ANNALS OF SURGERY

Vol. 154 October 1961 No. 4

Hypercalcemia Simulating Hyperparathyroidism Induced by XV-2 Carcinoma of Rabbit *

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HYPERCALCEMIA has been observed in a variety of human cancers both with and without demonstrable metastases to bone.[1, 2, 4, 9, 11, 12, 14, 18–21, 24, 27, 32, 34, 37] Myers [25] recently called attention again to abnormalities of calcium metabolism in patients with metastatic carcinoma of the prostate and breast. Connor *et al.*[7] noted hypercalcemia with carcinoma of the lung. They found that excision of the lesions in these patients was accompanied by a return of the serum calcium levels to normal. Similar observations have been made by Plimpton and Gellhorn; [26] in addition they have noted a rise in serum calcium concommitant with recurrence of the tumor. A syndrome resembling hyperparathyroidism was described by Schatten *et al.*[31] with metastatic squamous cell carcinoma of the vulva. It is interesting to note that calcium levels returned to normal upon excision of metastatic lesions on two separate occasions.

Observation of the terminal clinical state of rabbits with XV-2 carcinoma suggested that hypercalcemia may have been a significant factor in the demise of these animals. The terminal manifestations were not unlike these described for other species.[6] In our previous experience,[36] this tumor was found to be anaplastic in type, readily transplantable, and one which has remained stable in growth and in histopathological character throughout many generations. The XV-2 carcinoma developed from the naturally-occurring cutaneous papilloma of the cottontail rabbit, and was described by Shope,[33] in 1933. Kidd [15] in 1940, described the virus-induced papilloma-to-carcinoma sequence in the rabbit. Subsequently, Syverton *et al.*[35] and Kidd and Rous [30] reported the absence of the papilloma virus in rabbits with XV-2 carcinoma.

Method

Each of 13 New Zealand White rabbits with body weights ranging from 1.5 to 2.0 kg. received a transplant of XV-2 carcinoma. The donor tumor was obtained, using clean technic, immediately upon sacrifice of a donor animal. Two cc. of a milky suspension of tumor particles was injected into the right anterior thigh muscle,

* Presented before the American Surgical Association, Boca Raton, Florida, March 21–23, 1961.

This work has been supported in part by grants from the National Institutes of Health (#A-2372 and 2G-422 (Mr. Merrick)).

TABLE 1. *Plasma Calcium (mg./100 ml.)*

Animal No.	Weeks												
	0	1	2	3	4	5	6	6½	7	7½	8	8½	9
XV-2 Carcinoma untreated													
1	16.0	14.6	16.3	16.5	22.2	22.7							
2	14.2	14.4	16.3	17.3	14.4								
3	15.8	14.4	15.4	15.7	20.9								
4	16.6	15.6	16.5	15.9	18.4	22.6	14.1						
5	16.1	14.0	16.4	15.5	22.7	25.3	25.7		24.7		20.2		21.3
6	15.9	14.2	15.4	14.9	22.6	20.8	16.1						
7	16.0	13.8	16.0	15.1	18.2	21.2	19.3						
8	17.3	18.6	17.0	17.8	36.0	23.6	22.8						
9	17.6	17.9	18.9	16.2	16.2	21.1	27.0	25.6	30.0	33.0	27.4	22.4	17.6
10	17.3	17.1	17.0	29.0	38.1	23.2	25.6	24.0	24.8	27.4	24.8		
11	17.6	17.8	18.4	22.0	16.3	25.6	23.2	15.2					
12	15.1		16.3	15.2	16.6	19.2	17.4		15.4		14.1		14.8
13	17.4	17.1	17.7	16.8	13.2	18.0		19.0	21.6				
Control animals													
14		14.6				14.8							15.5
15		15.1				16.1			15.5				
16		17.2	17.1	16.5	15.2	18.4	18.0			21.0			
17	17.6	17.6	17.8		17.6	18.9		20.4		21.6			
18		18.4	18.6		18.4	18.4		20.2		22.0			
19		18.1	18.8	15.5	17.3	19.7		18.0					
20	17.7	17.9	17.7		18.3	18.5		19.4		20.8			

