ACTH-Induced Renal Glomerular Disease in Intact, Adrenalectomized and Castrate Male Mice.* (32389)

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Adrenocorticotropin (ACTH) produces characteristic renal glomerular lesions in intact and adrenalectomized female mice but of greater severity in the former(5). Early experiments suggested that ACTH affected the kidneys of male mice similarly but equally in adrenalectomized and intact males(5). This apparent difference with respect to sex in the action of ACTH on the kidneys of mice has been explored further. Clearly, earlier results were misleading and ACTH affects the kidneys of adrenalectomized mice of both sexes equally and less than those of intact mice in both sexes. This paper presents the results of recent experiments as well as those only summarized earlier, as inclusion of all pertinent data for a single analysis of variance provides the strongest basis for conclusions. Histological sections from the earlier experiment were re-evaluated using a multiple blind grading system. Also the ability was tested of a purer preparation of ACTH (Armour porcine ACTH, approx. 110 I.U./mg lot T-94303)† than previously used to induce renal disease.

Methods. A balanced experimental design was used (Table I). Brown house mice of a wild-derived out-bred strain were used in all experiments. Mice were castrated, adrenalectomized, both, or neither at 23-25 days of age as indicated in Table I. At this time daily injections subcutaneously of cortisol acetate (35 or 50 μg per mouse) were begun and continued until 24 hours before terminating the experiment. Daily injections of porcine ACTH, 4 units in 0.1 ml of gelatin vehicle per mouse as indicated in Table I, were begun a week after adrenalectomy and continued for 10 days. All mice were housed as groups of 4 to 6 per cage, depending on the experiment. Daily injections of 0.1 ml of a 16% solution of gelatin were given to mice not receiving ACTH. Mice were killed with ether a day after the last injection the gastrointestinal tract removed, and the rest of the animal put in 10% neutral buffered formalin. Each mouse was checked for completeness of adrenalectomy at autopsy with a binocular dissecting microscope. Results of this examination were further confirmed by body weight and weights of the thymus and spleen. After fixation the thymus, spleen, adrenals, kidneys and reproductive organs were removed, freed of fat and extraneous tissue, and weighed on torsion balances.

Every mouse was weighed at the time of adrenalectomy, again when injections of ACTH were begun, and when killed. Total length was measured at autopsy.

Kidneys were sectioned at 3-4 μ and stained with haematoxylin-eosin and with Lillie's allochrome stain (Lillie, 1954). Sections were examined by light microscopy and a number of morphological features graded as listed below.

The renal glomeruli were graded for cellularity and enlargement, hemorrhage, increase of intercapillary material and PAS-positive (PAS+) deposits in the tuft, peripheralization of the capillaries, changes in the capillary basement membranes, capillary dilation or thrombosis, adhesions, PAS+ deposits in and hyperplasia of the base of the tuft and juxtaglomerular region, and granulation of the juxtaglomerular cells. Renal papillae were examined for any abnormalities. Tubules were graded for casts, PAS+ droplets in the epithelial cells, vacuoles in the apical portion of proximal epithelial cells, dilation and PAS+ deposits at the bases of distal epithelial cells. Inflammatory lesions were looked for routinely. After initial examination, all numbers and identifying marks were covered, the slides thoroughly mixed, renumbered and regraded. The blind grading was repeated 3

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† Supplied through courtesy of Dr. J. D. Fisher, Armour & Co., Kankakee, Ill.

TABLE I. Data as Means and Their Standard Areas for Severity of Renal Glomerular Disease, for Organ Weights and for Body Weight. Details of procedure and design of the 3 experiments is shown also. All injections were administered subcutaneously. Mice were caged in groups of 4 or 6.

