Urinary Calcium Excretion of Women with Breast Cancer during Post-Hypophysectomy Polyuria, Spontaneous Interphase and Vasopressin-Induced Antidiuresis.* (27107)

BERNARD GARDNER, GILBERT S. GORDAN, AND JOSEPH WITT

Departments of Surgery, Medicine and Neurosurgery, University of California Medical Center, San Francisco

Thorn(1) has recently reported that small doses of porcine posterior pituitary extract increase urinary calcium excretion of normal dogs. Availability of patients undergoing hypophysectomy for objectively advancing disseminated breast cancer gave us the opportunity to measure urinary calcium excretion during spontaneous postoperative polyuria and after vasopressin administration, and thereby, to determine if a similar effect can be demonstrated in human subjects.

Methods. Eight patients with advanced breast cancer were studied before and after hypophysectomy. These patients had all received previous hormonal treatment with androgens, and subsequently, with a combination of prednisone, 30 mg/day and triiodothyronine, 50 μg/day, and were far along in the course of their disease. The patients received 200 mg of cortisone acetate daily, starting 24 hours before surgery. On the second postoperative day, the therapy was changed to hydrocortisone, 30 mg/day, which was maintained. Triiodothyronine, 50 μg/day, was introduced after the fourteenth day. Twelve-hour nocturnal urinary collections were used to avoid the non-specific effects of varying degrees of daytime immobilization.

Calcium was determined by the flame spectrophotometric method of Loken, Eisenberg and Teal(2), based on that of MacIntyre(3) in which the calcium emission at 422.7 mμ is measured, using an oxyacetylene flame, a Zeiss direct sprayer burner, a prism monochromator, and a IP21 photomultiplier tube, activated by 1200 volts. The method differs from that originally described by MacIntyre in that those ions present in urine and serum are added to the perchloric acid dilu-ent in amounts adequate to produce a plateau augmentation of the calcium emission (recovery is 99.5-100.5%).

Creatinine was determined by the method of Folin and Wu(4). Phosphorus was measured by the method of Taussky and Schorr (5). Tubular reabsorption of phosphate (TRP) was calculated by the method of Chambers, *et al.*(6), using 12-hour urine collections. No correction was made for protein binding of serum inorganic phosphate.

Results and discussion. The results are summarized in Tables I-VIII. Evaluation of the urinary calcium and creatinine clearance during the first 3 postoperative days was complicated by the augmenting effect of large doses of cortisone(7-9). Polyuria without interposition of an interphase developed in patient L.R. (Table I). A moderate, gradual decrease in urinary calcium excretion was noted in spite of the continued large urinary volume. Declines in calcium excretion and glomerular filtration following hypophysectomy have been noted previously(10,11), although Pearson has recently stated that calcium and phosphorus balance are undisturbed by hypophysectomy(12).

The mean creatinine clearance in these hypophysectomized breast cancer patients was 56.8 $\pm$ 3.5 ml/min. (mean $\pm$ S.E.) compared to 78.4 $\pm$ 4.4 in early untreated metastatic breast cancer patients(9). We have noticed the gradual return of urinary volume to normal levels after several months in our patients, confirming similar observations of Ray(13). Since a continuing decline in creatinine clearance occurs with advancing disease to a level of 40 $\pm$ 5.2 ml/min. (S.E.) in cancer patients within 6 weeks of death(9) it is possible that the return to normal of urinary volume may represent the effects of a markedly reduced glomerular filtration rate

* Supported in part by grant from Nat. Inst. Health, and an institutional grant from Am. Cancer Soc. allocated by the Cancer Coordinating Committee, Univ. of California.

(14,15) (for example, patient E.R., Table VI).

An interphase was observed in 5 of these patients (M.D., B.C., E.R., and L.P.). Patient M.D. (Table II) showed no significant change in urinary calcium excretion during the interphase but did manifest a gradual decline in calcium excretion, although remaining hypercalciuric throughout the period of observation.

