Relationship of Prostatic Serum Acid Phosphatase to Age and Size of the Prostate in Rats

By R. H. WOJNAS† and J. F. BESTER

Serum of freshly killed rats of various ages was treated with *p*-nitrophenyl phosphate in citric acid buffer solution pH 4.8. Total serum acid phosphatase was determined colorimetrically on the basis of *p*-nitrophenol formation. Residual serum acid phosphatase was determined on duplicate serum specimens similarly, but with the addition of L (+) tartrate. Prostatic serum acid phosphatase activity was computed by subtraction. This activity was found to increase markedly until prostate weight reached approximately 200 mg. after which there was little change in activity. Increases in prostatic weight continued as body weight and age increased. Hence, in young animals prostatic serum acid phosphatase activity is indicative of normal prostatic growth and not necessarily indicative of abnormality.

PROSTATIC enlargement is one of the more common afflictions of the elderly male, occurring in from 30–40 per cent of men over 60 years of age. In general, such enlargement is due to benign hypertrophy, carcinoma, or fibrosis. Despite the fact that the resulting symptomology, if any, will show many similarities regardless of cause, it is obviously important that early differential diagnosis be made so that appropriate corrective measures may be undertaken.

As early as 1935 Kutscher and Wolbergs (1) reported that normal prostatic tissue is extraordinarily rich in a phosphatase having an optimum activity at about pH 5.0. It is present principally in the Golgi region of prostatic epithelium, while a second prostatic phosphatase with an optimum activity at about pH 10.5 is found in the basement membrane of the acini of the gland (2). The former, with optimum activity at pH 4.9, has been designated "acid" phosphatase; the latter has been designated "alkaline" phosphatase.

Significant amounts of acid phosphatase appear in blood plasma as a result of malignant growth of the prostate and metastases of prostatic cancer in the bones (3). It has been shown that prostatic acid phosphatase can enter the circulation very readily (4). Gutman, Sproul, and Gutman (5) noted the presence of acid phosphatase at the site of skeletal metastases secondary to carcinoma of the prostate. Huggins and Hodges (6) observed marked increases in serum concentration of acid phosphatase in prostatic carcinoma. They reported that the concentration could be decreased by reducing the activity of androgens by castration or by estrogen administration and increased by the injection of androgens. Gutman and Gutman (7) reported that serum acid phosphatase activity exceeded maximal normal values in 12 of 15 cases of disseminated prostatic carcinoma, but that there was no significant increase in such prostatic diseases as benign hypertrophy and acute prostatitis, nor in uremia or lymphosarcoma.

Because of observations such as these, the determination of serum levels of prostatic acid phosphatase has become a routine step in the diagnosis of prostatic cancer. Indeed, Bonner, *et al.* (8), discovered the presence of unsuspected prostatic carcinomas in five patients by this technique. They also reported that in three cases diagnoses of prostatic cancer based upon detection of nodules by finger palpation were

TABLE I.—BODY WEIGHT, PROSTATE WEIGHT, AND PROSTATIC SERUM ACID PHOSPHATASE ACTIVITY

Log Body Weight Gm. × 1000	Log Enzyme[a] Activity O.D. × 1000	Log Prostate Weight Gm. × 1000
5.704	2.188	2.542
5.688	2.215	2.427
5.664	2.387	2.762
5.661	2.270	2.708
5.652	2.241	2.971
5.652	2.428	2.185
5.647	1.973	2.348
5.644	2.215	2.707
5.644	2.366	2.772
5.631	2.260	2.697
5.615	2.164	2.603
5.580	2.310	2.430
5.185	2.649	1.398
5.121	2.808	1.301
5.093	2.573	1.114
5.090	2.742	1.398
5.079	2.792	1.176
5.079	2.845	0.699
5.072	2.937	1.230
5.068	2.822	1.000
5.068	2.887	1.079
5.065	2.438	1.230
5.041	2.640	1.362

[a] Activity expressed as optical density of solution.

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† Present address: School of Medicine, University of California, San Francisco.