Exp No.	Sex	Test animal	No.	Age at start of ACTH inj's	Daily experimental treatment: Supportive	Daily experimental treatment: Units of ACTH	Severity of glomerular lesions	Wt of kidney, mg/10 g ± S.E.	Adrenal wt	Thymus wt	Splenic wt	Gonads	Sem. vesicles + coag. glands: Uterus	Final body wt, g ± S.E.	Gain in body wt
I	♂	Intact	12	32–34	0.1 ml gel.†	0	1.12 ± .24‖	163 ± 4	4.74 ± .20‖	49.8 ± 3.5‖	53.4 ± 4.9‖	169 ± 10‖	78.9 ± 9.1‖	19.3 ± .8	+6.4 ± .4‖
	"	"	10	"	0 "	4‡	3.22 ± .08	200 ± 6	9.87 ± .96	3.8 ± .3	14.8 ± 2.8	140 ± 12	45.2 ±10.9	14.3 ± .7	+ .2 ± .4
	"	Adrenalectomized	13	"	50 μg FA*	0	2.27 ± .17	199 ± 6		6.8 ± .7	34.0 ± 4.2	174 ± 5	90.4 ± 8.0	16.6 ± .5	+2.6 ± .4
	"	"	11	"	" "	4‡	2.83 ± .16	214 ± 5		5.8 ± .7	38.3 ± 3.0	172 ± 8	72.9 ± 6.4	17.4 ± .7	+4.0 ± .3
II	"	Intact	8	33	0.1 ml gel.	0	1.66 ± .08	169 ± 6	3.81 ± .17	51.2 ± 4.0	47.9 ± 1.8	167 ± 7	79.1 ± 6.3	20.0 ± .8	+4.1 ± .3
	"	"	8	"	" "	4‡	3.40 ± .10	208 ± 4	8.36 ± .35	4.2 ± .3	20.4 ± 1.7	182 ± 8	46.2 ± 5.1	17.2 ± .5	+1.2 ± .4
	"	Adrenalectomized	8	"	50 μg FA	0	1.44 ± .14	200 ± 4		5.3 ± .4	30.2 ± .8	161 ± 4	81.0 ± 9.8	16.0 ± .3	+1.0 ± .3
	"	"	7	"	" "	4‡	2.79 ± .10	206 ±10		8.4 ± 1.3	39.1 ± 3.8	168 ± 8	69.8 ± 6.4	16.9 ± .7	+3.2 ± .4
	"	Castrate	7	"	0.1 ml gel.	0	2.00 ±0	136 ± 3	5.40 ± .16	83.3 ± 1.4	60.5 ± 3.7		3.2 ± .3	19.6 ± .6	+3.3 ± .4
	"	"	8	"	" "	4‡	3.76 ± .14	167 ± 6	10.55 ± .27	4.1 ± .1	20.5 ± 1.5		2.9 ± .2	17.6 ± .3	+1.7 ± .7
	"	Castrate and adrenalectomized	6	"	50 μg FA	0	1.91 ± .24	158 ± 5		27.3 ± 9.1	41.8 ± 5.4		2.9 ± .2	16.1 ± .3	+1.9 ± .2
	"	Castrate and adrenalectomized	5	"		4‡	2.50 ± .29	164 ± 4		51.8 ± 7.4	51.3 ± 3.4		2.2 ± .3	15.7 ± .8	+2.9 ± .4
III	"	Intact	6	28–32	0.1 ml gel.	0	1.95 ± .17	158 ± 2	3.33 ± .32	53.7 ± 3.7	52.2 ± 5.6	169 ± 13	63.9 ± 9.0	19.0 ± .8	+6.6 ± .3
	"	"	5	"	" "	4§	3.50 ± .16	216 ± 6	7.16 ± .30	3.0 ± .3	20.5 ± 4.3	111 ± 18	15.4 ± 3.0	12.1 ± .6	+1.4 ± .1
	"	Adrenalectomized	6	"	35 μg FA	0	2.38 ± .15	174 ± 6		31.4 ± 6.4	41.3 ± 3.4	141 ± 13	56.9 ± 8.5	15.9 ± .9	+3.2 ± .5
	"	"	4	"	" "	4§	3.08 ± .25	197 ±12		37.0 ± 5.4	55.3 ± 9.4	127 ± 8	28.2 ± 4.8	14.6 ± .6	+4.9 ± .3
III	♀	Intact	6	28–32	0.1 ml gel.	0	1.58 ± .27	145 ± 3	5.86 ± .39	75.5 ± 5.5	38.2 ± 2.5	3.38 ±.35	20.2 ± 2.7	14.8 ± .8	+3.4 ± .4
	"	"	6	"	" "	4§	3.08 ± .08	184 ± 5	8.34 ± .37	6.2 ± .4	21.8 ± 1.6	3.69 ±.38	27.7 ±13.8	13.4 ± .7	+1.3 ± .4
	"	Adrenalectomized	6	"	35 μg FA	0	2.03 ± .21	158 ± 3		49.7 ± 4.7	48.7 ± 8.1	2.53 ±.11	11.3 ± .7	13.4 ± .4	+1.7 ± .3
	"	"	6	"	" "	4§	2.75 ± .17	162 ± 7		69.8 ±15.1	48.0 ± 3.7	2.70 ±.26	11.2 ± .7	14.3 ±1.2	+2.8 ± .4

