

# Studies on Prostatic Cancer

## I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate\*

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Carcinoma of the prostate gland is peculiarly favorable for endocrine investigation since frequent serial observations of the activity of phosphatases in serum were found to provide objective indices of activity of the neoplasm when the enzymes were increased in amount above normal. In the present paper data are given for the values of serum phosphatases in carcinoma of the prostate and in normal men. We shall demonstrate that the acid phosphatase of serum is reduced in metastatic carcinoma of the prostate by decreasing the activity of androgens through castration or estrogenic injections and that this enzyme is increased by injecting androgens. We have been unable to find previous observations indicating any relationship of hormones to carcinoma of the prostate gland.

An enzyme capable of hydrolyzing phosphoric esters was discovered by Grosser and Husler (4) in intestinal mucosa and kidney. Robison (16) found that this enzyme was particularly high in activity in growing bone and cartilage and that its activity was greatest at pH 9 to 9.5. This "alkaline phosphatase" was found by Kay (9) to be increased in the serum in certain bone diseases including metastasis of neoplasms to bone and later work has shown that among these conditions is carcinoma of the prostate.

Davies (3) and Bamann and Riedel (1) discovered that there occurs in the spleen and kidney of swine and cattle, in addition to the alkaline phosphatase, a phosphatase with an activity maximum at pH 4.8. An enzyme believed to be identical with this "acid phosphatase" was found by Kutscher and Wolbergs (11) to be present in very large amount in the human prostate gland. This finding of great activity of acid phosphatase in the prostate gland was confirmed and extended to include prostatic cancer by Gutman, Sproul, and Gutman (7). The serum of certain patients with disseminated prostatic carcinoma was found by Gutman and Gutman (6) and Barringer and Woodard (2) to exhibit increased acid phosphatase activity. Robinson, Gutman, and Gutman (15) summarized the acid phosphatase activity levels of 44 patients with carcinoma of the prostate. They concluded that a marked rise in acid phosphatase in serum is associated with the appearance or spread of roentgenologically demonstrable skeletal metastases and implies dissemination of the primary tumor and thus is of unfavorable prognostic significance.

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### METHODS AND MATERIALS

The phosphatase activity of serum was determined by the method of King and Armstrong (10) using 0.005 M disodium monophenylphosphate as substrate. The buffers used were 0.05 M barbital-sodium at pH 9.3, and 0.1 M Sørensen's citrate-HCl or Walpole's 0.2 N sodium acetate-acetic acid buffers at pH 5. All serums were tested in duplicate and were added directly to buffer-substrate solutions without dilution; they were incubated at 37.5° C. for 30 minutes. Precautions were observed that all solutions were at this temperature before testing. Blanks were run by adding the protein precipitant to the buffer-substrate solution before adding serum. Colorimetric procedures were carried out with the Evelyn photoelectric colorimeter using a 6600 Å filter. The results are expressed in King and Armstrong units, a unit being defined as that degree of phosphatase activity which at pH 9.3 (or pH 5.0, respectively) and 37.5° C. will liberate 1 mgm. of phenol from the specified buffer-substrate solution in one-half hour.

Phosphatase determinations at pH 5 and 9.3 were made on the serum of 40 normal men, of 21 men with benign prostatic hypertrophy, and of 47 men with carcinoma of the prostate. The diagnosis of carcinoma of the prostate gland was derived from one or more of the following procedures: rectal palpation, cystoscopic examination, transurethral resection with microscopic examination, or roentgenologic evidence of skeletal metastases. Necropsy was obtained in 2 cases. All patients had x-ray studies of the bony pelvis.

Eight patients who had carcinoma of the prostate with skeletal metastases and with moderate or great elevation of acid phosphatase of serum values above 20 units in 100 cc. were selected for intensive study in the hospital. Each patient also had elevation of alkaline phosphatase in the serum. Both of these enzymes were determined on the serum 3 times weekly for many weeks. Bilateral castration was carried out in all. Five patients were injected with stilbestrol, 1 mgm.

daily, or estradiol benzoate, 1.66 to 3.32 mgm. daily, before castration. Three patients were injected with testosterone propionate,<sup>1</sup> 25 mgm. daily, in sesame oil.