In patient B.C. (Table III) an interphase developed on the second postoperative day which progressed to permanent polyuria by the sixth postoperative day. A fall in urinary calcium excretion was observed on day 4 and again on day 6, at which time the poly-

TABLE I. Patient L.R. Progressive Osteolytic and Pulmonary Disease. Wt, 44.5 kg. Avg postoperative tubular reabsorption of phosphate, 73.4%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
8 days pre.-op.	Prednisone, 30 mg/day	825	1.013	4.25	50.1
Hypophysectomy	Cortisone, 200 "				
2	Cortisol, 30 "	2970	1.002	4.30	
3	*Idem*	1640	1.001	1.95	44
5	"	2160	1.002	3.40	43
6	"	2960	1.004	3.80	49
7	"	2510	1.003	2.75	33
8	"	2330	1.004	2.55	43

TABLE II. Patient M.D. Progressive Osteolytic and Pulmonary Metastases. Wt, 56.4 kg. Avg postoperative tubular reabsorption of phosphate, 82.2%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
4 days pre.-op.	Prednisone, 30 mg/day	1135	1.010	10.0	99
Hypophysectomy					
2	Cortisone, 200 "	3120	1.004	19.4	68.4
3	Cortisol, 60 "	1780	1.005	14.8	92.9
4	Cortisol, 30 "	845	1.015	15.0	88.1
5	*Idem*	1490	1.005	15.9	61
6	"	1320	1.009	17.4	69
7	"	1390	1.010	14.7	57
8	"	3675	1.004	13.9	48
12	" ; vasopressin, 10 I.U.	1210	1.013	12.9	54

TABLE III. Patient B.C. Progressive Pulmonary and Osseous Metastases. Wt, 77.9 kg. Avg postoperative tubular reabsorption of phosphate, 83.9%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
10 days pre.-op.	Prednisone, 30 mg/day	705	1.007	3.4	83.7
Hypophysectomy	Cortisone, 200 "				
2	Cortisol, 30 "	870	1.008	3.4	64
3	*Idem*	950	1.007	4.9	60
4	"	1220	1.006	1.9	56.0
6	"	2240	1.004	1.1	62.5
8	"	2450	1.005	2.7	63.6
9	"	1860	1.005	1.0	—
10	"	1785	1.003	3.7	—
179	"	540	1.002	.9	31.5

uria was fully manifested again. Further observations revealed some fluctuation in urinary calcium in spite of maintained polyuria.

Early interphases were similarly noted in patients E.B. (Table IV) and L.P. (Table V). Patient E.B. showed no fall in urinary calcium during the interphase until day 7. However, this decreased level of calcium excretion was maintained 6 weeks later at a time when polyuria was again noted. Patient L.P. and E.R. (Table VI) showed a decline in calcium excretion during the interphase which increased again during the permanent phase. Patient E.R. showed the greatest correlation between calcium excretion and urinary volume of the patients studied, but op-

TABLE IV. Patient E.B. Progressive Osteolytic and Pulmonary Metastases. Wt, 50 kg. Avg postoperative tubular reabsorption of phosphate, 82.2%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
20 days pre.-op.	None	465	1.010	4.6	73
Hypophysectomy	Cortisone, 200 mg/day				
3	Cortisol, 30 "	1075	1.010	3.2	85
5	*Idem*	1245	1.008	4.1	71
7	"	925	1.004	1.2	38.7
50	" ; triiodothyronine, 50 μg/day	1895	1.005	1.3	48.3

TABLE V. Patient L.P. Progressive Osteolytic, Cerebral and Pulmonary Metastases. Wt, 46 kg. Avg postoperative tubular reabsorption of phosphate, 90.8%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
1 day pre.-op.	Cortisone, 200 mg/day	890	1.004	1.32	
Hypophysectomy	Cortisone, 200 mg/day				
2	Cortisol, 30 "	670	1.008	1.05	55
3	*Idem*	475	1.013	1.30	63.2
4	"	1785	1.004	2.85	49
5	"	3235	1.003	2.95	67.5
6	" + vasopressin, 5 I.U.	1235	1.008	1.65	67.1
7	*Idem*	1155	1.008	2.65	67.7