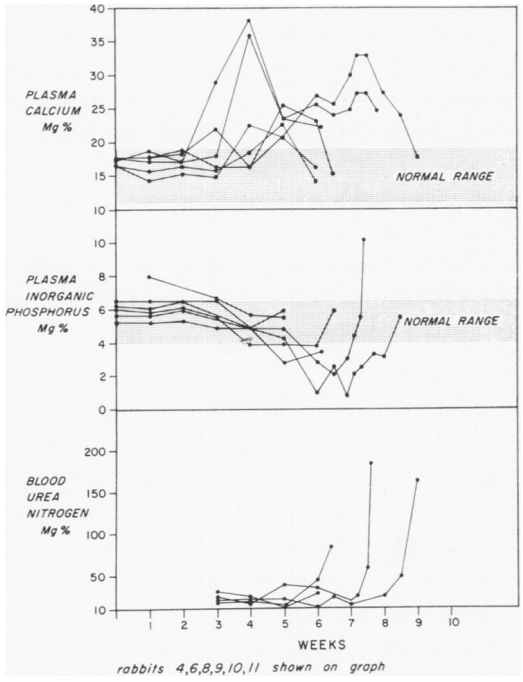

FIG. 1. Rabbits bearing XV-2 carcinoma.

The size of the tumor particles was controlled only by filtering the suspension through 12-ply gauze. Seven control rabbits were maintained throughout the experiment. Whole blood was collected into heparinized centrifuge tubes via ear vein drip method. Blood samples were obtained from one to three times per week, for an average of about six weeks. Plasma calcium and phosphorus concentrations were obtained on each specimen. The plasma calcium levels were determined by a chelating method [5] in which the end point was determined photoelectrically, and by a calcium phosphate precipitation method.[10, 28, 29] Plasma inorganic phosphorus was determined by a modification of the method of Fiske and Subbarow.[10] Blood urea nitrogen concentrations [13] were noted on specimens from four tumor and five control rabbits. Postmortem examination was performed on each animal immediately after

death and appropriate tissues were obtained for histopathologic study.

Results

In 11 of the tumor-bearing rabbits there was an increasing degree of hypercalcemia which usually occurred after the third week postimplantation (Table 1). Two of the animals had plasma calcium levels as high as 36–38 mg.% during the period of most rapid tumor growth. Within a 14 to 21-day period prior to death, there was a decrease in the concentration to within normal range in the majority of animals. Rabbits 4, 6, 9, 10 and 11 demonstrated a sustained hypercalcemia with a sudden return to control levels within one to two days prior to death (Fig. 1). The control rabbits did not manifest a significant change in the calcium concentration. The normal range of calcium was found to be 14 to 18 mg.%. The plasma inorganic phosphorus concentrations generally revealed a decrease after the third week postimplantation (Table 2). Several animals had levels as low as 1.0 to 3.0 mg.%. Within one to two days prior to death the palsma phosphorus was found to be at or above control levels in four rabbits.[1, 12, 18, 19] Of these four, No. 1, 18 and 19 demonstrated the striking simultaneous reversal of hypercalcemia which has been previously described by Collip.[6]

The blood urea nitrogen concentrations increased from 85 to 185 mg.% in three of the four tumor-bearing animals in which determinations were obtained one week prior to death. The animals (1, 18 and 19) which demonstrated the marked elevation of BUN also showed the significant elevation in phosphorus and the decrease in calcium concentrations which has been described above. The control rabbits maintained levels of 15 to 25 mg.%.

Two animals did not support a sustained growth of the XV-2 carcinoma after the third and fourth week postimplantation.

TABLE 2. *Plasma Inorganic Phosphorus (mg./100 ml.)*

Animal No.	Weeks												
	0	1	2	3	4	5	6	6½	7	7½	8	8½	9
XV-2 Carcinoma untreated													
1		5.4		6.5	5.7								
2		6.1		5.9	9.1								
3		6.2		8.0	5.7								
4		8.0		6.7	5.7	5.5							
5		6.3		6.6	5.7	4.9	5.1		5.1		5.3		5.3
6		6.5		6.6	4.9	5.9							
7		6.2		6.5	6.0	7.5	6.1						
8	5.3	5.2	5.3	4.9	4.9	2.8	3.4						
9	5.6	5.7	6.0	5.5	5.0	4.3	1.0	2.5	0.8	2.5	3.3	3.1	5.5
10	6.2	6.1	6.5		4.8	4.8	2.8	2.1	3.0	5.5	10.5		
11	5.9	5.7	6.1	5.6	3.8	3.9	3.8	5.9					
12			6.0	7.1	6.3	5.4							
13	6.4	6.3	7.1	5.6	4.1	5.5		2.8	5.7	5.1	5.9		5.7
Control animals													
14		5.6	5.6	5.9	5.7	6.2	4.1		5.1				
15	5.9	6.3	6.6	5.2	5.2	5.6			5.7				
16		5.9	4.7	6.7	5.6	6.0			5.9				
17		5.4	6.3	5.9	5.5	6.0							
18	6.3	6.2	6.7	5.6	5.8				5.8				
19			6.2			6.6							
20			7.9			9.8							

