tained by the Clark-Collip procedure but this bias was not significant at $p = 0.05$ by the t test(6). In a similar study of 21 urines, results by the chloranilate method averaged 1.3 mg/100 ml greater than those yielded by the Clark-Collip procedure. This difference was significant at $p = 0.05$ but not at $p = 0.01$ (t test). Similarly, results of fecal calcium were about 5 to 10% higher by the chloranilate method. The cause for these discrepancies is not apparent and it is impossible at this time to tell which method is more accurate. For most purposes, however, this difference is unimportant.

Reproducibility. The standard deviations (mg/100 ml), calculated from duplicate analyses, were as follows: 0.16 and 0.59 for the Clark-Collip method applied to serum and urine, respectively; 0.17 and 0.16 for the chloranilate method applied to serum and urine, respectively. This means that by the chloranilate method the precision (95% limits) of a single analysis of serum calcium was about $\pm$ 3.2% and that for a single analysis of urine calcium at a level of 25 mg/100 ml was about $\pm$ 1.5%.

Discussion. It is the authors' opinion that the chief advantage of the chloranilate method for the determination of serum calcium is the working time saved when many samples are to be run. The advantage of direct application to urine without preliminary ashing is obvious. The precision of the technic for both serum and urine is quite good and the results, in the case of serum, appear identical to those obtained by the Clark-Collip procedure. With urine and fecal samples, the results by the chloranilate method are slightly higher than those obtained by the Clark-Collip procedure.

Summary. The Ferro-Ham chloranilate procedure for determination of serum calcium has been investigated. Modifications of this procedure are presented for determination of urine and fecal calcium and ultramicro modification for determination of serum calcium is described. Presence of turbidity, apparently lipid in nature, in final solution constitutes a source of significant positive error in the chloranilate method for determination of calcium in serum. This turbidity can be removed by ether extraction. Results obtained by the chloranilate method have been compared with the Clark-Collip procedure.

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Effect of Some Hormones on Calcium Balance in Elderly Subjects.* (23889)

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Our earlier studies on calcium and phosphorus balance in elderly subjects have shown that calcium requirements are higher for such subjects than other workers found for young adults, with little change in phosphorus requirements(1,2). We later noted that addition of Vit. D to a supposedly adequate diet increased calcium retention in these subjects (3). Increased calcium requirements in the aged make it more likely that such subjects will be in negative calcium balance on an ordinary diet. The increase in incidence of osteoporosis in the aged has been noted by many workers(4). We have been studying the ef-

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fects of various hormones on nitrogen balance in elderly subjects for some time. Some cause an increase in nitrogen retention and definite clinical improvement(5,6). Use of these hormones has found a definite place in treatment of debilitated elderly subjects(7). In view of the precarious state of calcium equilibrium in such individuals, we felt it advisable to make further studies on the effect of these hormones on calcium balance, as any effect in decreasing calcium retention would be undesirable.

We present results on effect of thyroid, insulin, cortisone and ACTH on calcium balance. We also give a revised summary of some earlier work on the effect of sex hormones(8) and Vit. D(3) so that the data may be compared.

Material and methods. The subjects had been used in our earlier studies on calcium balance(1). All were free of acute or infectious diseases. Their ages were: DE—88 years, OS—70 years, KO—69 years, KI—72 years, and MC—76 years. Details of clinical status and general procedure of calcium balance determinations have been given(1). Details of diets, adjustment of intake and collection of specimens were the same as in our earlier work on nitrogen balance(9). The diet was similar to Diet No. 3 of this reference. Duplicate portions, each equivalent to all food offered for that day, were analyzed separately each day. Urine specimens, stool specimens and food left by each patient were pooled separately for 5-day periods for analysis. Calcium determinations were made with the Beckman Flame photometer using the procedure previously outlined(3). Calcium balance was determined for 6 to 8 weeks prior to administration of each hormone, and average calculated for this period. The hormone was then administered and calcium balance again determined. After administration of hormone was discontinued, an adjustment period, usually 2 weeks, was allowed before the base period for the next hormone administration was started. Dosages of hormones were: thyroid, 30 mg/day for 60 days; protamine zinc insulin, 10 units/day for 30 days; cortisone, 100 mg/day for 40 days; and ACTH, 30 mg/day for 30 days.

