estrogen plus androgen—20 mg testosterone/day plus 1 mg estradiol benzoate twice weekly for 30 days.

There are no marked changes in calcium retention with any of the hormones used. With thyroid and cortisone administration the average balance became less negative, while there was no change with administration of insulin and ACTH. Even with administration of Vit. D there was no large change in calcium retention. The latter figures show that changes in calcium balance need not be large to be detected by the methods used.

Discussion. As summarized from our earlier studies, at the dosage used, estrogen caused only a slight increase in calcium retention. Only administration of androgen (or androgen plus estrogen, which was probably largely an androgen effect) caused any marked increase in calcium retention. There are some changes in calcium intake in individual subjects during different periods, but these are not large, and average intakes were very close, during periods in which each hormone was studied. There are also some variations in calcium balance for individual subjects during various base periods, which are in some instances nearly as great as changes caused by hormone administration. Accordingly, it cannot be said that any hormone except testosterone will cause a significant increase in calcium retention. However, it can be definitely stated that no hormone used will cause decrease in calcium retention.

1. Ackermann, P. G., Toro, G., *J. Gerontol.*, 1953, v8, 289.
2. ———, *ibid.*, 1954, v9, 446.
3. ———, *ibid.*, 1953, v8, 451.
4. Snapper, I., *Medical Clinics on Bone Disease,* Ed. 2, N. Y., 1949.
5. Kountz, W. B., Ackermann, P. G., Kheim, T., *J. Clin. Endocrin. and Metab.*, 1953, v13, 534.
6. Kountz, W. B., *Ann. Int. Med.*, 1951, v35, 1055.
7. Kountz, W. B., Kheim, T., Ackermann, P. G., Toro, G., *J. Am. Ger. Soc.*, 1956, v4, 1005.
8. Ackermann, P. G., Toro, G., Kountz, W. B., Kheim, T., *J. Gerontol.*, 1954, v9, 450.
9. Kountz, W. B., Hofstatter, L., Ackermann, P. G., *ibid.*, 1951, v6, 20.

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Experimental Production of Nephrotic Syndrome Following Renal Vein Constriction in Rats.* (23890)

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Development of a nephrotic syndrome as a result of renal vein thrombosis has been recognized since 1840. However, recurrent, isolated case reports since have made no lasting impressions until recently, when the clinical syndrome has been ably described and relevant literature reviewed in at least 3 excellent articles(1,2,3). The syndrome does not seem to have been reproduced experimentally by occlusion of renal veins in dogs and rabbits. This may be because many of these experiments were of brief duration and performed under severe conditions(1,4). The present report describes a syndrome of massive proteinuria, hypoproteinemia and hypoalbuminemia, hypercholesterolemia, mild hypertension and ascites elicited in rats by sub-total ligation of one renal vein and subsequent contralateral nephrectomy.

Methods. Male Sprague-Dawley rats were used. Experiments are summarized in Table I. The first set consisted of effect of unilateral complete occlusion of left renal vein. The second consisted of the effect of varying degrees of incomplete occlusion of this vein, at a site between kidney and entry of adrenal vein. Partial occlusion was effected by tying a silk ligature firmly around the vein, enclosing a stylet, subsequently withdrawn. The

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TABLE I. Outline of Experiments.

Series	Group	No. rats	Operation	Result
I	1	10	Complete occlusion left renal vein	Partial or complete necrosis
II		14	Partial occlusion	Moderate atrophy, mild proteinuria (Groups 1, 2)
	1	6	Stylet .45 mm	
	2	4	" .7 "	
	3	4	" .85 "	
III			Partial occlusion, later contralateral nephrectomy	
	1	13	Stylet .45 mm; mean wt 94 g	5 survivors: nephrotic syndrome
	2	28		
	a.	9	Stylet .7 mm; mean wt 89 g	6 survivors: 3 with severe proteinuria
	b.	9	" .7 " " 143 g	In effect, "sham-operations"
	c.	10	" .7 " " 244 g	5 survivors: 2 with severe proteinuria

third set of experiments consisted of incomplete occlusion of left renal vein as above and right nephrectomy, 11 to 21 days later. The observations included estimates of body weight, fluid intake, urine flow, proteinuria, arterial pressure and, at end of experiment in Series III, collection of blood for analysis of serum proteins (total and differential), total cholesterol and blood urea nitrogen. At autopsy, kidney and heart were removed for weighing and histological studies.

Results. Complete occlusion of one renal vein (Series I). This operation caused transient proteinuria (to 450 mg /24 hours) with concurrent massive hematuria which subsided on 3rd day; some rats showed mild fluctuant hypertension. The animals were killed on 15th post-operative day; the left kidney was atrophic (mean ratio left/right kidney weight 0.45). Kidneys which had developed collateral venous circulation showed partial cortical necrosis with signs of tubular regeneration; however, medullae were diffusely necrotic and kidneys not functional. There was no mortality. No blood chemical analyses were done.