The phosphatases of cerebrospinal fluid in 3 cases and of spermatocele fluid in 1 case were determined concurrently with those of the serum on 1 occasion. *In this paper, units of enzyme refer to 100 cc. of the specified body fluid.*

In 4 normal adult male dogs, serial observations of the phosphatases of the serum were made during 1 week before and 4 weeks after bilateral castration. Castration was done under ether anesthesia.

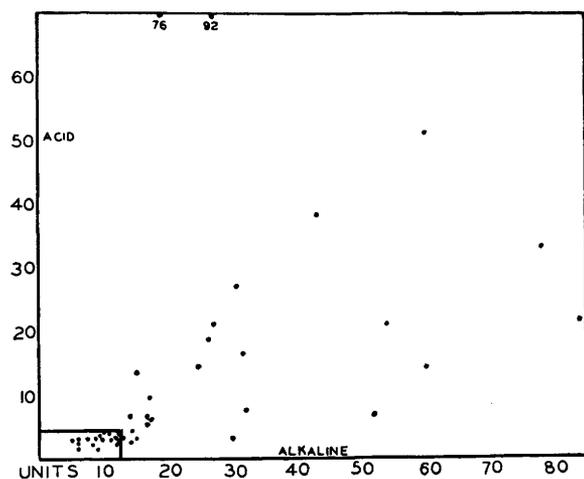


FIG. 1.—Scatter diagram of acid and alkaline phosphatase values in serum in carcinoma of the prostate. Ordinates, acid phosphatase; abscissae, alkaline phosphatase.

## RESULTS

*Values in carcinoma of the prostate and in controls.* The values with standard deviation in the control groups were: in the serum of 40 normal men, acid phosphatase  $3.25 \pm 1.37$  units, alkaline phosphatase  $8.15 \pm 1.74$  units. The upper limit for acid phosphatase is slightly higher than that of Gutman and Gutman (6) who used citrate buffer, whereas most of our determinations were made with acetate buffering. The nature of the buffer is not the cause of the difference obtained, however, since determinations made in parallel using citrate and acetate buffer yielded identical results.

The values in 47 cases of carcinoma of the prostate with and without demonstrable metastases are shown in Fig. 1. Of these 47 patients, 23 had acid phosphatase below 4.5 units and alkaline phosphatase below 12.5 units; of the remaining 24 patients all had elevation

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of alkaline phosphatase above 12.5 units, and 21 had acid phosphatase values above 5 units.

*Correlation between roentgenologic evidence of skeletal metastases and serum phosphatases.*—Positive x-ray evidence of metastasis of prostatic carcinoma to the bony pelvis was obtained in 25 cases. Both acid and alkaline phosphatase activities of the serum were increased above normal in 19 cases. Alkaline phosphatase alone was increased in 2 cases while acid phosphatase was within normal limits, and neither enzyme was increased in 4 cases. There was no evidence of metastasis on x-ray examination of the pelvis of 20 patients; in 16 men both acid and alkaline phosphatase were normal; in 3 men alkaline phosphatase was elevated but in none of this group was the acid phosphatase increased in amount. No deductions could be made from enzyme study of the serum with respect to the osteoplastic or osteolytic nature of the metastatic lesion.

*Effect of castration. Acid phosphatase.*—In each of 8 patients with prostatic cancer this activity rapidly decreased following bilateral orchietomy.

Following operation, in the case of P. R., there was a fall from 26 to 5 units in 7 days (Fig. 2); in the case of J. R. a decrease from 35 to 3 units in 9 days (Fig. 3); in the case of O. A. there was a fall from 25 to 5 units in 12 days; in the case of C. B. a decrease from 39 to 9 units in 12 days.

Following castration the decrease usually persisted in a range slightly above or below the upper limits of the normal values; it was maintained at this value with slight fluctuation during the period of observation which in the longest case has been 180 days.

The values observed in the case of P. R. varied from 2.5 to 7.5 units during 150 days; in the case of J. R. between 3 and 6 units in 104 days; in the case of C. B. between 7 and 9 units in 125 days; and in M. M. the values fluctuated between 9 and 15 units per 100 cc. in 180 days.

In one patient, S. R., acid and alkaline phosphatase levels of serum were 90 and 30 units respectively 163 days after orchietomy. No other enzyme studies were made on this man, who was the only patient with high enzyme levels after orchietomy.

In one patient, J. F., with benign prostatic hypertrophy who was subjected to castration, as previously reported by Huggins and Stevens (8), acid and alkaline serum phosphatases were 3 and 9.25 units, respectively, 2 years after orchietomy.

