the meeting of antigen and antibody in the tissues. The appearance of this inflammatory process probably further delays antigen elimination.

In our experiments, when serum was tested for radioactivity due to subcutaneously injected antigen, it was found that radioactivity in the serum of immune animals was always less than that of the control. This finding might have been anticipated from reports (7,8) demonstrating an accelerated rate of disappearance of intravenously injected antigen in immune animals. Data reported above show that less subcutaneous antigen reaches the blood stream in immune animals during the first 24 hours after injection.

*Summary.* An Arthus phenomenon delays removal of the antigen from the area. This is believed to be primarily due to the presence of antibodies. However, a non-specific effect due to inflammation must also be contributory to this slower removal of antigen because removal of a non-antigen from the Arthus reaction area is decreased.

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## Prostatic Fraction of Acid Phosphatase in Human Serum.* (26842)

P. ANGELETTI, B. MOORE, V. SUNTZEFF AND R. GAYLE
(Introduced by E. W. Dempsey)
*Wernse Cancer Research Laboratory, Washington University School of Medicine, St. Louis, Mo.*

There is evidence for non-identity of acid phosphatase from different tissues of the same species(1). Heterogeneity of this enzyme has been observed also within the same tissue where it may exist in several distinct forms(2,3). In serum, the level of enzyme activity represents the sum of several components, the characterization of which may be of diagnostic interest. Thus, the Fishman-Lerner method to measure that part of the activity in human serum which derives from the prostate gland has proved very useful in diagnosis of prostatic cancer. In fact, many clinical investigations demonstrate that most of the acid phosphatase of serum, which can be inhibited by L-tartrate, originates from the prostate gland(4,5,6). In this paper it will be shown that the tartrate sensitive fraction of acid phosphatase can be separated from the non-sensitive one by chromatography of serum proteins on anion exchange cellulose columns, and that the tartrate sensitive fraction has chromatographic properties similar to the enzyme extracted from prostate tissue.

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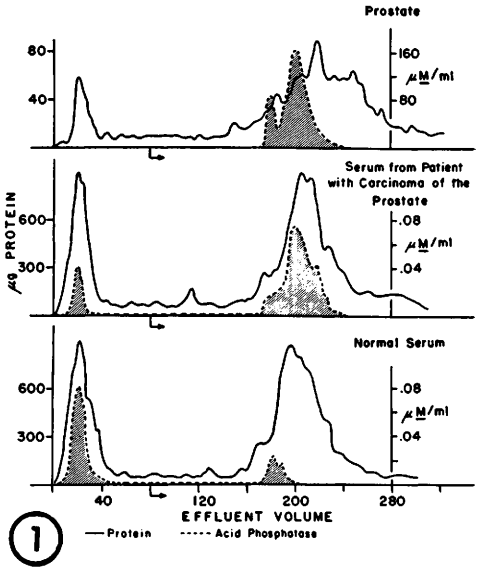
*Materials and methods.* Three samples of normal serum from young healthy males, 4 samples of serum from patients with benign



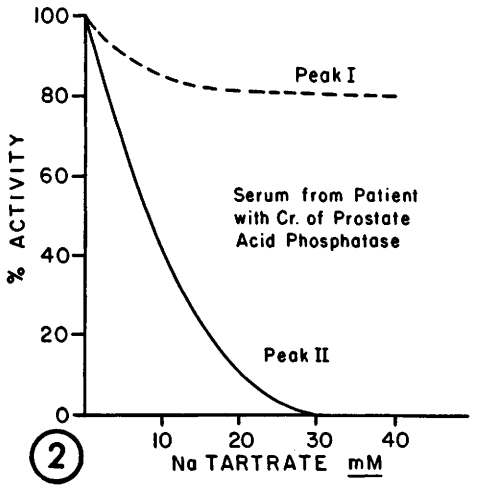




FIG. 1. Typical chromatograms on DEAE cellulose columns of normal serum, serum from patients with prostatic cancer and of soluble proteins extracted from prostatic tissue. Solid line is protein and broken line is acid phosphatase activity in μmoles/ml/hr of p-nitrophenol liberated from substrate at 38°C.