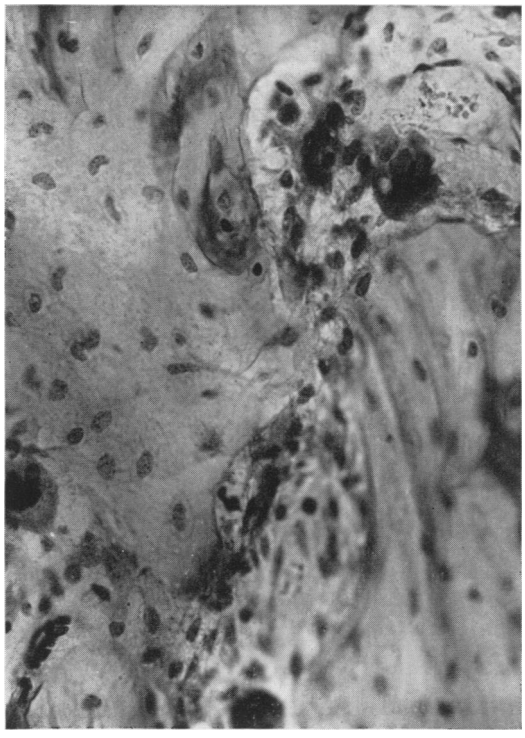

FIG. 2. Bone resorption showing giant cells

The calcium and phosphorus concentrations in these animals failed to show striking changes noted in the other tumor-bearing rabbits.

Roentgenograms and histopathological examination of specimens of ribs, vertebral and femurs showed no metastic lesions. Extensive bone resorption was consistently found, and was associated with a large number of giant cells (Fig. 2). A marked hyperplasia of bone marrow with a significant increase in percentage of white cell forms was noted. Histological evaluation of the kidney specimens revealed marked hyperemia, degeneration and necrosis, predominately in the proximal tubules, and accompanied by massive proteinaceous exudate within the tubules.

Discussion

The hypercalcemia associated with the growth of the anaplastic XV-2 carcinoma correlated well with the data previously reported in certain human neoplasms. No conclusive evidence has been presented which indicates whether this effect occurs directly as a result of substances introduced into the circulation by tumor growth or whether it occurs secondarily through increased parathyroid gland activity. As early as 1923, Klemperer [17] noted parathyroid hyperplasia and extensive bone destruction in generalized carcinomatosis. However, other authors [2, 6, 7, 31] have found no alterations in the parathyroid glands in the presence of hypercalcemia associated with malignant disease. Their investigations suggest that the malignant tumor is directly responsible in some way for the serum abnormalities. A reduction in hypercalcemia associated with certain human neoplasms has been obtained upon removal of the neoplasm. This suggests that the tumor may produce a substance which behaves like extract of the parathyroid.

The occurrence in tumor-bearing animals of a specific protein possessing a marked calcium affinity is another possible explanation. This need not necessarily be reflected in a change in total protein concentration, and might require more precise biochemical fractionation. Another possibility is that these tumors may produce a vitamin D-like substance and thus cause an excessive absorption of dietary calcium similar to that postulated in Boeck's sarcoid.[3] However, if we consider that the tumor produces a substance similar to extract of parathyroid gland, then we might anticipate a deficiency in calcium absorption from the gastrointestinal tract.[8]

The reduction in plasma phosphorus generally observed is not easily explained. Alterations in dietary phosphorus did not appear to be an important factor in the determination observed in this experiment. If the tumors do produce a parathormone-like substance the reduction could be explained on the basis of decreased tubular reabsorption of phosphorus.

