with 2% cholestyramine was 13.2 g in 4 weeks compared with a loss of 5.4 g in the control group. Only in this group were fatty acids of dietary origin found in the feces in increased amounts during cholestyramine administration.

In the mature rats, 2% cholestyramine decreased fat retention by about 5% in animals on the chow diet. In animals switched to synthetic diets, the loss of fat during the first week on cholestyramine was quite marked, but there was little effect during the second week. These results may have been due in part to adjustment to the synthetic diets, since little effect of cholestyramine on fecal fat loss was found in those groups fed the synthetic diets for 2 weeks before cholestyramine was added to the diets.

The incorporation of cholestyramine as 2% of the diet provided 2 and 1 g of cholestyramine per kg of body weight per day in the young and mature rats, respectively. In man, the daily dose of 12 to 15 g of cholestyramine (used in lowering plasma cholesterol) provides about 0.2 g of cholestyramine per kg per day. Thus, fat utilization is decreased by cholestyramine both in the young rat and in man(4) when given at high dose levels and especially with high levels of dietary fat.

Summary. In the young weanling rat, 2% cholestyramine in the diet increased the loss of fecal fat, particularly in diets of high fat content. Administration of cholestyramine to the mature rat resulted in a moderate decrease in fat retention, but after one week the effect was minimal.

1. Carey, J. B., Jr., J. Clin. Invest., 1961, v40, 1028.
2. Bergen, S. S., Jr., Van Itallie, T. B., Tennent, D. M., Sebrell, W. H., Circulation, 1959, v20, 981.
3. Tennent, D. M., Siegel, H., Zanetti, M. E., Kuron, G. W., Ott, W. H., Wolf, F. J., J. Lipid Res., 1960, v1, 469.
4. Hashim, S. A., Bergen, S. S., Jr., Van Itallie, T. B., Proc. Soc. Exp. Biol. and Med., 1961, v106, 173.
5. Hyun, S. A., Vahouny, G. V., Treadwell, C. R., ibid., 1963, v112, 496.
6. Kelley, K. L., Lehman, E. G., Robinson, H. J., Fed. Proc., 1963, v22, 434, abstr.
7. Assn. of Official Agricultural Chemists Official Methods of Analysis, edit. 9, Washington, D. C., 1960, p161.
8. Smith, L. M., J. Dairy Sci., 1961, v44, 607.
9. Saret, H. P., Snipper, L. P., J. Nutrition, 1954, v52, 525.
10. Jones, J. H., Foster, C., ibid., 1942, v24, 245.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Effect of Aldosterone on Juxtaglomerular Index and Zona Glomerulosa of Normotensive and Hypertensive Rats.* (29857)

EDWIN R. FISHER AND M. TAMURA
Departments of Pathology, University of Pittsburgh and VA Hospital, Pittsburgh, Pa.

Evidence has been accumulated which indicates that renal hypertension results from increased activity of the renin-angiotensin-aldosterone mechanism. The precise mechanism of action of angiotensin II and aldosterone in this regard is less certain and in some instances increased secretion of both substances has not been accompanied by hypertension(1,2). Feedback control of this system is considered to be mediated by the depressor effect of aldosterone's sodium retaining properties on the juxtaglomerular apparatus of the kidney, the site of renin formation(3). However, rabbits pretreated with aldosterone exhibit a lesser pressor response to angiotensin infusions than controls(4). Although angiotensin has been noted to cause an increase in renal renin in rats, this effect is prevented when aldosterone is also administered(5). This has suggested that the latter may have a direct blocking effect on angiotensin(4). In an attempt further to explore this possibility, it was considered worthwhile

* Supported by USPHS Grant C-5195.

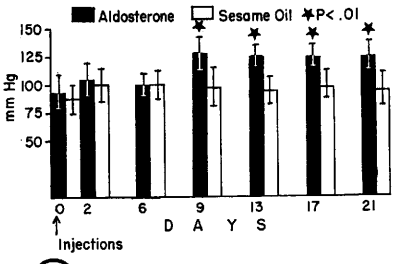
to investigate the effect of aldosterone on certain parameters of the renin-angiotensin-aldosterone mechanism in rats with renal hypertension induced by unilateral renal artery constriction. This model appeared pertinent since it has been demonstrated previously that renal hypertension in the rat appears unaffected by alteration in sodium state although the degree of granularity of juxtaglomerular cells, a reliable index of renal renin content, reflects electrolyte composition in this situation(6).

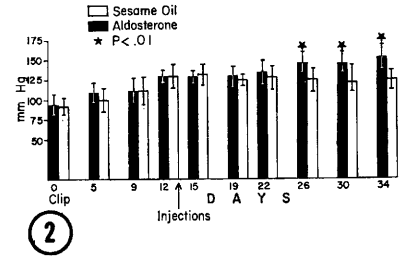
Methods. Fifty-two adult female Wistar rats weighing 115-125 g survived the experiment. Thirty-four were subjected to partial constriction of the left renal artery. All exhibited elevation of blood pressure within 12 days following operation, at which time 19 received daily subcutaneous injections for 3 weeks of 0.5 mg of crystalline d-aldosterone acetate (Ciba) suspended in sesame oil. The remaining 15 were injected with sesame oil only. Groups of 9 unoperated normotensive rats received similar injections of aldosterone and sesame oil.