Partial occlusion of one renal vein (Series II). These experiments were done primarily to discover a degree of partial occlusion which would be compatible with survival after contralateral uninephrectomy. Fourteen rats each weighing 157-200 g were divided into 3 groups in which stylets respectively of .45, .7 and .85 mm diameter were used (Table I). The procedure provoked transient mild proteinuria in most animals of the 2 groups with greater degrees of occlusion; some had persistent proteinuria to 150 mg daily to the time of sacrifice on 15th day. Means of arterial pressures were not significantly increased, although individuals among the first 2 groups showed transient hypertension to 175 mm Hg. The ratios of left/right kidney weight were respectively 0.43, 0.7 and 1.04 in the 3 groups. Correspondingly, kidneys of the 0.85 mm group were histologically normal, whereas kidneys of groups with more intense venous occlusion showed varying degrees of focal necrosis, tubular atrophy and thickening of basement membranes.

Partial occlusion of one renal vein with subsequent contralateral nephrectomy (Series III). Results of Series II indicated that stylets of 0.45 and 0.7 mm were presumably optimal. Rats in Group 1 of Series III weighed 60-104 g. Functional changes after venous occlusion (0.45 mm) corresponded to those described in above paragraph. Eleven days later contralateral nephrectomy was done; 8 rats became oliguric and died during the first post-operative week, showing intense renal atrophy. The 5 survivors showed no immediate symptoms; urine flow and proteinuria persisted at preoperative levels for 4 days; then there began a rise in proteinuria to maxima ranging from 200 to 300 mg daily at 8th to 10th day. The experiment was terminated on 13th day after nephrectomy (Fig. 1). Arterial pressure rose immediately after nephrectomy and hypertension persisted at

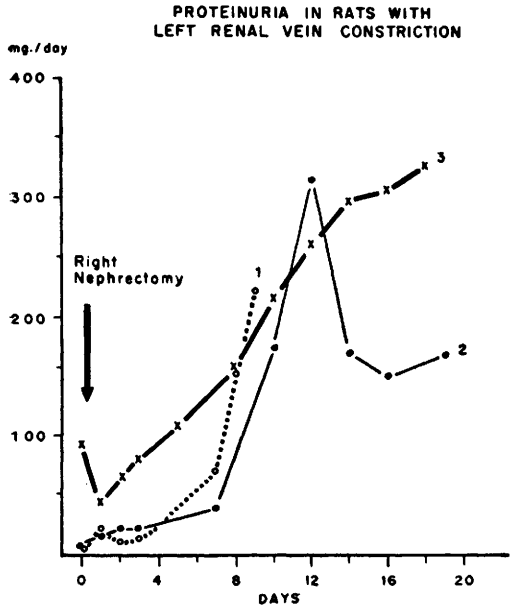

FIG. 1. Proteinuria in Series III. Curve 1, group 1; curve 2, group 2a; curve 3, group 2c.

levels of 188 and 195 mm Hg in 2 rats, while in 3 the hypertension remitted. Serum urea nitrogen and cholesterol increased, while serum protein concentration decreased (Table II). Electrophoretic analyses of serum proteins demonstrated hypoalbuminemia, with increases in alpha and beta globulins but no definite changes in gamma globulin. At autopsy 3 rats had gross ascites, but no peripheral edema. Kidneys of all were large, pale and adherent to abdominal wall, with formation of venous collaterals through the capsule and from the segment of vein lying between ligature and kidney. Increased average heart weight reflected the pre-existing hypertension.

Group 2 consisted of 28 rats divided into 3 sub-groups of different body weights. In *sub-group 2a* contralateral nephrectomy was performed 21 days after partial venous occlusion, at which time urine flow, proteinuria and blood pressure were substantially normal. Three rats died of uremia during the following week; among survivors, 3 showed rapid increases of urinary protein at about 7th day, to maxima of about 300 mg daily at about 12th day; proteinuria persisted at lower levels to end of experiment on 23rd day after nephrectomy (Fig. 1). Blood pressure was not significantly affected. Serum urea nitrogen and protein concentrations were normal, but cholesterol was increased (Table II). At autopsy there was no ascites; the kidneys, although not enlarged, were pale.

In sub-group 2b nephrectomy was performed on 12th day after venous occlusion. Animals were observed for 20 days afterwards.

TABLE II. Chemical and Morphological Changes after Renal Vein Occlusion (Series III).