The rise in the blood urea nitrogen concentration in animals with hypercalcemia and hypophosphatemia can very likely be explained on the basis of hypercalcemic nephropathy. Hypercalcemia in a variety of diseases has been found to produce characteristic pathologic changes in the kidneys.[16] Although the histopathologic changes observed in the present study were not completely characteristic, the terminal azotemia and elevation of phosphorus were seen. The latter is presumably on the basis of phosphate retention. McLean [22, 23] noted that when the phosphate concentration of the blood is augmented in normal animals a subsequent fall in total calcium occurs. This mechanism may be present in the tumor rabbit during the terminal illness and result in a reduction in the hypercalcemia.

Conclusion

1. Hypercalcemia, hypophosphatemia, and bone resorption simulating hyperparathyroidism is found associated with anaplastic XV-2 carcinoma of the rabbit.

2. Terminal hypercalcemic nephropathy is probably a factor in the demise of rabbits with XV-2 carcinoma.

Bibliography

1. Adair, F. E., R. C. Mellors, J. H. Farrow, H. Q. Woodard, G. C. Escher and J. A. Urban: The Use of Estrogens and Androgens in Advanced Mammary Cancer. Clinical and Laboratory Study of 105 Female Patients. J. A. M. A., **140**:1193, 1949.
2. Albright, F. and E. C. Reifenstein, Jr.: The Parathyroid Glands and Metabolic Bone Diseases: Selected Studies. Baltimore, Williams and Wilkins Co., 1948.
3. Anderson, J., C. E. Dent, C. Harper and G. R. Philpot: Effect of Cortisone on Calcium Metabolism in Sarcoidosis with Hypercalcemia. Lancet, **11**:720, 1954.
4. Aub, J. C., D. M. Tibbetts and I. T. Nathanson: Metabolic Effects of Treatment of Carcinoma of the Prostate. Cancer Res., **7**:723, 1947.
5. Campbell, W. R.: The Estimation of Calcium and Magnesium. Canad. J. Biochem. and Physiol., **35**:1033, 1957.
6. Collip, J. B.: The Parathyroid Glands. Medicine, **5**:1, 1926.
7. Connor, J. B., W. C. Thomas, Jr. and J. E. Howard: The Etiology of Hypercalcemia Associated with Lung Carcinoma. J. Clin. Investig., **35**:697, 1956.
8. Eiseman, B. and W. Silen: Endocrinologic Factors in the Pathogenesis of Peptic Ulcer. Adv. Int. Med., **X**:107, 1960.
9. Farrow, J. H. and H. Q. Woodard: The Influence of Androgenic and Estrogenic Substances on the Serum Calcium in Cases of Skeletal Metastases From Mammary Cancer. J. A. M. A., **118**:339, 1942.
10. Fiske, C. H. and Y. Subbarow: The Colorimetric Determination of Phosphorus. J. Biol. Chem., **66**:375, 1925.
11. Gutman, A. B., T. L. Tyson and E. B. Gutman: Serum Calcium Inorganic Phosphorus and Phosphatase Activity; In Hyperparathyroidism, Paget's Disease, Multiple Myeloma and Neoplastic Disease of Bone. Arch. Int. Med., **57**:379, 1936.
12. Jackson, H., Jr. and F. H. L. Taylor: Calcium, Potassium and Inorganic Phosphate Content of the Serum in Cancer Patients. Am. J. Cancer, **19**:379, 1933.
13. Karr, W. G.: A Method for the Determination of Blood Urea Nitrogen. J. Lab. and Clin. Med., **9**:329, 1924.
14. Kennedy, B. J., D. M. Tibbetts, I. T. Nathanson and J. C. Aub.: Chemical and Systemic Changes in Patients with Advanced Carcinoma of the Breast Following Hormonal Therapy and Castration. Cancer Res., **9**:551, 1949.
15. Kidd, J. G. and P. Rous: A Transplantable Rabbit Carcinoma Originating in a Virus-induced Papilloma. J. Exp. Med., **71**:813, 1940.
16. Klatskin, G. and M. Gordon: Renal Complications of Sarcoidosis and Their Relationship to Hypercalcemia. Am. J. Med., **15**:484, 1953.
17. Klemperer, P.: Parathyroid Hyperplasia and Bone Destruction in Generalized Carcinomatosis. Surg., Gynec. & Obst., **36**:11, 1923.
18. Laszio, D.: Mineral Metabolism in Metastatic Bone Cancer. Cancer Res., **9**:614, 1949.
19. Laszio, D., C. A. Schulman, J. Bellin, E. D. Gottesman and A. Schilling: Metabolic Studies of Patients with Carcinoma of the Breast and the Effects of Testosterone Therapy. Cancer Res., **10**:230, 1950.
20. Laszio, D., C. A. Schulman, J. Belling, E. D. Gottesman and A. Schilling: Mineral and

