Experimental Congenital Adrenal Cortical Hyperplasia: Prevention of Adrenal Hyperplasia and Clitoral Hypertrophy by Corticosterone.* (31363)

ALLEN S. GOLDMAN AND WILLIAM C. YAKOVAC (Introduced by T. N. Harris)
The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia

A single dose of a synthetic steroidal inhibitor (2α-cyano-4,4,17α-trimethyl-androst-5-en-17β-ol-3-one) of 3β-hydroxysteroid dehydrogenase (3β-enzyme), administered on various days of gestation to pregnant rats produces anatomic changes in their offspring identical to those of human infants born with deficient activity of this enzyme (1-4). The anatomic changes consist of adrenal cortical hyperplasia, clitoral hypertrophy in females, and hypospadias in males. Activity of the 3β-enzyme is essential in steroidogenic endocrine tissues in the early biosynthesis of all biologically active steroid hormones(5). The 16th day of gestation is optimal for production of hypospadias by the inhibitor and corresponds to the early appearance of strong activity of the 3β-enzyme in the fetal Leydig cells. The 19th day is optimal for the production of adrenal cortical hyperplasia and of clitoral hypertrophy, and corresponds to the time of maximal adrenal cortical activity of the 3β-enzyme. Testosterone prevents the production of hypospadias but does not affect the degree of adrenal cortical hyperplasia or clitoral hypertrophy produced by the inhibitor. Production of adrenal cortical hyperplasia in mature female rats by the inhibitor is dependent upon the presence of the pituitary, and is prevented by concurrent administration of glucocorticoid(6-8). This paper reports the effects of corticosterone, the major steroidal secretory product of the rat adrenal cortex, on the fetal effects of the inhibitor.

A total of 25 timed-pregnant rats, obtained from the supplier (Charles River Breeding Laboratories) according to procedures previously described(9), were divided at random into 4 groups of 20 experimental and a group of 5 control animals. The maximum variation in time of conception was about 15 hours. Half of the experimental females were given a single intramuscular injection of the inhibitor dissolved in propylene glycol at a dose of 5 mg/kg on the 16th day, while the remaining 10 experimental females were similarly treated on the 19th day. Each experimental female also was given daily intramuscular injections of either corticosterone dissolved in propylene glycol at a dose of 10 mg/kg, or an equivalent volume of propylene glycol alone from the day of the injection of inhibitor to the 20th day. Control females were treated in identical manner as experimental animals with the exception that the inhibitor and corticosterone were omitted from the propylene glycol. The fetuses were delivered on the 21st day by Caesarian section and fixed in formalin. The gonadal sex of each fixed fetus was determined by dissection after the anogenital distance was measured with micrometer calipers. The adrenals of each fixed fetus were obtained by microdissection and weighed. The adrenals of each pregnant animal were dissected and weighed at time of delivery.

The inhibitor produced maternal adrenal hyperplasia of equal magnitude on day 16 or 19 (Table I). In the fetuses, the inhibitor produced a highly significant degree of hypospadias in gonadal males only on the 16th day. Fetal adrenal cortical hyperplasia in either sex, and clitoral hypertrophy in gonadal females were produced maximally on the 19th day. Corticosterone prevented the production of fetal (and maternal) adrenal hyperplasia, and significantly reduced the percent of gonadal females having clitoral hypertrophy, but did not alter the production of hypospadias by the inhibitor.

These data, in light of our previous findings, suggest the following explanation of the abnormal genital differentiation occurring in

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TABLE I. Effects of Corticosterone on Action of Inhibitor.

Group	Daily dose of corticosterone (mg/kg) 16th to 20th days	19th to 20th days	Single dose of inhibitor (mg/kg) 16th day	19th day	Maternal effects: Adrenal gland wt	P compared to group A	B	D	Fetal effects: Adrenal gland wt (mg)	P compared to group A	B	D	Males: Anogenital distance (mm)	P compared to group A	B	D	Females: Clitoral hypertrophy (%)	P compared to group A	B	D
A	—	—	—	—	91.3 ± 4	—	—	—	2.0 ± .32	—	—	—	2.98 ± .08	—	—	—	0	—	—	—
B	—	—	5	—	111.3 ± 8.6	.04	—	—	2.79 ± .48	.001	—	—	2.54 ± .11	.0001	—	—	26.7 ± 11.8	—	—	—
C	10	—	5	—	74.2 ± 7.5	.014	.035	—	2.11 ± .25	NS	.03	—	2.47 ± .16	.0001	NS	—	0	NS	.046	—
D	—	—	—	5	128.7 ± 24.5	.04	NS	—	3.18 ± .59	.001	—	—	2.95 ± .07	NS	—	—	92.0 ± 13.8	.0001	—	—
E	—	—	—	5	93 ± 17	NS	—	NS	2.1 ± .36	NS	—	.01	2.93 ± .08	NS	—	NS	50.0 ± 22.6	.01	—	.02

experimental or human congenital adrenal cortical hyperplasia due to deficient activity of the 3β-enzyme. In affected male fetuses, normal differentiation of the penis is blocked by insufficient testicular production of testosterone or testosterone-like products due either to genetically deficient or to inhibited activity of the testicular 3β-enzyme. This explanation is supported by the observation that in the rabbit, testosterone prevents the feminization of male genitalia caused by fetal orchiectomy, and that male fetal decapitation (hypophysectomy) produces fetal testicular impairment and hypospadias(10). In the affected female fetus, on the other hand, clitoral hypertrophy presumably results from the virilizing influences of excess adrenal secretion of 3β-hydroxysteroidal androgens (dehydroepiandrosterone?) in response to the block in adrenal corticoid biosynthesis and subsequent excessive adrenocorticotrophic secretion(1). Hence, corticosterone administration suppresses virilization of females with deficient activity of the 3β-enzyme.

