

PROSTATIC ANTIBACTERIAL FACTOR*

Identity and Significance

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ABSTRACT — Normal human prostatic fluid possesses pronounced antibacterial activity. This activity is absent or markedly diminished in the fluid of men with chronic bacterial prostatitis. Ion-probe and mass spectrographic analysis of this antibacterial factor has shown it to be a zinc salt. Prostatic fluid zinc levels in 15 men with chronic bacterial prostatitis averaged 50 µg. per milliliter (range 0 to 139 µg. per milliliter). The zinc level in the expressed prostate secretion (EPS) of 49 control men averaged 448 µg. per milliliter (range 150 to 1,000 µg. per milliliter). There was no overlap in the range of zinc values between the two groups. The decrease in EPS zinc concentration in the patient population was not secondary to a decreased serum zinc level. Exogenous zinc given orally did not increase the EPS zinc level. The decrease in EPS zinc was not limited to infected prostatic fluid cultures and may precede the entry of bacteria into the prostate. The data presented suggest that zinc may serve as an *in vivo* defense mechanism against prostatic invasion and subsequent urinary tract infections in men.

Urinary tract infections occur more frequently in adult females than males. The reason for this difference is unknown. In adult males the majority of urinary tract infections are secondary to an initial prostatic infection which later ascends to infect the bladder urine.¹ For several years, we and others have been investigating the biochemical properties of prostatic fluid and have demonstrated that the normal human, canine, and rat prostatic secretions possess pronounced antibacterial activity.²⁻⁶ These observations have led to the purification and isolation of PAF (prostatic antibacterial factor) from prostatic fluid and semen.⁷ Positive identification of PAF by mass spectroscopy and ion-probe analysis has confirmed that the bactericidal activity of PAF is directly related to the total zinc concentration of the fluid and no other antibacterial agent was identified.

Studies involving patients with bacteriologically documented chronic bacterial prostatitis demonstrated a marked diminution or total absence of zinc in the EPS (expressed prostate secretion) of these patients as compared with normal males. This suggests that the cation may serve as an *in vivo* antibacterial defense mechanism against prostatic and urinary tract infections in humans.

In vitro studies of zinc, at concentrations normally found in prostatic fluid, have confirmed its bactericidal activity against a variety of gram-positive and gram-negative bacteria.

Material and Methods

Isolation, purification and identification of PAF

Canine and human prostatic fluid specimens were fractionated by linear gradient elution of 0-1N hydrochloride on a Dowex 50WX2 (H⁺) column.⁷ Crystallization of the PAF fraction occurred readily by raising the pH of the solution

*This study was supported in part by Research Grant No. 5 RO1 A110668, National Institute of Allergy and Infectious Disease, National Institutes of Health, U.S. Public Health Service.

above pH 9 with 1N sodium hydroxide, causing a fine precipitate to form. The crystals were separated by centrifugation for ten minutes at 2,000 g., the supernatant discarded, and the crystals dried in a vacuum desiccator over phosphorous pentoxide.

Ion-probe analysis of the crystals was performed by Dr. Kenneth Williams* in the department of geology (earth sciences) using an electro probe analyzer (micro) Model ARL EMX-SM.

Mass spectrographic analysis was performed by Dr. Alan Duffield* in the department of genetics using a Varian-MAT 711 high resolution mass spectrometer.

Amino acids were determined by Dr. Wilfred Pereira* of the genetics department using a Varian GC gas chromatograph with an OV-17 gas-chrom column. For this analysis, the PAF fraction from the Dowex column⁷ was evaporated to dryness, reconstituted with 6N hydrochloride, and hydrolyzed under nitrogen for eighteen hours at 100°C.

Zinc determinations

All glassware used for these determinations was soaked in 2N nitric acid for at least twenty-four hours, rinsed three times with deionized water, and stored in plastic containers to minimize contamination. A zinc nitrate standard was diluted with deionized water prior to analysis. Zinc measurements were made using an atomic absorption spectrophotometer calibrated for digital concentration readout.

All prostatic fluid and semen samples were diluted with deionized water prior to analysis. Dilutions of 1:100 to 1:500 were used for prostatic fluid and 1:100 or 1:200 for semen samples. All samples were analyzed in duplicate.

Clinical material

All patients in this study had culture-documented bacterial prostatitis.⁸ To minimize urinary contamination of prostatic secretions, all prostatic fluid specimens were collected after thorough stripping of the urethra to eliminate residual urine, or before urine specimens were obtained.