Protein Metabolism in Osteolytic Metastases. J. A. M. A., **148**:1027, 1952.
21. Mason, R. L. and S. Warren: Metastatic Carcinoma Simulating Hyperparathyroidism. Am. J. Path., **7**:415, 1931.
22. McLean, F. C. and M. A. Hinrichs: The Inverse Relationship Between Calcium and Phosphorus in the Blood. J. Biol. Chem., **109**:lxiii, 1935.
23. McLean, F. C. and L. Leiter: The State of Calcium in the Blood in Nephritis and Uremia. J. Clin. Investig., **14**:705, 1935.
24. Myers, W. P. L.: Hypercalcemia in Neoplastic Disease. Cancer, **9**:1135, 1956.
25. Myers, W. P. L.: Calcium Studies in Malignant Tumors. 11 Applications of Simplified Measurements to Clinical Problems. Cancer, **12**:494, 1959.
26. Plimpton, C. H. and A. Gellhorn: Hypercalcemia in Malignant Disease Without Evidence of Bone Destruction. Am. J. Med., **21**: 750, 1956.
27. Reifenstein, E. C., Jr., J. Albright and S. L. Wells: The Accumulation, Interpretation and Presentation of Data Pertaining to Metabolic Balances, Notably Those of Calcium, Phosphorus, and Nitrogen. J. Clin. Endocrinol., **5**:367, 1945.
28. Roe, J. H. and B. S. Kahn: The Colorimetric Determination of Blood Calcium. J. Biol. Chem., **67**:585, 1926.
29. Roe, J. H. and B. S. Kahn: The Colorimetric Determination of Blood Calcium. J. Biol. Chem., **81**:1, 1929.
30. Rous, P., J. G. Kidd and W. E. Smith: Loss by the XV-2 Carcinoma to Immunize Hosts Against the Papilloma Virus. J. Exp. Med., **96**:159, 1952.
31. Schatten, W. E., A. G. Ship, W. J. Pieper and F. C. Bartter: Syndrome Resembling Hyperparathyroidism Associated with Squamous Cell Carcinoma. Ann. Surg., **148**:890, 1958.
32. Schilling, A., J. Bellin, E. D. Gottesman and D. Laszlo: Metabolic Studies of Patients with Carcinoma of the Prostate and the Effects of Stilbestrol Therapy. Cancer Res., **10**:239, 1950.
33. Shope, R. E.: Infectious Papillomatosis of Rabbits. J. Exp. Med., **58**:607, 1933.
34. Swyer, A. J., J. S. Berger, H. M. Gordon and D. Laszlo: Hypercalcemia in Osteolytic Metastatic Cancer of the Breast. Am. J. Med., **8**:72, 1950.
35. Syverton, J. T., E. B. Wells, J. Kooman, H. E. Dascomb and G. P. Berry: The Virus-induced Rabbit Papilloma-to-carcinoma Sequence. Cancer Res., **10**:474, 1950.
36. Wilson, J. R. and K. Grimson: XV-2 Carcinoma of Rabbit Treated with Cytotoxic Agents. (To be published.)
37. Woodard, H. Q.: Changes in Blood Chemistry Associated with Carcinoma Metastatic to Bone. Cancer, **6**:1219, 1953.

Correction

Reference is made to the article, The Influence of Dietary Antibiotics on a Surgical Experiment, Ann. Surg., **153**:585 (April) 1961, Isidore Cohn, Jr., M.D. The following statement which appears on page 586 is incorrect:

"If one assumes that the average dog eats one pound of food a day, the daily intake of antibiotic in the antibiotic-containing food is 2.5 micrograms of tetracycline per day."

This should read 2.5 milligrams of tetracycline instead of 2.5 micrograms.