crease in total serum protein as initial alterations in nephrosis. In all instances, a definite interval of from one to 4 days following proteinuria occurred prior to development of hypercholesterolemia. The sequence of hypoproteinemia and then hypercholesterolemia, though established here, does not necessarily indicate a causal relationship between the two.

Summary. The sequence of development of hypoproteinemia and hypercholesterolemia was studied in 15 aminonucleoside nephrotic animals. Hypercholesterolemia succeeds rather than precedes hypoproteinemia.

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The Juxtaglomerular Apparatus as an Extra-Adrenal Site of ACTH Action.*† (26188)

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We have shown that a variety of hypotensive drugs are able to produce morphological changes suggestive of hyperfunction in the renal juxtaglomerular apparatus(1). These responses occur within minutes to a few hours, in contrast to the weeks or months required for appearance of changes reported by others(2,3,4) and summarized by Tobian(5, 6). In attempting to explain the rapid responses to drugs which we have seen, we studied the relationship between the adenohypophysis and the juxtaglomerular apparatus (JGA). This report will indicate that JGA responds quickly to ACTH in absence of the adrenals and that it might be considered as an extra-adrenal target of this hormone.

Materials and methods. All experiments were performed on young male albino rats of the Harlan strain, weight approximately 150 g. Hypophysectomized rats were supplied by the Hormone Assay Laboratories. Adrenalectomized animals were maintained on 1% saline drinking water in addition to their regular rations. Some groups of rats were adrenalectomized 3 days after hypophysectomy, and were maintained on 1% saline drinking water plus their regular rations. In acute experiments, animals were used on the fourth post-operative day.

Drugs were administered intraperitoneally in doses indicated in the tables. The commercial ACTH used was Parke-Davis' brand of corticotropin. A specially purified ACTH which was virtually free of other endocrine activity was also used.‡ Other drugs used were from regular commercial sources. For hydralazine the solvent was 10% propylene glycol. Other drugs were dissolved in saline.

Radial wedges of kidney were removed at time of sacrifice, fixed in formol-sublimate-acetic acid fixative, and embedded in paraffin. Sections were cut at 6 μ and were stained by a combined Alcian blue-PAS technic. The method consisted in staining first

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‡ Purified ACTH virtually free of other endocrine activity was supplied by research division of Parke-Davis Co.

with 0.15% aqueous Alcian blue for 30 minutes, followed by an alkaline alcohol wash for 1.5 hours. Next a conventional, periodic acid-Schiff stain was applied to the section. The method results in nuclei and cytoplasm stained faint green, brush border and basement membranes purple, and JG granules bright red. This provides better visualization of the granules than the PAS stain applied alone.

Degree of granulation was estimated in the juxtaglomerular apparati associated with 100 glomeruli from each animal. A weighted granulation index was calculated according to the method of the Hartrofts(7). Normal, solvent-injected, and sham-operated control groups were used. Each experimental and control group consisted of 6 animals. Experimental and appropriate control groups were compared statistically and reported as not significant if the P-value of the difference between means was 5% or more.

Results. The weighted granulation indices of 3 groups of control animals (Exp. 1, 2, 3) of Table I are not very different from one another, and agree closely with the control values reported by others(3). Removal of the adrenals produces significant elevation of JG granulation, as was reported by Dunihue(2), but this elevation does not occur in hypophysectomized animals (Exp. 4 and 5, Table I). Hypophysectomy alone produces no important change in the functional appearance of the JGA in a few days, but if the animals are maintained for 6 weeks a significant hypogranulation results, as shown in Exp. 6 and 7. The remaining experiments listed in Table I demonstrate that ACTH, both commercial and specially purified, produces marked hypergranulation of the JGA in intact rats, in adrenalectomized rats, and in hypophysectomized-adrenalectomized rats.

TABLE I. Effects of ACTH on Renal Juxtaglomerular Granulation in the Presence and Absence of Adrenals.

Exp. No.	Treatment	WGI*	P
1	Normal control	34 ± 3.5	
2	Sham-operated	39 ± 5.6	n.s.
3	Saline injected	37 ± 4.5	"
4	Adrenalectomized 4 days	51 ± 4.6	<5%
5	Hypophysectomized, then adrenalectomized 4 days	37 ± 1.3	n.s.
6	Hypophysect. 4 days	31 ± 5.2	"
7	" 6 wk	19 ± 4.2	<5%
8	ACTH 5 units i.p., 1.5 hr	95 ± 3.4	<1%
9	Adrenalect. 4 days then ACTH 5 units i.p., 1.5 hr	86 ±12	<2%
10	Adrenalect. 4 days, pure ACTH 5 units i.p., 1 hr	68 ± 4.2	<5%
11	Hypophysect.-adrenalect., then ACTH 5 units i.p., 1.5 hr	80 ±12	<1%

* Weighted granulation index ± stand. error.
n.s. = not significant.