* Cortisol acetate (Merck).
† 16% gelatin vehicle for ACTH.
‡ Commercial highly purified ACTH in 16% gelatin vehicle (Armour "ACTHAR"). 60-80 units per mg. Necessary dilutions were made with 16% Knox gelatin solution.
§ Armour Porcine ACTH, Lot T-97308, approx. 110 U/mg and negligible contamination with the remaining anterior pituitary trophins, courtesy of Dr. J. D. Fisher.
‖ Mean ± S.E.

times and an average grade determined for each kidney. Glomerular changes generally were the most pronounced and conspicuous of the renal changes observed and accurately reflected the over-all effects of ACTH on the kidneys. Therefore the mean degree of morphological change in the glomeruli was used to summate and quantify the renal effects of ACTH. The glomeruli were graded on a basis of 0 for completely normal, or unaltered, to 5 for the most severely scarred, obsolete glomeruli.

Results. Data from the 3 experiments are summarized in Table I which gives means and standard errors of the means for various measurements made. Severity of renal changes is similarly expressed. The renal lesions produced in mice by ACTH have been described elsewhere(1,2,5,6) and will be only summarized here. ACTH produces marked sclerosis of the renal glomeruli in intact mice. There is a marked scarring of the glomerular tuft with loss of capillary patency resulting from a great increase in intercapillary weakly PAS+ fibrillar-appearing material. Deposits of intensely PAS+, fibrinoid-like material occur in the intercapillary areas and sometimes in capillary lumens. Similar material is deposited in the juxtaglomerular region. Patent capillaries, usually at the periphery of the glomerular tuft, often are greatly dilated. Ultimately the glomerular tuft is destroyed. Typically there is not interstitial inflammation in the kidney. Characteristic tubular changes consist of vacuolation of the apical portions of the proximal epithelial cells, deposits of intensely PAS+ material at the bases of distal epithelial cells in proximity to glomeruli, and often large numbers of PAS+ granules, presumably resorbed protein, in the proximal epithelial cells. Other less consistent and less conspicuous alterations also occur(5).

Analyses of the data on severity of renal lesions are summarized in Table II. Several points of interest emerge from these analyses. There was a difference between experiments of dubious significance (Table IIA), and there were no over-all differences between adrenalectomized and intact males. ACTH produced marked glomerular damage in contrast to its effects on kidneys of mice not given ACTH. The mean severity of renal damage for each treatment group and its standard error for 3 experiments combined are shown in Fig. 1. When these means are

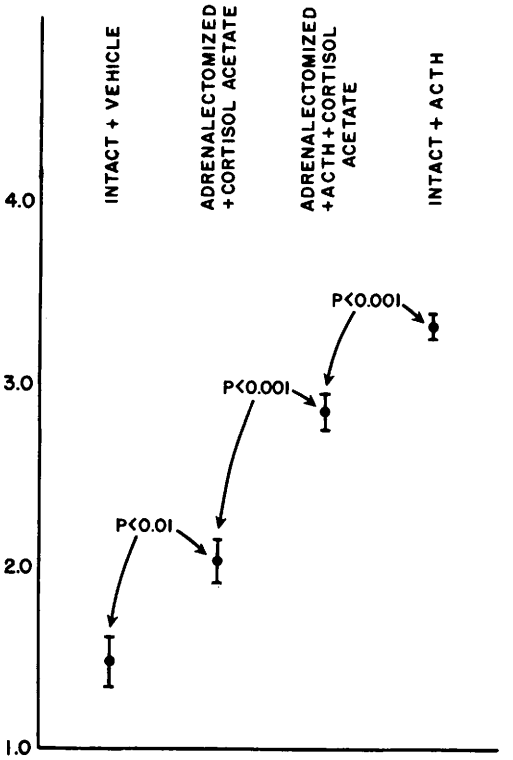

FIG. 1. Means of severity of renal disease and their standard errors in male mice from all 3 experiments. Only comparable treatment groups were used.

compared by "t" tests, the mean of each treatment group differs from every other at a level of significance of 1% or better.

Analysis of the data on renal lesions from only the ACTH-treated males from the 3 experiments (Table IIB, Fig. 1) indicated that there were no significant differences between experiments with respect to the effects of ACTH on the kidneys, but that those of intact mice were more severely affected than those of adrenalectomized males ($P<0.0001$). Analysis of data from mice not given ACTH (Tables I and IIC) indicates that despite a significant difference between experiments glomerular involvement was significantly greater in adrenalectomized mice given hydrocortisone than in intact controls. These re-

sults concur with those from females(5). Thus there was a stepwise progressive increase in glomerular damage going from inconsequential in intact controls, to perceptible in adrenalectomized males given F, thence to greater in adrenalectomized mice given F and ACTH, and finally to severe in intact mice given ACTH (Fig. 1). The renal lesions

TABLE II. Summaries of Analyses of Variance of Renal Glomerular Lesions Induced in Mice by ACTH for Each Source of Variance in Each Analysis of Variance. The one of the pair associated with the greater severity of renal glomerular disease is italicized.