Animals were housed in individual cages and maintained on Wayne Lab Blox and tap water *ad lib.* Blood pressure was estimated by a microphonic method prior to and twice weekly after operation and/or injections of aldosterone or sesame oil.

All rats were sacrificed 3 weeks after the initiation of injections by aortic puncture. Kidneys were fixed in Helly's fluid and sections stained for estimation of juxtaglomerular index (JGI) according to the method of Hartrofts(7). Heart and adrenals were blotted and weighed. Frozen sections of the latter were stained with oil red O after formalin fixation and width of zona glomerulosa expressed as percentage of cortex (ZGI). Portions of liver, heart and gastrointestinal tract were also fixed in formalin, dehydrated and imbedded in paraffin in the usual manner. Sections were stained with azure-eosin. Serum Na, Cl, K and hematocrit were determined as previously described(6).

Results. Aldosterone-treated normotensive, unoperated rats exhibited moderate elevation of blood pressure (25-30 mm Hg) after 9 injections (Fig. 1). Comparable elevation of





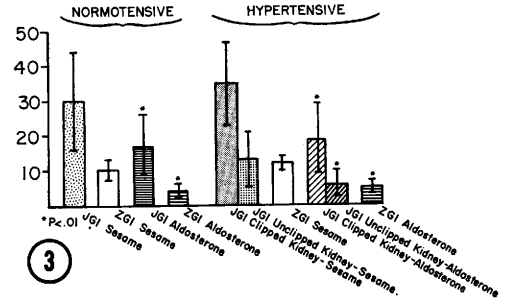


FIG. 1. Effect of aldosterone on blood pressure of normotensive rats.

FIG. 2. Effect of aldosterone on blood pressure of rats with renal hypertension.

FIG. 3. Effect of aldosterone on JGI and ZGI of normotensive and hypertensive rats.

blood pressure was noted after 13 injections of aldosterone in rats with renal hypertension (Fig. 2). Injections of sesame oil were without significant effect on blood pressure in both groups.

JGI and ZGI were significantly less in aldosterone-treated normotensive rats than in controls receiving sesame oil only (Fig. 3). Similarly, JGI of clipped and untouched kidneys and ZGI of aldosterone-treated rats with renal hypertension were significantly less than that of hypertensive members treated only with sesame oil (Fig. 3).

Heart and adrenal weights were comparably elevated in aldosterone-treated and control hypertensive rats only (Table I). Vascu-

TABLE I. Effect of Aldosterone on Serum Na, K, Hematocrit (Hct) and Adrenal and Heart Weights of Normotensive and Hypertensive Rats.

	No.	Na	K	Hct	Adrenal wt (mg)	Heart wt (mg)
Normotensive						
Aldosterone	9	154 ± 4*†	4.1 ± .4*	44 ± 3	47 ± 9	652 ± 70
Sesame oil (control)	9	144 ± 4	5.5 ± .7	45 ± 6	51 ± 9	616 ± 65
Hypertensive						
Aldosterone	19	145 ± 8	4.3 ± .7*	43 ± 6	63 ± 10	810 ± 30
Sesame oil (control)	15	140 ± 6	5.0 ± .8	45 ± 4	63 ± 15	850 ± 35

* P <.01 compared to control. † Standard deviation.

lar lesions were qualitatively and quantitatively similar in both aldosterone and sesame oil-treated hypertensive rats consisting of arteritis of hepatic hilar and intramural coronary arteries in approximately 20% of hypertensive rats. No morphologic alterations were noted in aldosterone-treated, normotensive animals.

Serum Na was significantly elevated in aldosterone-treated, normotensive rats and K was slightly, but significantly, decreased in these latter as well as aldosterone-treated hypertensive members (Table I). No significant change in hematocrit or serum Cl was noted in any group.

Discussion. This study indicates that aldosterone administration results in moderate, but significant, elevation of blood pressure in rats not sensitized to steroid activity by unilateral nephrectomy and salt overload. Similar doses of aldosterone have been observed by Gross *et al*(8) to produce more marked hyperpiesis in rats primed by these modalities. The comparable elevation of blood pressure observed following aldosterone administration in rats with renal hypertension is unlike the experience with angiotensin. Gaskell *et al*(9) observed rats with renal hypertension to be more sensitive to its pressor effects than normotensive controls. This finding militates against the possibility that aldosterone directly antagonizes angiotensin or stimulates angiotensinase activity in this situation. This contention is substantiated by the decreased JGI observed in aldosterone-treated, normotensive and hypertensive rats. Hyperplasia of juxtaglomerular cells has been observed in humans with hyperaldosteronism and angiotensinemia without hypertension(1). Renal renin and JGI were considered unaffected in rats in which a blocking action of aldosterone on angiotensin activity has been considered(5). We have no certain explanation for the lack of significant elevation of serum sodium in aldosterone-treated hypertensive rats except to note that this parameter of sodium state in other situations has not invariably coincided with apparent salt overload or depletion(6). Nevertheless, it appears more likely that the decreased JGI observed in rats treated with aldosterone results from the salt retaining properties of this hormone. This effect of sodium retention on JGI has been repeatedly noted. These interpretations are consonant with the view that aldosterone influences renin production in this form of renal hypertension by a "negative feedback" mechanism. Depression of ZGI noted in these experiments represents another instance in which this parameter and JGI exhibit parallel changes and is considered as further evidence concerning the mechanism of action of aldosterone in the renin-angiotensin-aldosterone system.