TABLE VI. Patient E.R. Progressive Pulmonary Metastases. Wt, 57.1 kg. Avg postoperative tubular reabsorption of phosphate, 73.4%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
5 days pre.-op.	Prednisone, 30 mg/day	720	1.011	3.5	74
Hypophysectomy	Cortisone, 200 "				
2	Cortisol, 30 "	4000	1.001	1.50	95
3	*Idem*	2485	1.002	1.65	91
4	"	1495	1.005	3.15	44
5	"	985	1.004	.76	16
6	"	2975	1.006	2.20	60
7	"	1325	1.007	.98	59
8	"	890	1.010	.9	32
9	"	760	1.007	.49	50
29	" + triiodothyronine, 50 μg/day	3260	1.003	2.75	79.5
78	*Idem*	2750	1.005	1.5	115
113	"	1020	1.007	.5	44

posite to that which should be expected from analysis of Thorn's experiments. This patient had no osseous disease and was undergoing objective regression of her pulmonary lesions at the time that the urinary calcium excretion and glomerular filtration were increasing.

Patient L.P. was the only other patient in this study who obtained an objective regression from the procedure, and it can be seen that she similarly had an increased calcium excretion compared to her earlier levels.

Administration of exogenous vasopressin was investigated in 4 patients (M.D., H.W., L.P., and F.O., Table II, V, VII, and VIII), accounting for 9 observations. The calciuric effect of vasopressin noted by Thorn in his investigations on dogs could not be verified in these patients. In fact several patients showed a moderate fall, and one a marked fall in urinary calcium excretion, which was not maintained or reduplicated by subsequent injections of vasopressin.

The tubular reabsorption of phosphate for this group of patients was 81.4 ± 2.3, which is not significantly different from our normal females (85 ± 0.5), although 3 patients had subnormal values.

TABLE VII. Patient H.W. Progressive Chest Wall Metastases. Wt, 68.8 kg. Avg postoperative tubular reabsorption of phosphate, 77.6%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
2 mo pre.-op.	Delta-1 Testololactone	1050	1.010	3.15	
Hypophysectomy	Cortisone, 200 mg/day				
30	Cortisol, 30 mg/day; triiodothyronine, 50 μg/day	2795	1.004	3.4	66
52	*Idem*	2010	1.001	4.6	39
94	" + vasopressin, 10 I.U.	450	1.018	3.8	71.6
136	*Idem*	445	1.015	4.3	52.5
178	Cortisol, 30 mg/day; triiodothyronine, 50 μg/day	1845	1.004	2.5	46.8
220	*Idem* + vasopressin, 10 I.U.	375	1.022	.7	68

TABLE VIII. Patient F.O. Progressive Osteolytic and Pulmonary Metastases. Wt, 57.8 kg. Avg postoperative tubular reabsorption of phosphate, 74.0%.

Postoperative days	Therapy	Urinary vol (ml/12 hr)	Specific gravity	Urinary calcium (meq/12 hr)	Creatinine clearance (ml/min.)
1 day pre.-op.	Cortisone, 200 mg/day	570	1.019	6.3	54
Hypophysectomy	*Idem*				
3	Cortisol, 30 mg/day	1585	1.007	.40	65
6	*Idem*	1890	1.010	2.40	37
7	"	1970	1.008	2.10	38.4
11	"	2505	1.004	2.80	55
17	" ; triiodothyronine, 50 μg/day + vasopressin, 10 I.U.	325	1.022	.09	40
25	*Idem*	200	1.020	1.65	43
26	Cortisol, 30 mg/day + triiodothyronine, 50 μg/day	1110	1.005	.85	51
27	*Idem*	2180	1.002	1.55	53
28	"	2680	1.003	1.25	41
29	" + vasopressin, 10 I.U.	271	1.021	1.20	40.7
32	Cortisol, 30 mg/day + triiodothyronine, 50 μg/day	3785	1.005	1.45	47

Summary. (1) Urinary calcium excretion and creatinine clearance were measured in 8 patients before and after hypophysectomy for advanced breast cancer. (2) Mean creatinine clearance for the group was lower than in patients in an earlier stage of metastatic breast cancer. (3) No consistent changes in urinary calcium excretion could be correlated either with polyuria, spontaneous interphase, or vasopressin-induced reduction in urinary volume.