Source of variance	d.f.	Mean squares	F	P
A. Intact and adrenalectomized males: all three experiments combined				
Between experiments	2	.912	2.59	.20>P>.005
No ACTH *vs ACTH*	1	44.367	126.04	<<.001
Intact *vs* adrenex* & cortisol	1	.079	.22	N.S.
Residual	94	.352		
B. Same, but only ACTH-treated males				
Between experiments	2	.28	1.90	N.S.
Intact vs adrenex & cortisol	1	2.62	17.82	<.0001
Residual	43	.15		
C. Same, but only non-ACTH-treated males				
Between experiments	2	1.39	3.61	<.05
Intact *vs adrenex & cortisol*	1	4.10	10.57	<.01
Residual	48	.39		
D. Exp. II only; intact, castrate and adrenalectomized males				
Non-castrate *vs castrate*	1	.999	6.75	<.05
Non-adrenex vs adrenex	1	4.522	30.55	<<.0001
No ACTH *vs ACTH*	1	28.981	195.82	<<.00001
Residual	51	.148		
E. Exp. II only, only ACTH-treated males				
Non-castrate *vs* castrate	1	.24	1.67	N.S.
Non-adrenex vs adrenex	1	4.90	34.03	<.0001
Residual	23	.144		
F. Exp. II only, adrenex males only				
Non-castrate *vs* castrate	1	.04	.14	N.S.
No ACTH *vs ACTH*	1	6.65	23.83	<.001
Residual	22	.28		
G. Exp. II only, non-adrenex males only				
Non-castrate *vs castrate*	1	.933	14.09	<.001
No ACTH *vs ACTH*	1	22.707	343.00	<<.00001
Residual	27	.066		
H. Exp. III only, male and female mice combined				
Males vs females	1	.973	4.05	=.05
No ACTH *vs ACTH*	1	13.508	56.28	<<.00001
Non-adrenex *vs* adrenex	1	.008	.03	N.S.
Residual	41	.240		
I. Exp. III only, males and females, ACTH-treated only				
Males vs females	1	.800	6.32	<.05
Intact vs adrenex	1	.808	6.36	<.05
Residual	18	.127		
J. Exp. I & II (50 μg cortisol acetate per day) *vs* III (35 μg cortisol acetate) males only				
Exp. I *vs* Exp. II & III combined	1	.69	2.30	N.S.
No ACTH *vs ACTH*	1	8.19	27.30	<<.0001
Residual	46	.30		

* Adrenalectomized.

produced by ACTH in intact males were significantly more severe than those in adrenalectomized males. Also, ACTH produced a very similar degree of renal damage in intact males and females (Tables I and IIH and I). The mean severity of renal damage in adrenalectomized mice was similar for both sexes. The small, but significant, differences between males and females in the degree of glomerular involvement (Tables IIH and I) result from a difference in the base line or control levels of glomerular alteration. Increments in severity of renal disease over control values were nearly identical for both sexes in every treatment group (Table I).

A separate analysis of data from Experiment II only (Tables I and II, D-G) suggests that castration adversely affected the kidneys. However, this was true only in non-adrenalectomized males (Table IIG) and castration had no effect on the severity of renal disease in adrenalectomized mice (Table IIF) or in ACTH-treated mice (Table IIC). Other results from Experiment II paralleled those from the 3 experiments combined.

The ability of a very highly purified preparation of porcine ACTH (110 I.U. per mg, 3d International Standard) to produce renal disease (Tables I, Exp. III, and IIJ) in male and female mice was explored in Experiment III. The 110 unit preparation resulted in renal lesions of severity equal to those produced by the 60-80 unit preparation. The purer 110 unit preparation has "neglibible contamination with the remaining anterior pituitary tropins" (J. D. Fisher, personal communication). The similarity of action of 60-80 and 110 I.U./mg ACTH on the kidneys suggests that these effects are an inherent property of the ACTH molecule itself. This interpretation is supported further by the fact that β^{1-24} synthetic corticotrophin has similar activity in approximately equivalent dosages(6).