The patient group was comprised of 15 men ranging in age from thirty-two to sixty-three years, with a mean age of forty-nine years. These men all gave a history of recurrent urinary tract infections and had bacteriologic proof of chronic bacterial prostatitis.⁸ The normal group consisted

of men seen in the urology clinic for conditions other than bacterial prostatitis (hypertension, sterile calculi, and so on). An individual was not considered to be normal unless he had a negative past history for genitourinary tract infection. Also, all men had lower tract localization studies performed confirming the absence of bacterial prostatitis. In this group were 26 men, twenty-five to seventy-four years of age, with a mean of forty-eight years. The third group of men in the study were 23 patients with minimal to moderately symptomatic benign prostatic hypertrophy who were being evaluated prior to the start of antidiuretic therapy. These men ranged in age from fifty-one to eighty-one years, with a mean of sixty-eight years. As in the normal group, these men also had a negative history of infection, and the prostatic secretion was sterile on culture. Thus, a total of 49 men were proved to be free from prostatic bacterial infection and served as the control group.

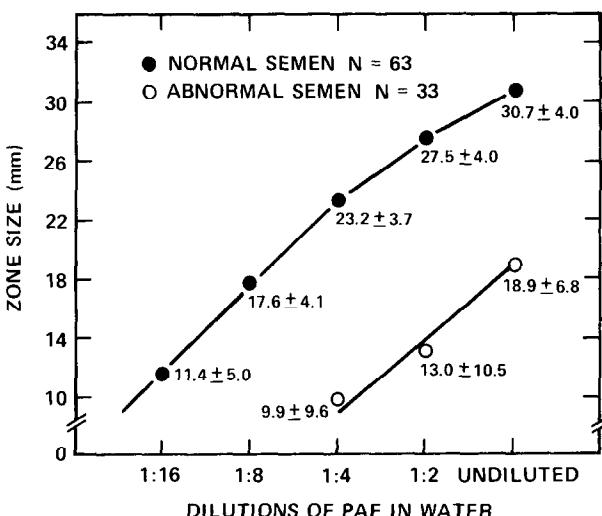


FIGURE 1. Diameter of zones of inhibition obtained with various dilutions of PAF in water. Closed circles indicate semen samples from men with no history of prostatic infections and negative prostatic fluid cultures. Open circles are values measured in semen of men with documented bacterial prostatitis.

Serum zinc levels were obtained by venipuncture in all cases prior to prostatic manipulation. In patients with prostatitis taking exogenous zinc supplementation no zinc tablets were taken for at least forty-eight hours before the blood was drawn. Ten ml. of blood were collected in a plastic syringe and transferred immediately into a glass tube previously washed with nitric acid as described previously.

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Cultures of known urinary tract pathogens obtained from the urology bacteriology laboratory were subcultured and grown in MOPS (morpholinopropone sulfonic acid) medium. This medium was previously shown to have minimal inhibitory effects on the PAF.⁷ Doubling dilutions of zinc sulfate in concentrations ranging from 7×10^{-5} M to 1×10^{-3} M were made in MOPS medium. The test organisms were incubated at 37°C. for eighteen hours; appropriate dilutions were made in MOPS medium and added to the tubes containing the zinc standard. The final concentration of bacteria was 1×10^3 to 1×10^5 bacteria per milliliter. A 0.1-ml. aliquot was taken from each tube immediately after mixing and placed on blood agar to determine the bacterial count at time zero. The tubes were then incubated at 37°C. and a similar aliquot removed and plated at four hours, six hours, and twenty-four hours later. The tubes were kept in an incubator at 37°C. between platings.

The Student *t* test was used to determine the significance between the experimental groups.

Results

Using the PAF fraction obtained via ion-exchange chromatography,⁷ the antibacterial activity of human semen or EPS in normal males and patients with documented bacterial prostatitis was quantified. Early in the study observations were made on secretions obtained following prostatic massage. Ten of thirteen samples (77 per cent) obtained from 5 patients documented as having chronic bacterial prostatitis had no PAF demonstrable; when bactericidal activity was present, the diameter of the zone of inhibition averaged only 12.4 mm. Six samples of EPS from normal males all had evidence of antibacterial activity with an average of 21.2 mm. diameter zone of inhibition. Because of the small volume of fluid obtained on prostatic massage, the quantitative difference in PAF activities between patients and controls was more easily and more accurately assessed using seminal plasma. In Figure 1 the diameter of the zone of inhibition shown by the PAF fractions from 63 seminal plasma specimens obtained from 49 controls is compared with the PAF activity of 33 seminal plasmas obtained from 15 men with chronic bacterial prostatitis. The diameter of the zone of inhibition obtained with the undiluted PAF fraction, isolated from the seminal plasma of the controls, averaged 30.7 ± 4.0 mm. Eight of 33 "patient" seminal plasma specimens had no demonstrable antibacterial ac-

tivity. Even in samples in which antibacterial activity was seen, the diameter of the zone of inhibition was much less (mean = 18.9 ± 6.8 mm.) than that found in the control specimens. A comparison on the dilution curve enabled us to quantitate PAF activity in the seminal plasma of patients (Fig. 1). Hence, a 1:4 dilution of a "normal" seminal plasma gave a larger zone size than the undiluted sample from the patient group, indicating that the mean PAF level in the patient's semen was less than 25 per cent of that expected to be present in normal subjects. However, serial semen samples from the same normal individual assayed over a period of several months varied by as much as 5 mm. from sample to sample, indicating a moderate degree of fluctuation in the amount of PAF normally present. Successive assays on the same sample over a two-month period did not vary by more than 4 per cent.