1. Thorn, N. A., *Danish Med. Bull.*, 1960, v53, 110.
2. Loken, H. F., Eisenberg, E., Teal, J. S., in preparation.
3. MacIntyre, I., *Biochem. J.*, 1957, v67, 164.
4. Folin, O., Wu, H., *J. Biol. Chem.*, 1919, v30, 81.
5. Taussky, H. H., Schorr, E., Kurzmann, G., *ibid.*, 1953, v202, 675.
6. Chambers, E. L., Jr., Gordan, G. S., Goldman, L., Reifenstein, E. C., Jr., *J. Clin. Endocrinol. and Metab.*, 1956, v16, 1507.
7. Roberts, K. E., Pitts, R. P., *Endocrinol.*, 1952, v50, 51.
8. Laake, H., *Acta Endocrinol.*, 1960, v34, 60.
9. Gardner, B., Gordan, G. S., Loken, H. F., Thomas, A. N., Teal, J. S., in preparation.
10. Matson, D., In *Hypophysectomy,* Pearson, O. H., editor, Springfield, Ill., Charles C Thomas, 1957, p33.
11. White, H. L., Heinbecker, P., *Am. J. Physiol.*, 1938, v123, 566.
12. Pearson, O. H., Ray, B. S., in *Endocrine Aspects of Breast Cancer,* Proc. of Conference held at Univ. of Glasgow, Edinburgh, E. & S. Livingstone, 1957, p92.
13. Ray, B. S., *Arch. Neurol.*, 1960, v3, 121.
14. Kleeman, C. R., Maxwell, M. H., Rockney, R., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 189.
15. Olivecrona, H., Luft. R., *Ann. Roy. Coll. Surg. Eng.*, 1957, v20, 267.

Received September 5, 1961. P.S.E.B.M., 1962, v109.

Secondary Serological Response to Infectious Canine Hepatitis Virus Produced by Adenovirus Type 4.* (27108)

LELAND E. CARMICHAEL AND JAMES A. BAKER
Veterinary Virus Research Institute, New York State Veterinary College at Cornell University, Ithaca, N. Y.

Comparison of infectious canine hepatitis (ICH) virus with human adenoviruses indicates that ICH virus is a member of the adenovirus group(1,2,3). Dogs have been shown to be susceptible to human adenovirus infection, and they develop complement-fixing and neutralizing antibodies following a single inoculation(3,4). These findings actuated further studies on the relationship between ICH virus and human adenoviruses, with the specific aim of determining the clinical and immunological response of dogs to adenovirus type 4 and to ICH virus following adenovirus inoculation. The results of these studies are presented below.

Virus used. Adenovirus type 4 stock was obtained from Lt. R. R. Gutekunst (Naval Medical Research Unit 4, U. S. Naval Training Center, Great Lakes, Ill.) Stock virus was transferred once in HeLa cell cultures maintained in a media composed of medium 199 (Difco), 15% tryptose-phosphate broth, 5% agammaglobulin calf serum and antibiotics. When complete cell degeneration occurred, the cultures were frozen and thawed 6 times and centrifuged at 1500 rpm for 10 minutes to remove cellular debris. The clarified virus suspension was then distributed in sealed vials and stored at –50°C. The virus titer of the pooled suspension was $10^{4.0}$ $TCID_{50}$ per 0.1 ml.

For animal inoculation Cornell strain 1 ICH virus was stored at –50°C as small portions of liver from an infected dog. In the preparation of inoculum, a portion of stored liver was made into a 10% suspension with 0.15 M phosphate-buffered saline (pH 7.4). The virus content of the suspensions ranged from $10^{5.5}$ to $10^{5.8}$ per ml.

For neutralization tests, Cornell 1 strain

* The authors acknowledge with appreciation support from the Office of Naval Research.