table II).

Discussion. Competitive inhibition would be suggested as the mechanism of DCA-blocking effect for testosterone if the Na/K data are used for interpretation. Increasing doses, *e.g.*, cause greater blocking of DCA to suggest importance of dosage ratio of testoster-

TABLE II. Effects of Progesterone Alone on Urinary Ratio of Na/K in Adrenalectomized Rats.

Treatment/rat, progesterone, mg	No. of measurements	Mean ratio Na/K
—	8	1.76
2	4	1.56
4	"	1.43*
8	"	1.46*
20	"	1.16*

* $P < .05$, relative to untreated controls; least significant difference for P of .05 = ± .27; 5 animals/measurement.

one/DCA. When given alone, the steroid did not affect Na/K in a manner opposing DCA action, to rule out physiological antagonism as the possible mechanism of effect. Thus, it appears that the compound exerts electrolyte effects by competing with DCA for receptor sites in target tissues, as described for other blockers(2,3,6). Inhibition is believed to be at the renal level, the primary site of action of mineralocorticoids for effects on electrolyte excretion(10-12). Unlike progesterone, testosterone did not possess DCA-like effects on Na/K when given alone; it produced for this reason an increasing blocking response over a relatively wide range of doses. The steroid appeared to be as potent as progesterone for minimal blockade; at large doses, it gave significantly greater blocking effects than progesterone. If the relative potencies in our study extrapolate to man, it would appear that testosterone would be at least as effective as progesterone in antagonizing mineralocorticoids in Addisonians(7-9). The steroid as produced endogenously under normal conditions would not be expected to influence electrolyte metabolism if body fluid titers of the hormone are gauged by doses required for replacement therapy in hypogonadal states (13-15). Potentially, however, it may influence electrolyte excretion by representing one member in a pool of structurally related blocking agents.

Summary. Large doses of testosterone blocked urinary electrolyte effects of desoxycorticosterone acetate (DCA) in adrenalectomized rats. The steroid reversed the Na/K response to DCA by simultaneously blocking Na retention and K loss. It did not affect Na/K in a manner opposing DCA action when given alone. These observations suggest competitive inhibition as the mechanism of effect for testosterone. Unlike progesterone, it was devoid of DCA-like effects on Na/K given alone, and it gave increasing DCA-blocking effect over a relatively wide range of doses.

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