In addition to its effects on the histology of the kidneys, ACTH affected other organs and somatic growth (Table I). It decreased growth as indicated by lower terminal body weight ($P<0.001$). Adrenalectomy plus F in the doses used had no significant over-all effect on body weight, but resulted in a significant decrease in weight in Exp. II ($P<0.001$). Testicular weight was unaffected by ACTH or by adrenalectomy and F, although ACTH significantly and appreciably reduced the weight of the seminal vesicles ($P<0.001$), suggesting that it has some degree of inhibitory action on reproductive function in male mice. To this limited extent ACTH affects reproductive function in male mice as it does in females(5). Adrenalectomy with F-treatment significantly increased weight of the seminal vesicles ($P<0.001$) (Table I).

As expected ACTH decreased thymic and splenic weights ($P<0.0001$ and <0.001, respectively) and increased adrenal weight ($P<0.00001$) (Table I), but it had no significant effect on the reproductive organs of females. Histology of the vaginae, uteri and ovaries was no help in explaining its lack of action on these organs.

Discussion and conclusions. Contrary to earlier results, ACTH clearly has less effect on the kidneys of adrenalectomized male mice maintained on hydrocortisone than on those of intact males. Furthermore, the degree to which ACTH affects the kidneys of intact or adrenalectomized male mice is the same, respectively, as in intact or adrenalectomized females. It is clear that ACTH produced marked glomerular lesions in intact and adrenalectomized mice of both sexes.

Castration did not alter the effect of ACTH on the kidneys of males, but resulted in slightly more glomerular pathology in intact non-ACTH-treated mice. Castration had no effect in adrenalectomized or ACTH-treated intact males. Possibly the operative trauma of castration and sham-adrenalectomy increased endogenous ACTH secretion sufficiently to account for the increase in severity of glomerular lesions in sham-adrenalectomized male mice. Castration could have had an effect in otherwise intact ACTH-treated mice that was overridden by the greater effects of ACTH. The analyses (Table IID, E, F) suggest such an interpretation, as castration had an over-all effect of increasing the severity of renal disease ($P<0.05$), significantly increased severity of non-adrenalectomized

males ($P<0.001$) and approached having a significant effect in ACTH-treated mice (Table IIE). There can be no question that castration reduced renal weight ($P<0.0001$).

It is difficult to explain the apparent lack of effect of ACTH on the female reproductive tracts in Exp. III, particularly the significantly lower ovarian and uterine weights in adrenalectomized mice, since these results are contrary to those from a large number of earlier experiments(3-5). The failure of ACTH to affect the female reproductive tract cannot be attributed to the lower daily dose of hydrocortisone for two reasons: First, the failure occurred in intact, as well as in adrenalectomized females, and second, hydrocortisone in the doses used does not affect female reproductive function(5).

Contrary to its lack of effect on reproductive organs of females, ACTH decreased reproductive function in males, in this experiment as indicated by the seminal vesicles, presumably by decreasing testicular androgen production. However, this action apparently is limited as testicular weight was unaffected. ACTH may have acted centrally to inhibit gonadotrophin secretion, as suggested by extrapolation from data for females(5).

Summary. In 3 experiments of balanced design the ability of ACTH to produce renal glomerular disease in intact and adrenalectomized male mice was determined. The effects of castration on ACTH-induced glomerulnephritis were studied and the disease in males and females compared. Adrenalectomized mice were given 35 or 50 μg of hydrocortisone-acetate daily. Four units of ACTH daily for 10 days was used. ACTH-induced glomerular lesions exhibit marked increases in intercapillary mesangial material and deposition of intensely PAS+ material in the mesangial matrix and axial capillaries, and peripheralization and ballooning of capillaries. Four I.U. of ACTH daily induced the severest glomerular disease in non-adrenalectomized males, less in adrenalectomized mice and was unaffected by the presence or absence of the testes. ACTH affected the glomeruli of intact and adrenalectomized male and female mice similarly with respect to control values. Two preparations of ACTH, one assaying 60-80 and the other 110 I.U. per mg, produced similar renal disease. It was concluded that ACTH affected both sexes equally; that its effect was greatest in intact and significantly less in adrenalectomized mice.

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Comparison of Biological Activity of Injected and Orally Administered L-Thyroxine, L-Triiodothyronine and Thyroprotein in Fowls.* (32390)

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A method for determination of the thyroxine secretion rate in fowls was presented some years ago(1). It involved the graded injection of L-thyroxine (L-T_4) until the release of thyroidal-I^{131} was blocked, indicating that the L-T_4 had stopped the further release of thyrotropic hormone (TSH). This

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