In 4 dogs, the values of the serum phosphatases were within the range found in normal men; bilateral orchietomy resulted in no essential changes in their levels.

*Alkaline phosphatase.*—A rise was always observed in the value of this enzyme following castration in this series of patients. This rise was then succeeded by a decrease to or towards the normal value.

In the case of J. R., the value at the time of castration was 19 units. The maximum value, 41 units, was attained 39 days following orchietomy, and at 104 days the value was 20 units. In the case of P. R. the pre-castration value was 22 units, the maximum value found was 27 units 10 days after castration, and

this value decreased to 9 units at 150 days. In the case of C. B. the precastration value was 82 units, the maximum rise to 94 units was seen 12 days following orchietomy, and the last value obtained was 35 units 125 days following testis removal.

**Effect of estrogen injection.**—Estrogen was given during periods of 8 to 23 days; all patients developed small palpable areas of subareolar breast tissue. The results were similar to those observed following castration; namely, a sharp decrease of acid phosphatase

**Effect of androgen injection.**—Testosterone propionate caused an increase of serum acid phosphatase above the pre-injection level in these patients. Following the cessation of injections, there was a decline to the preliminary level. In one patient this decrease was succeeded by a marked increase in acid phosphatase. There were no changes in alkaline phosphatase during the period of injection, but a rise occurred subsequent to treatment.

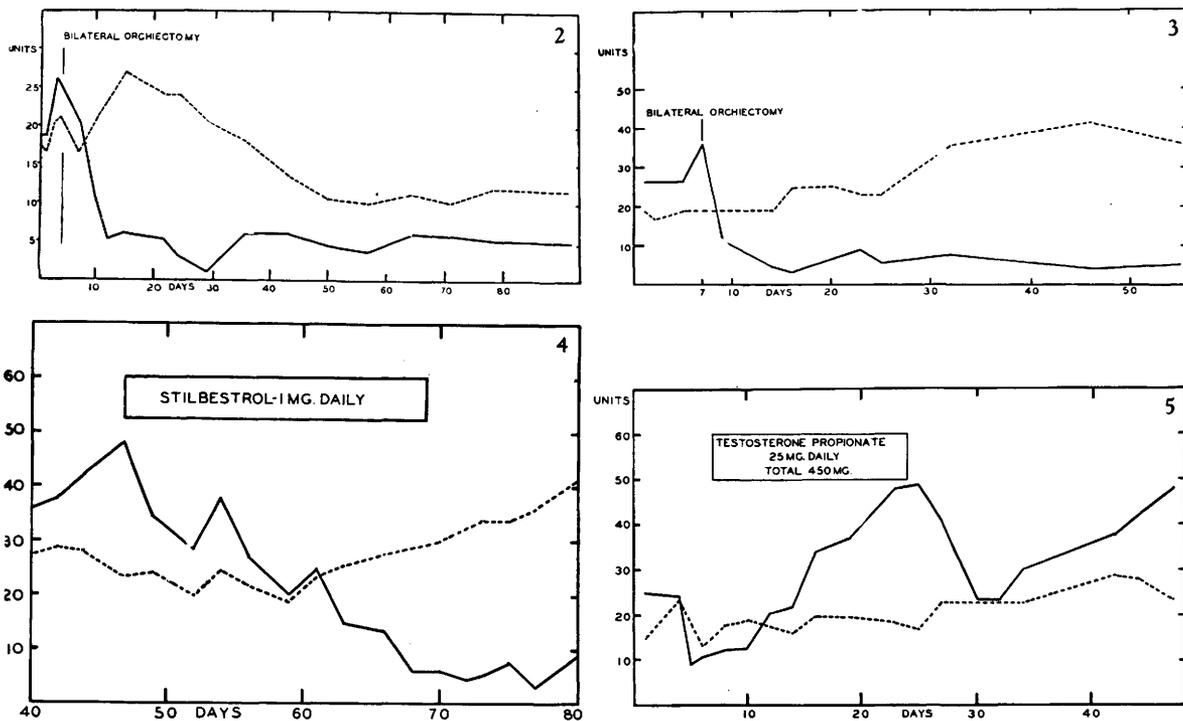


FIG. 2.—The effect of castration on serum phosphatases in metastatic carcinoma of the prostate in the case of P. R. Acid phosphatase —————; alkaline phosphatase - - - - -.