FIG. 2. Effect of L-tartrate on activity of the 2 peaks of serum acid phosphatase.

hyperplasia of the prostate and 4 samples of serum from patients with prostatic carcinoma were chromatographed. In 2 of the carcinoma cases serum was taken immediately before and one month following surgical operations. Three samples of prostatic tissue, one with prostatic carcinoma and 2 with benign hyperplasia, were also chromatographed.

Prostatic tissue was homogenized in 0.005 M tris-phosphate buffer pH 8.0 with a "Virtis" homogenizer and was then centrifuged at 13,000 rpm for 30 minutes. The clear supernatant from this extract and the samples of serum were dialyzed for 24 hours against the same buffer and stored frozen until used. Column chromatography was carried out on DEAE cellulose as previously described(7). A parabolic gradient elution to a final concentration of 1 M NaCl was used. Proteins were assayed in the fractions by the Lowry method(8). Acid phosphatase was measured in the fractions using p-nitro-phenylphosphate as the substrate(9). Protein recovery was about 80% and recovery for the enzyme activity after chromatography ranged between 70% and 80%.

*Results.* Fig. 1 shows typcial chromatograms of prostate tissue extracts, normal serum and serum from patients with prostatic carcinoma. Part of the protein was not bound to the column and was eluted directly by the starting buffer. The rest of the protein was then eluted by the gradient and separated into a number of peaks. The acid phosphatase activity in prostate tissue chromatograms was localized in 2 peaks between 0.07 and 0.16 M NaCl. In the chromatograms of normal serum the enzyme was fractionated into 2 main components. One corresponding to about 70%-80% of total activity was found with the protein fraction not bound to the DEAE, and a second one appeared after the gradient elution in a zone between 0.07 and 0.10 M NaCl. Two major components were also found in the serum from patients with prostatic carcinoma. In all these cases, however, the second fraction was spread out in a larger zone and represented from 60% to 80% of total activity. Localization of this more tightly bound fraction of serum acid phosphatase corresponded to the localization of the enzyme in the prostatic extract chromatogram (Fig. 1). As has been reported, prostatic acid phosphatase is completely inhibited by 20 mM sodium tartrate. Fig. 2 shows that the 2 chromatographically separated fractions of serum acid phosphatase have different sensitivities to tartrate. Where-

TABLE I. Distribution of Acid Phosphatase Fractions in Serum.

| | % of total activity | |
|---|---|---|
| | 1st peak | 2nd peak |
| Normal serum | 83 | 17 |
| | 72 | 28 |
| | 77 | 23 |
| Serum from patients with benign hyperplasia of prostate | 72 | 28 |
| | 58 | 42 |
| | 70 | 30 |
| Serum from patients with carcinoma of prostate | 32 | 68 |
| | 27 | 73 |
| | 41 | 59 |

as the first fraction was only slightly inhibited, the second was completely inhibited at concentration of 30 mM sodium tartrate.

The pattern of distribution of acid phosphatase in serum from patients with benign hyperplasia of the prostate was similar to that of normal serum. Two major fractions were found and most of the enzyme activity was associated with the first unbound protein peak, except for one case in which activity was evenly distributed between the 2 peaks (Table I). After rechromatography under the same conditions, each of the 2 peaks of enzyme activity appeared at the same position as during the first fractionation. In 2 cases of patients with prostatic carcinoma, samples of serum were taken one month after surgical removal of the prostate and subsequent estrogen treatment. In both cases the chromatographic pattern of acid phosphatase returned to normal. That is, the relative amount of activity in the second peak decreased to the normal proportion. The similar chromatographic behavior of the prostatic enzyme and of the second serum component, as well as their equal sensitivity to tartrate inhibition, indicate the probable prostatic origin of this fraction of the enzyme present in the serum. The marked increase of the second peak of serum acid phosphatase in patients with prostatic cancer, followed by a sharp fall soon after prostatectomy, gives further evidence that this peak corresponds to the prostatic fraction of serum phosphatase as determined by the Fishman-Lerner method.

*Summary.* Serum acid phosphatase represents a mixture of different molecular forms of the enzyme which can be separated and characterized. At least 2 major components can be separated by column chromatography with high recovery of the enzyme activity. The prostatic fraction of acid phosphatase appears, on the basis of its chromatographic behavior and its sensitivity to tartrate inhibition, to be localized in the second chromatographic peak.

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