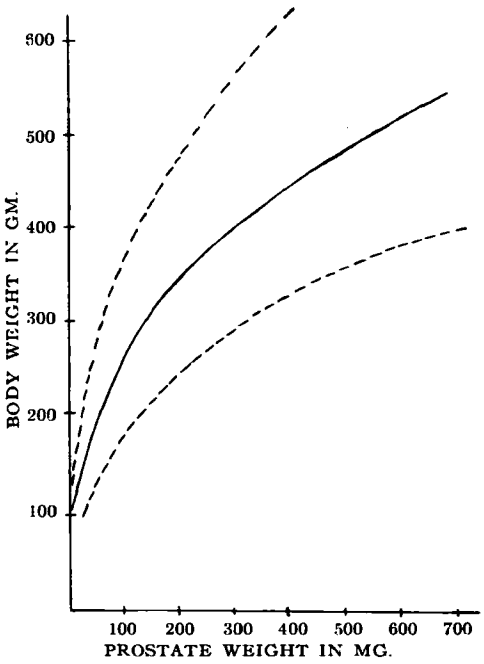

Fig. 1.—Relationship of body weight and prostate weight, showing mean values and 90% confidence limits.

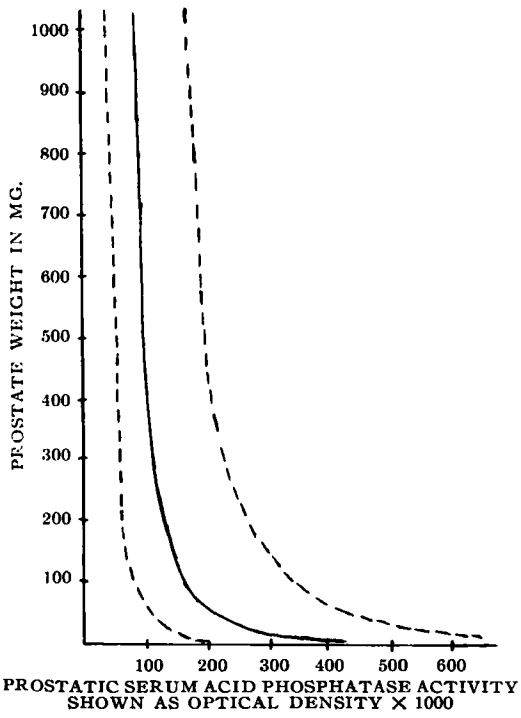

Fig. 2.—Relationship of prostate weight and prostatic serum acid phosphatase activity, showing mean values and 90% confidence limits.

shown to be incorrect by this test; the error was further shown by subsequent biopsy indicating benign hypertrophy.

Earlier work in our laboratory indicated that in some respects the prostate was markedly influenced by age (9). It therefore seemed important to determine whether prostatic serum acid phosphatase level and activity change appreciably at various age levels.

Various investigators have used *p*-nitrophenyl phosphate as a reagent for measuring phosphatase activity (10, 11). It is colorless, but the *p*-nitrophenol liberated by phosphatase hydrolysis is yellow and has a maximum absorbance between 400–420 mμ. The substrate is thus the indicator in the test. However, it is not specific for prostatic acid phosphatase in serum. As reported by Fishman and Lerner (12), erythrocytes increase serum phosphatase levels upon hemolysis. The same investigators showed that L-tartrate will inhibit prostatic serum acid phosphatase but will not inhibit other serum phosphatases. Thus, it is possible to determine total serum phosphatase activity of a specimen and then, by L-tartrate inhibition, to determine "residual" phosphatase activity. The difference between these will be prostatic serum acid phosphatase activity. This procedure formed the basis of the method used in the study being reported.

EXPERIMENTAL

Equipment and Reagents.—Wistar strain rats were used in the study. No restrictions in diet were observed. The rats were of two general age groups—about 6 weeks and about 18 months. The *p*-nitrophenyl phosphate reagent and the buffer solutions were obtained commercially as a prepared kit for phosphatase determination.[1] All other reagents used were Baker and Adamson reagent grade chemicals. Colorimetric determinations were made in a Beckman DU spectrophotometer.