Summary. Corticosterone prevents the production of adrenal cortical hyperplasia and clitoral hypertrophy in rat gonadal female fetuses by a selective inhibitor of 3β-hydroxysteroid dehydrogenase (3β-enzyme), but does not affect the production of hypospadias in gonadal male fetuses. These findings suggest that clitoral hypertrophy results in female fetuses with deficient activity of the 3β-enzyme from excess androgenic steroidal precursors of adrenal origin produced in response to the block in adrenal corticoid biosynthesis; while hypospadias in male fetuses with deficient activity of the 3β-enzyme results from testicular secretion of androgenic steroidal products insufficient for normal penile differentiation.

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1. Goldman, A. S., Yakovac, W. C., Bongiovanni, A. M., Proc. Soc. Exp. Biol. and Med., 1966, v121, 757.

2. ———, Endocrinology, 1965, v77, 1105.

3. Goldman, A. S., J. Clin. Endocrinol., in press.

4. Goldman, A. S., Prader, A., ibid., 1964, v24, 894.

5. Samuels, L. T., Greenberg, D. M., eds., in Me-

tabolic Pathways, Academic Press, New York, 1960, Chap. 11.

6. Potts, G. O., Burnham, D. F., Beyler, A. L., Fed. Proc., 1963, v22, 166.

7. ———, ibid., 1963, v22, 270.

8. Harding, H. R., Potts, G. O., ibid., 1964, v23, 356.

9. Goldman, A. S., Yakovac, W. C., J. Pharmacol. Exp. Ther., 1963, v142, 351.

10. Jost, A., Hermaphroditism, Genital Anomalies and Related Endocrine Disorders, H. W. Jones, Jr., W. W. Scott, eds., Williams & Wilkins Co., 1958.

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Conditions Affecting the Stability of a Simian Adenovirus in the Presence of Magnesium Chloride.* (31364)

BRUCE C. CASTO AND WILLIAM McD. HAMMON

Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.

A number of enteroviruses have been shown to be protected from thermal inactivation by suspension in one or two molar concentrations of certain divalent cations(1,2,3). Contrariwise, the thermal inactivation of certain DNA viruses (adenovirus, herpes virus, pox virus, and papova virus groups)(4,5,6) and certain other RNA viruses (arbovirus and myxovirus groups)(5) has been shown to be enhanced in the presence of these concentrations of divalent cations.

In all of the above cited reports, the inactivation experiments were conducted using virus suspended in cell culture medium, either undiluted or diluted 10-fold in a serum-free, balanced salt solution. Such experiments fail to take into consideration the influence of medium components on either the enhancement of, or the stabilization against, thermal inactivation of virus infectivity in the presence of divalent cations.

This paper describes those conditions under which the thermal inactivation of a DNA virus, Simian adenovirus Type 15 (SV15), was not enhanced in the presence of 1M $MgCl_2$ at 50°C.

Materials and methods. The Simian adenovirus, SV15, used in these studies was obtained from Dr. J. L. Melnick and had been passaged 3 times in African green monkey kidney in his laboratory. The original source and previous passage history of this virus are unknown to us; however, we have reported(7) that this virus stock does not contain adeno-associated virus (AAV).

Monkey kidney cell cultures (MKCC) for virus assays were prepared according to the technique described by Wallis *et al*(8). Cultures were maintained in 0.5% lactalbumin hydrolysate (LA) in Earle's balanced salt solution (EBSS), which was supplemented with 0.5% agamma calf serum (Hyland).

Thermal inactivation studies at 50°C in 1M $MgCl_2$ were performed in the manner described by Wallis and Melnick(1). Virus titrations were done on the samples immediately after removal from heating. All virus dilutions (log_{10}) were made in LA medium free from serum and bicarbonate. Two-tenths ml from appropriate virus dilutions was inoculated into each of 4 tubes of MKCC, after which all inoculated tubes were placed in a roller drum and incubated at 36°C. Final readings for cytopathic effects (CPE) were made after incubation for 10-12 days.

Results. Effect of 1M $MgCl_2$ on SV15. Cell culture fluids containing SV15, as received from Dr. J. L. Melnick, were diluted 10-fold in serum and bicarbonate-free Hanks' balanced salt solution (HBSS). Portions of these diluted samples were further mixed with an equal amount of 2M $MgCl_2$ or deionized, distilled water (DDH_2O) and heated at 50°C. As can be seen from Table I, only slight inactivation of virus occurred (0.7 log_{10}) over the

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