Group	No. rats	Serum proteins: Total, g/100 ml	Differential in %: Albumin	α-globulin	β-globulin	γ-globulin
1	4	3.01 (2.00–3.65)	29.1 (26.3–32.6)	24.1 (23.8–24.4)	27.4 (23.2–31.2)	18.8 (15.7–24.8)
2a.	3	6.30 (5.59–6.97)	49.9 (43.6–55.8)	16.8 (14.4–19.9)	18.2 (15.4–20.5)	15.1 (14.4–16.0)
b.	7	6.52 (6.25–7.00)	42.4 (39.2–47.3)	16.8 (13.2–21.9)	24.1 (18.8–29.6)	16.7 (12.5–20.2)
c.	2	6.58 (6.40–6.75)	50.3 (49.7–50.8)	12.8 (11.6–13.9)	19.0 (16.6–21.3)	18.1 (17.4–18.7)

Group	No. rats	Cholesterol, mg/100 ml	BUN, mg/100 ml	Kidney, % of body wt	Heart, % of body wt
1	4	91 (81–113)	106 (39–208)	.84 (.69–1.00)	.34 (.28–.40)
2a.	3	100 (83–119)	35 (34– 38)	.62 (.57– .71)	.27 (.26–.29)
b.	7	67 (61– 71)	33 (29– 37)	.64 (.55– .73)	.31 (.22–.36)
c.	2	136 (111–160)	28 (27– 28)	.61 (.60– .61)	.27 (.26–.27)

None showed significant changes. In effect, these represent unintentional "sham-operations."

Sub-group 2c was subjected to right nephrectomy 14 days after partial renal venous occlusion. Five rats died during following week. Of survivors, 3 showed no significant changes. Starting about 3rd day after nephrectomy, 2 developed proteinuria, with maxima of 250 and 660 mg daily at 13th day; it persisted until end of experiment 20 days later. Neither of these showed hypertension, azotemia or significant changes in serum total protein fractions; both showed hypercholes-