Procedure.—After recording the age and weight of the rat, it was sacrificed in a closed jar of ether. An incision was made along the ventral midline of the body and 5–10 ml. of blood were removed from the aorta with a syringe. The blood was centrifuged in a refrigerated clinical centrifuge for approximately 10 minutes or until complete separation of serum had been obtained. The serum was then removed by pipet and frozen until used in the determination of its enzyme content.

The viscera of the rat were then pushed to one side and the prostate gland was teased from its surrounding organs and fat deposits. The lobes of the prostate were excised with a minimum of connective tissue. The gland was placed in a refrigerated calcium chloride desiccator overnight. The following day the gland was weighed and assayed for its total iodine content by the method of Menschenfreund (13). This procedure was dictated by the concurrent study (in this laboratory) of the

[1] Sigma Chemical Co., St. Louis, Mo.

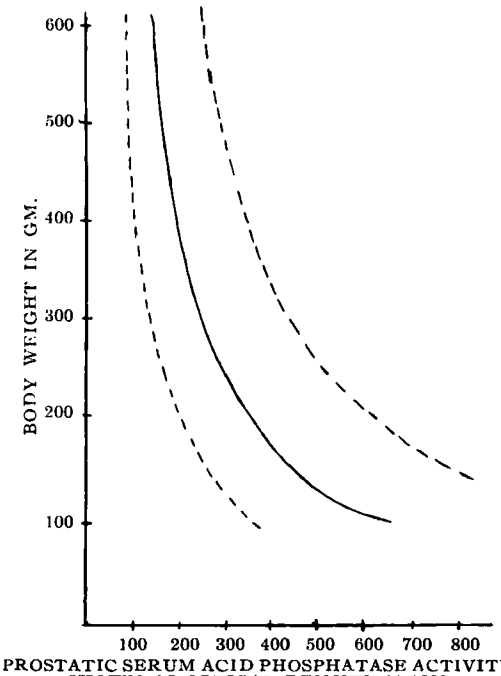

Fig. 3.—Relationship of body weight and prostatic serum acid phosphatase activity, showing mean values and 90% confidence limits.

possible role of iodine in prostatic function. However, this study is not part of the present report.

Total serum acid phosphatase and residual serum acid phosphatase activities were determined by standard procedures (14). The activity of prostatic serum acid phosphatase was obtained by subtraction.

It was unnecessary to prepare a standard curve for the Beckman DU spectrophotometer since relative rather than absolute values for phosphatase activity were being studied.

RESULTS AND DISCUSSION

Data obtained in the study appear in Table I. Logarithmic transformation of the data was necessary to reduce skewness. In all cases the data were multiplied by 1000 before determining their respective logarithms in order to avoid the use of negative computations.

Ages of the rats are not shown. Obviously, animals of approximately 18 months of age were much heavier than those of approximately 6 weeks of age. From examination of Table I, it is apparent that age is paralleled by body weight with an evident separation between the groups.

The data in Table I were then transformed to their respective arithmetic values; these were then used to show the relationships of interest in Figs. 1, 2, and 3.

The relationship between body weight and prostate weight shown in Fig. 1 has a t value of 15.9 with $p < 0.001$. As expected the greatest rate of increase in prostatic weight occurred in animals whose body weights were less than 200 Gm.

Figure 2 indicates the relationship between prostatic serum acid phosphatase activity and prostate weight. Here the t value was 6.64 with $p < 0.001$. This is rather remarkable correlation, and as Fig. 2 clearly shows, enzyme activity is greatest in those rats whose prostates weigh less than 200 mg., *i.e.*, in young rats. This is also indicated by Fig. 3; enzyme activity is markedly greater in young rats with body weights of less than 200 Gm. In this latter relationship, a t value of 8.59 with $p < 0.001$ was obtained.

Elevation of prostatic serum acid phosphatase activity has been accepted as an indication of prostatic cancer. Results of this study indicate that care must be exercised to prevent incorrect interpretation of the test in the young because activity of that enzyme is considerably higher in young animals.

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