FIG. 3.—The effect of castration on serum phosphatase values in metastatic carcinoma of the prostate in the case of J. R. Acid phosphatase —————; alkaline phosphatase - - - - -.

FIG. 4.—The effect of estrogen injection, 1 mgm. stilbestrol for 23 days, on serum phosphatases in metastatic carcinoma of the prostate in the case of O. A. Acid phosphatase —————; alkaline phosphatase - - - - -. Ordinates, units per 100 cc. of serum; abscissae, time in days.

FIG. 5.—The effect of androgen injection, testosterone propionate, 25 mgm. daily for 18 days, on serum phosphatases in metastatic carcinoma of the prostate in the case of O. A. Acid phosphatase —————; alkaline phosphatase - - - - -.

accompanied by an increase of alkaline phosphatase in the serum.

The patient P. M. was injected with 7 mgm. stilbestrol and 46.5 mgm. estradiol benzoate in 27 days. The acid phosphatase of his serum decreased from 50 to 19.5 units while the alkaline phosphatase rose from 55 to 95.5 units. J. C. received 8 mgm. stilbestrol in 8 days. His acid phosphatase fell from 32 to 4.5 units while the alkaline phosphatase rose from 27.5 to 64.5 units. O. A. received 30 mgm. stilbestrol in 23 days (Fig. 4). Acid phosphatase of his serum decreased from 48 to 4.5 units while alkaline phosphatase increased from 23 to 41.5 units.

In the case of C. B., previously castrated, injected with 25 mgm. testosterone propionate daily for 15 days, acid phosphatase rose from 9 to 27 units; cessation of androgen injection was followed by a decrease to 9 units 9 days following injection. In the case of O. A., injected with 25 mgm. testosterone propionate daily for 18 days, acid phosphatase rose from 11 units to a high value of 48.75 units reached the day following completion of androgen injection. This value decreased to 23.5 units 6 days following cessation of androgens, whereupon it rose to 46 units in the next 27 days (Fig. 5).

**Distribution of phosphatases in body fluids in carcinoma of the prostate.**—The phosphatases were found

to be low in cerebrospinal fluid and in spermatocele fluid although the values were concurrently high in blood serum.

In the case of P. M., acid and alkaline phosphatases of the spinal fluid were 0.75 and 0 units at a time when these enzymes in the serum were 51 and 64 units respectively. Similarly in O. A., acid and alkaline phosphatases of spinal fluid were 0 and 0 at a time when the findings in serum were 23 and 22 units respectively. In the case of J. C., acid and alkaline phosphatases of spinal fluid were 0 and 1.5 units when these levels in serum were 19.5 and 46 units respectively. In the case of C. R., acid and alkaline phosphatases of spermatocele fluid were 0 and 1.5 when the serum levels were 49 and 62 units, respectively.

#### DISCUSSION

In disseminated carcinoma of the prostate the lability of acid phosphatase in serum as influenced by androgen supply or withdrawal indicates that this malignancy is influenced by the activity of male hormones in the host. With respect to serum enzymes, prostatic cancer is inhibited by decreasing androgenic hormonal function and activated by increasing the androgens. Moreover, our data, as yet unpublished, show that inhibition is not limited to the enzyme content of serum.

Certain bone diseases, as shown by Kay (9), are associated with increased alkaline phosphatase activity of serum and Roberts (14) discovered that certain liver diseases cause a similar increase. At the present time elevation of alkaline phosphatase of serum is regarded as indicating only bone or hepatic disease. There is no evidence to show that hepatic damage occurs with frequency in carcinoma of the prostate while extensive bone involvement by metastasis is often observed. It can be assumed with little risk that elevation of alkaline phosphatase of serum in carcinoma of the prostate is usually due to bone lesions, in the absence of gross evidence of hepatopathy.

Marked increases in acid phosphatase in serum have been observed only in prostatic carcinoma. Robinson, Gutman, and Gutman (15) found slight but definite elevations of this enzyme (the highest 8.7 units) in osteitis deformans and several other diseases where the bones were involved. The data in the present paper indicate that carcinoma of the prostate with skeletal metastasis is sometimes present with serum acid phosphatase values within the normal range. However, we found that when there was a marked rise of acid phosphatase (above 10 units) disseminated prostatic carcinoma was always present.