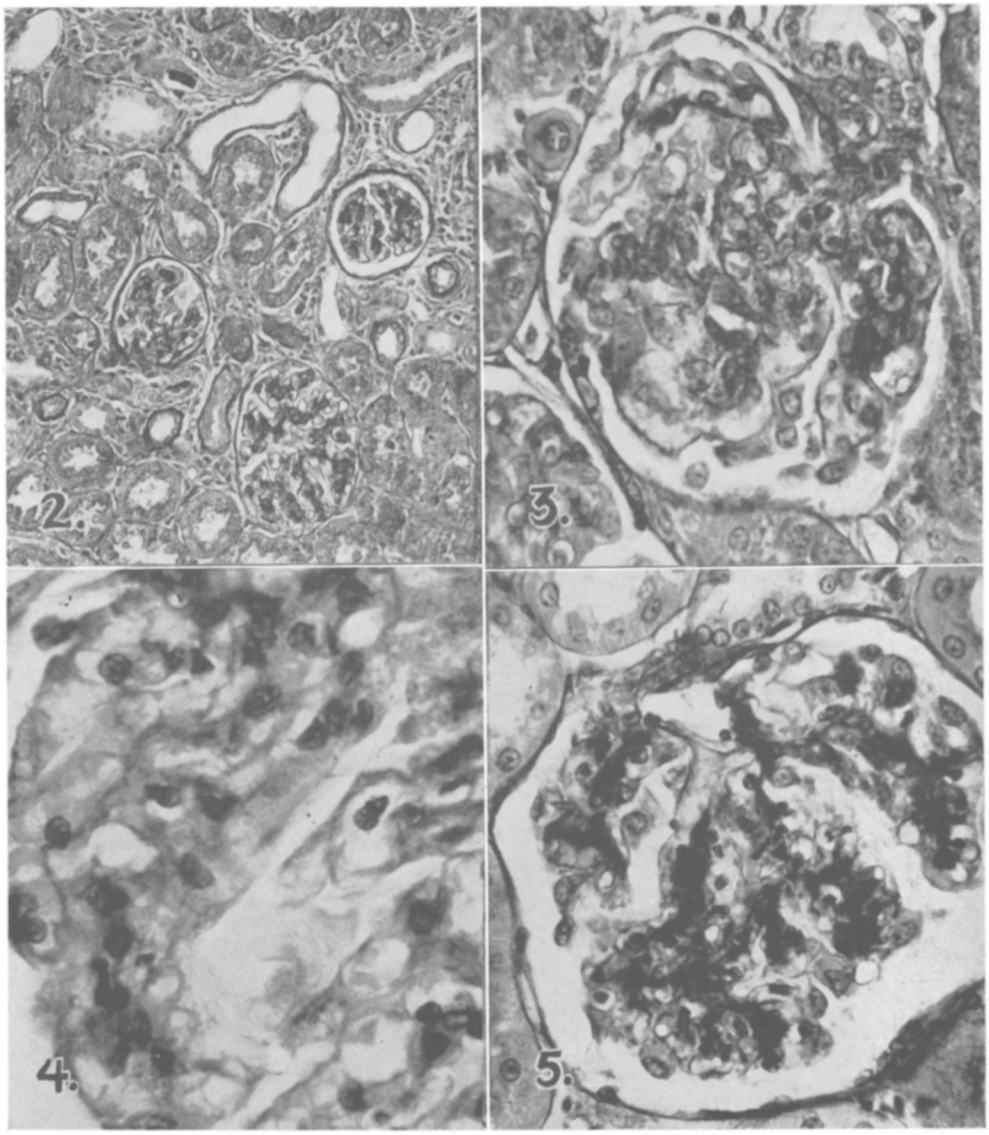

FIG. 2. Kidney showing interstitial fibrosis, tubular atrophy and thickening of basement membranes. PAS, × 160.

FIG. 3. Glomerulus from above kidney showing coalescence of tufts and proliferation of endothelial cells. PAS, × 475.

FIG. 4. Glomerulus showing disruption of normal architecture associated with thickening and fraying of membranes. PAS, × 1000.

FIG. 5. Glomerulus showing marked deposition of PAS-positive material and compactness of tufts. PAS, × 475.

terolemia (111 and 160 mg/100 ml). At autopsy there was no ascites; kidneys were pale.

Histologic findings. The following summarizes observations in the 8 rats which developed severe proteinuria and other aspects of nephrotic syndrome. With one exception, in which there was evident interstitial fibrosis and tubular atrophy (Fig. 2) kidneys appeared normal under low power, showing only a few hyalin or granular tubular casts. Under high power, the only significant changes were found in the glomeruli. These showed condensation and simplification of tuft patterns, coalescence of tufts and fraying and thickening of basement membranes (Fig. 4); glomeruli from animal with interstitial fibrosis showed in addition endothelial proliferation (Fig. 3). Some glomeruli contained deposits of "Schiff-positive" material (Fig. 5). These changes resemble those in clinical cases of nephrotic syndrome, occurring idiopathically or in association with renal vein thrombosis.

Summary. Among 41 albino rats subjected to partial occlusion of left renal vein with subsequent contralateral nephrectomy, 8 developed severe proteinuria and hypercholesterolemia, while 4 had also ascites and hypoalbuminemia. The kidneys were pale, with histological changes limited to glomeruli. These consisted of simplification of tufts, with coalescence, fraying and thickening of basement membranes. This technic requires further standardization, but seems to reproduce the clinical syndrome associated with renal venous thrombosis. To elicite the syndrome, venous occlusion must be incomplete and involve all functioning renal tissue.

ADDENDUM: Since completion of this manuscript, we found that G. Lagrue, B. N. Halpern, P. Milliez and A. Brannelec, *C. R. Soc. Biol.*, 1957, v151, 81, described a similar syndrome, although without noting edema or describing histologic appearance of kidneys, in rabbits subjected to bilateral sub-total occlusion of renal veins.

1. Pollak, V. E., Kark, A. M., Pirani, C. L., Shafter, H. A., Muehrcke, R. C., *Am. J. Med.*, 1956, v21, 496.
2. Harrison, C. V., Milne, M. D., Steiner, R. E., *Quart. J. Med.*, 1956, v25, 285.
3. Hasson, J., Berkman, J. I., Parker, J. G., Rifkin, H., *Ann. Int. Med.*, 1957, v47, 493.
4. Leger, L., George, L. A., *Presse Med.*, 1954, v62, 721.

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Physiologic Chloride Levels in Human Whole Saliva. (23891)

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Chloride concentration of human saliva has been shown to vary in certain systemic disease states(1,2). Dental research interest in salivary chlorides in relation to dental caries has stemmed primarily from the knowledge that chlorides enhance amylase activity and from reports that amylase activity of saliva from caries-active subjects is significantly higher than that from subjects free from carious lesions(3-6). There is diverse opinion as to significant differences in salivary amylase and chloride in subjects with caries activity(7-11) when compared to caries resistant subjects.

Our investigations involve multiple chemical analyses on saliva specimens collected from subjects presenting various oral pathologic states. Since there is considerable disagreement in the literature as to physiologic levels of some constituents under study, we instituted concurrent studies under identical experimental conditions to establish physiologic baselines.

The present study was designed to determine physiologic chloride levels under 2 different forms of stimulation. Also studied were effects of age and sex on chloride concentration and salivary flow rate and the effect of volume on chloride levels.

Procedure. All subjects had recently undergone thorough physical examination and each found to be fit. Times of arising and retiring,