TABLE I. Changes in Calcium Balance with Hormone Administration. All values expressed as mg Ca/kg body wt/day.

	Before hormone admin.				During hormone admin.			
Patient	Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Bal.
	Thyroid admin., 30 mg/day for 60 days							
DE	15.3	1.3	16.9	−2.9	15.3	1.2	16.1	−2.0
KO	18.1	3.1	15.7	− .7	18.1	1.8	16.6	− .3
MC	15.6	1.4	18.1	−3.9	15.5	1.5	15.9	−1.9
KI	14.4	1.5	15.9	−3.0	15.5	1.6	15.0	−1.1
OS	14.6	1.9	13.7	−1.0	15.1	1.9	13.3	− .1
Avg	15.8	1.8	16.1	−2.3	15.9	1.6	15.4	−1.1
	Insulin admin., 10 units/day for 30 days							
DE	15.3	1.3	16.7	−2.7	14.8	1.4	15.3	−1.9
KO	18.8	1.6	16.4	+ .8	18.4	1.9	15.5	+1.0
MC	15.8	1.4	15.9	−1.5	16.0	1.4	16.6	−2.0
KI	15.1	1.5	14.7	−1.1	14.6	1.5	12.9	+ .2
OS	14.9	1.6	13.5	− .2	15.1	1.6	14.3	− .8
Avg	16.0	1.5	15.4	− .9	15.8	1.6	14.9	− .7
	Cortisone admin., 100 mg/day for 40 days							
DE	15.5	2.0	15.8	−2.3	15.9	2.7	15.5	−2.3
KO	17.9	4.0	15.3	−1.4	18.2	4.6	12.6	+1.0
MC	15.6	2.2	16.9	−3.5	15.2	2.6	15.1	−2.5
KI	14.9	1.9	17.0	−4.0	14.0	2.5	14.5	−3.0
OS	12.8	1.6	13.0	−1.8	13.5	2.2	12.3	−1.0
Avg	15.3	2.3	15.6	−2.6	15.4	2.9	14.1	−1.6
	ACTH admin., 30 mg/day for 30 days							
DE	14.8	2.1	14.1	−1.4	14.5	2.2	14.7	−2.4
KO	18.1	3.2	15.6	− .7	18.3	3.0	16.1	− .8
MC	15.5	2.3	16.6	−3.4	15.0	2.0	15.3	−2.3
KI	16.1	1.9	16.5	−2.3	15.5	1.8	15.6	−1.9
OS	12.8	1.6	13.0	−1.8	13.3	1.5	13.3	−1.5
Avg	15.5	2.2	15.2	−1.9	15.3	2.1	15.0	−1.8
	Vit. D admin., 600 units/day for 40 days							
"	15.4	1.6	15.3	−1.5	15.2	1.5	13.6	+ .1
	Estrogen admin. for 60 days							
"	16.5	2.5	14.1	− .1	16.5	2.0	13.7	+ .8
	Androgen admin. for 30 days							
"	16.4	3.0	13.7	− .3	16.1	1.7	12.3	+2.1
	Estrogen + androgen admin. for 30 days							
"	16.2	2.7	13.7	− .2	16.4	2.0	12.3	+2.1

Results are presented in the Table. All values are expressed as mg of calcium/kg body weight/day averaged over the entire balance period. For comparison, we present the summary of our earlier work on administration of Vit. D (600 units/day for 60 days) on the same 5 subjects, and of our other work on the effect of sex hormones. Details have been presented(8) but the dosages used were as follows: estrogen—1 mg estradiol benzoate in oil, I.M. twice weekly for 60 days; androgen—30 mg testosterone/day for 30 days;

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.

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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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