TABLE II. Effects of Hypophysectomy on JGA Changes Produced by Drugs.

Exp. No.	Treatment	WGI*	P
1	Normal control	34 ± 3.5	
2	Hydralazine solvent control 0.5 cc i.p., 35 min.	39 ± 6.3	n.s.
3	Hypophysectomized, 4 days	31 ± 5.2	"
4	Hydralazine 25 mg/kg i.p., 35 min.	77 ± 3.2	<5%
5	Hypophysect. 4 days then hydralazine 25 mg/kg i.p., 35 min.	38 ± 5.2	n.s.
6	Vasopressin 1.25 units i.p. q 2 days × 3	77 ± 4.5	<5%
7	Hypophysect. 4 days then vasopressin 1.25 units i.p. q 2 days × 3	30 ± 3.2	n.s.

* Weighted granulation index ± stand. error.
n.s. = not significant.

Table II gives additional evidence for participation of the pituitary in the responses leading to morphological changes in renal JGA. The hypergranulation produced by such varied drugs(1) as hydralazine (Exp. 4) and vasopressin (Exp. 6) is seen to be completely prevented by prior hypophysectomy (Exp. 5 and 7).

Discussion. The action of ACTH in producing alterations of the JGA was first shown by Dougherty(8), who attributed this to an effect of the hormone on the adrenal cortex. It has also been shown that removal of the adrenals produces a hyperfunctional state of the JGA(2). Our results show that the effect of adrenalectomy upon the JGA cannot be a direct one, since it is prevented by prior removal of the pituitary. The effect is better explained by the increased circulating corticotropin level that follows adrenalectomy.

Garber *et al.*(1) have also shown that a variety of methods of raising ACTH level produce rapid alteration of the JG granulation.

The results of the experiments on the effects of ACTH, hydralazine, and vasopressin in the adrenalectomized and/or hypophysectomized animals indicate that ACTH has a direct tropic effect on the renal juxtaglomerular apparatus that is independent of the presence of the adrenals. The possibility that additional adenohypophyseal principles also affect the JGA has not been ruled out.

These observations may help to explain the diversity of procedures that have been shown to produce significant morphological changes in the JGA(2,3,4). These include alteration of salt balance, of adrenal function, of blood pressure. Finally, in connection with the recent suggested localization of renin to the JGA(9), Haynes *et al.*(10) found that ACTH stimulates renin release from the kidney, and that this action of ACTH also occurs in adrenalectomized animals. This is consistent with our suggestion that the JGA may be a target structure for the direct action of ACTH.

Summary. ACTH produces hypergranulation of the renal juxtaglomerular cells even in absence of pituitary and adrenal glands, and the chronic absence of ACTH depletes juxtaglomerular granules. The renal effect of vasopressin and of hydralazine, substances which produce hypergranulation of juxtaglomerular cells, is blocked by hypophysectomy. These findings support the concept that ACTH mediates many of the responses of the juxtaglomerular cells, and that the juxtaglomerular apparatus is an extra-adrenal site for the direct action of ACTH.

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An Improved *in vitro* Method for Determination of Serum "Insulin-Like" Activity. (26189)

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Accurate measurement of the insulin or "insulin-like" activity of biological fluids has been one of the major problems confronting endocrinological investigators. Much progress has been made since the introduction of the method utilizing uptake of glucose by the rat diaphragm. Vallance-Owen and Wright (1) have recently reviewed the available procedures. Martin *et al.*(2) have reported that the $C^{14}O_2$ production from glucose-1-C^{14} by the rat epididymal fat body was useful. Recently Beigelman(3) and Humbel(4) have utilized uptake of glucose by the epididymal fat body to measure insulin-like activity of human serum. In our studies we have modified the experimental design of the method using glucose uptake by epididymal tissue. This has resulted in a marked increase of accuracy and made the method readily amenable to statistical analysis. Although the present method was primarily designed to study the effects of various agents on the action of insulin, it should be extremely useful in determining insulin or "insulin-like" activ-