Gutman and Gutman (6) observed that there was no correlation between the height of serum acid and alkaline phosphatases in carcinoma of the prostate, and in general our data agree with their findings. This lack of correlation can now be explained. The elevated phosphatase levels in carcinoma of the prostate

are due to the action of two different types of cells, prostatic epithelium and osteoblasts. The activity of prostatic epithelium (as deduced from the height of serum acid phosphatase) in disseminated cancer of the prostate depends at least in part on the degree of activity of androgens present in the body, whereas elevation of alkaline phosphatase is dependent on increased osteoblastic function as a result of invasion of the bones by tumor. It is thus apparent that increased acid and alkaline phosphatase activities in serum may be considered separately since they indicate activity of different types of cells. The function of those cells contributing to the greater part of the moiety whose maximum occurs at pH 5 is dependent on androgen levels, and can be increased or decreased by adjusting the male sex hormone level.

Having established a relationship of androgenic hormones to carcinoma of the prostate, the findings in the case of O. A. (Fig. 5) can be interpreted. In this man the serum acid phosphatase had been greatly increased by androgen injections and, following cessation of androgen, the enzyme decreased for 1 week when it was succeeded by an abrupt spontaneous secondary rise. The decrease following cessation of androgen injection may be assumed to be the result of the depression of gonadal activity caused by androgen injections as indicated by Moore and Price (12). The subsequent rise is explained by postulating that the cancer cells had been functionally expanded by androgen and therefore responded in an augmented way to hormones produced by the recovering testis.

Castration caused a marked decrease of serum acid phosphatase but in some cases the enzyme level did not reach normal while in others it lay in a range at the upper limit of normal values. Much proof has been adduced that androgen is produced in extragonadal loci especially in the suprarenal gland. The most ready explanation for variations in serum acid phosphatase levels following orchiectomy as well as the failure of some values to reach the normal range may be postulated by assuming varying amounts or activity of androgens produced in extragonadal sources in different individuals. It must be pointed out however that urinary assays of hormone excretion were not done in this study. From the evidence one cannot exclude the possibility that foci of metastatic carcinoma as well as the original site might affect the levels of phosphatase and so maintain this enzyme at an abnormally high range even though there were no androgens produced in extragonadal areas. Against this alternative explanation is the fact that atrophic prostatic tissue contains merely traces of acid phosphatase as Gutman and Gutman (5) found in the infantile human prostate gland.

None of the phosphatases has been isolated as yet

in a pure state. The findings of none or small amounts of these enzymes in cerebrospinal or spermatocele fluids show that the enzymes are molecules which do not cross certain membranes readily from the blood and are not inconsistent with the evidence of Kutscher and Pany (12) that phosphatase is a protein.

#### SUMMARY AND CONCLUSIONS

1. The content in serum of phosphatase with activity maximum at pH 5 in normal men and in men with benign prostatic hypertrophy was  $3.25 \pm 1.37$  King and Armstrong units in 100 cc. Serum phosphatase with activity maximum at pH 9.3 in this control group was  $7.9 \pm 2.1$  units in 100 cc.

2. In a group of 25 men with roentgenologic evidence of metastatic carcinoma to the bony pelvis, both acid and alkaline phosphatases were increased above normal in 19 cases, only alkaline phosphatase was increased in 2 cases, and both values were within normal limits in 4 cases. Metastatic carcinoma of the prostate may be present in bones when the phosphatases of serum are normal, but when acid phosphatase is present in activity greater than 10 units in 100 cc., disseminated prostatic cancer is present.

3. In prostatic cancer with marked elevation of acid phosphatase, castration or injection of large amounts of estrogen caused a sharp reduction of this enzyme to or towards the normal range. Alkaline phosphatase values rose following castration and then decreased, but more slowly than acid phosphatase. In certain cases, these values reached and were maintained in a normal range during the period of observation, 180 days, while in other patients the values were slightly above normal.

4. In 3 patients with prostatic cancer, androgen injection caused a sharp rise of serum acid phosphatase. In 1 case following cessation of androgen there was a decrease of the acid phosphatase followed by a secondary spontaneous rise.

5. Acid phosphatase does not readily cross membranes from the blood, since it was present only in traces in cerebrospinal and spermatocele fluids when concurrent serum level was high.

6. Prostatic cancer is influenced by androgenic activity in the body. At least with respect to serum phosphatases, disseminated carcinoma of the prostate

is inhibited by eliminating androgens, through castration or neutralization of their activity by estrogen injection. Cancer of the prostate is activated by androgen injections.

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