The lack of influence of aldosterone on the vascular lesions of renal hypertension indicates that this humoral factor plays little or no direct role in the pathogenesis of these changes.

Summary. Injections of aldosterone produced moderate, but significant, elevation of blood pressure in normotensive rats. A comparable elevation of blood pressure was noted in rats with existing renal hypertension produced by unilateral renal artery constriction. JGI and ZGI were decreased in kidneys

and adrenals from both aldosterone-treated groups. These findings are interpreted to indicate the indirect role of aldosterone in regulating renin production. Aldosterone failed to influence the vascular lesions of renal hypertension.

1. Bartter, F. C., Pronove, P., Gill, J. R., MacCardle, R. C., Am. J. Med., 1962, v33, 811.
2. Hickler, R. B., Lauler, D. P., Thorn, G. W., J. Clin. Invest., 1963, v42, 635.
3. Davis, J. O., J.A.M.A., 1964, v188, 1062.
4. Katz, Y. J., Moore, R. S., Velasquez, A. M., Tamosaitis, I. T., Science, 1963, v141, 725.
5. Katz, Y. J., Bernick, S., Patek, P. R., deHoyos, R., Moore, R. S., Physiologist, 1962, v5, 165.
6. Fisher, E. R., Klein, H. Z., Proc. Soc. Exp. Biol., and Med., 1963, v113, 37.
7. Hartroft, P. M., Hartroft, W. S., J. Exp. Med., 1953, v97, 415.
8. Gross, F., Loustalot, P., Meier, R., Acta Endocrinol., 1957, v26, 417.
9. Gaskell, J. R., Jones, R. K., Shapiro, A. P., Proc. Soc. Exp. Biol. and Med., 1964, v115, 179.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Pepsinogen I in Semen. Chromatographic Separation of Pepsinogen I from Human Semen.* (29858)

MAX J. SEIJFFERS,† LEON L. MILLER AND HARRY L. SEGAL
Departments of Medicine and Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Investigation of proteolytic enzymes in human semen led Lundquist and Seedorff to the discovery of a proteolytic enzyme active at low pH in seminal fluid(1). This enzyme was thought to be a pepsinogen and could be activated with acid to a pepsin with kinetics analogous to those described by Herriott(2) for porcine pepsinogen. The activity of the resulting pepsin resembled the peptic activity of human gastric juice very closely in proteolytic activity *vs* pH curve, in milk clotting activity, in lability towards weak alkali, and in stability at acid reaction. The unactivated pepsinogen in semen showed no milk clotting activity; such activity was readily elicited after prior incubation with acid. Lastly, from semen the above investigators crystallized a material which was indistinguishable from crystalline swine pepsin. Investigation pointed to the seminal vesicles as the organ of secretion(3).

Until recently it was generally assumed that peptic activity in human gastric juice was ascribable to a single pepsin, derived from a single pepsinogen. However, column chromatographic fractionation on DEAE (diethylaminoethyl)-cellulose has enabled us to show recently that at least 3 closely related pepsins(4), derived from separate pepsinogens(5), account for the proteolytic activity in human gastric juice. In view of the reported closely similar behaviour of the proteolytic enzymes secreted by gastric mucosa and by seminal vesicles(4) it was of interest to determine whether this resemblance could be confirmed by chromatographic separation of the pepsinogens from seminal plasma. This report presents a comparison of the chromatographic behaviour of human seminal pepsinogen with that of human gastric mucosal pepsinogens and of human gastric pepsins with that of seminal pepsin, respectively.

Methods. Human semen was obtained by masturbation from 5 healthy unmarried individuals, aged 22-24 years. The five specimens were pooled and diluted to 150 ml with 0.1 M acetate buffer pH 5.3. This solution was dialyzed *vs* 0.1 M acetate buffer pH 5.3 at 4°C and thereafter filtered through filter paper (Eaton and Dikeman #615). The filtrate was applied to a DEAE-cellulose column (v. infra).

* This study was supported by USPHS Grant CS-9546 (C-4) and USPHS Training Grant 5177-06.

† Present address: Dept. of Medicine A, Hadassah Hebrew Univ. Hosp. and Med. School, Jerusalem, Israel.