The PAF fraction, purified at least five thousand fold on a weight basis,⁷ was readily crystallized by increasing the pH to above 9 by adding 1N sodium hydroxide. After separation, no antibacterial activity was observed in the supernatant fraction. The resulting crystals were submitted for ion-probe and mass spectrographic analysis. Both analytical methods indicated that the crystals were predominantly zinc chloride. The PAF fraction was not composed entirely of zinc salts as shown by the results of amino acid analysis and gas chromatography on the acid hydrolyzed fraction. Under these conditions the following amino acids were also identified: alanine, glutamine, glycine, isoleucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine.

The experiments done following identification of zinc salts in the PAF fraction proved that the fraction of canine and human prostatic fluid possessing antibacterial activity and a zinc chloride standard solution were eluted from ion exchange and gel filtration chromatography columns in an identical volume of eluate. Those substances previously demonstrated as inhibitors of PAF activity, namely, agar, casein, tartrate, or other anionic agents,³ also inhibited the normal bactericidal action of zinc salts in solution. The zones of inhibition measured on the PAF assay of both canine and human prostatic fluid were directly proportional to the zinc concentration of these fluids. The addition of a zinc chloride solution in varying concentrations to the prostatic fluid gave an increase in the bactericidal action which was proportional to the amount of zinc added (Fig. 2). Additional indirect evidence presented in favor of

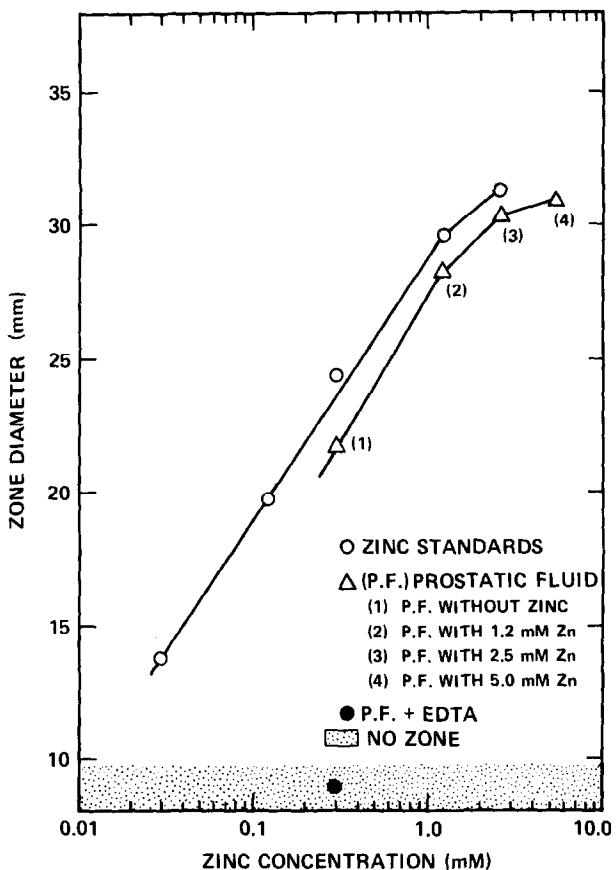


FIGURE 2. Diameter of the zone of inhibition of various concentrations of zinc, and prostatic fluid (P.F.) with increasing concentrations of added zinc. Shaded area represents diameter (9 mm.) of cylinder containing test solution.

the antibacterial action of prostatic fluid being directly related to its zinc content is the abolishment of the antibacterial effect when equimolar amounts of EDTA (ethylenediaminetetraacetic acid), a cation chelating agent, was added to the prostatic fluid (Fig. 2).

Expressed prostatic secretions, collected from control males and patients with chronic bacterial prostatitis, were analyzed for zinc. The zinc concentration of the prostatic fluid showed a wide variation among individuals. The range of prostatic fluid zinc level in 65 EPS specimens obtained from 49 men free from bacterial prostatic infection (normals plus benign prostatic hypertrophy group) was 150 to 1,000 μg . per milliliter with a mean value of 448 μg . per milliliter (Fig. 3). In sharp contrast, the zinc concentration in 61 specimens of EPS obtained from 15 patients with documented chronic bacterial prostatitis averaged only 50 μg . per milliliter. Several of the specimens had no detectable zinc present by this sensitive atomic absorption spectrophotometric

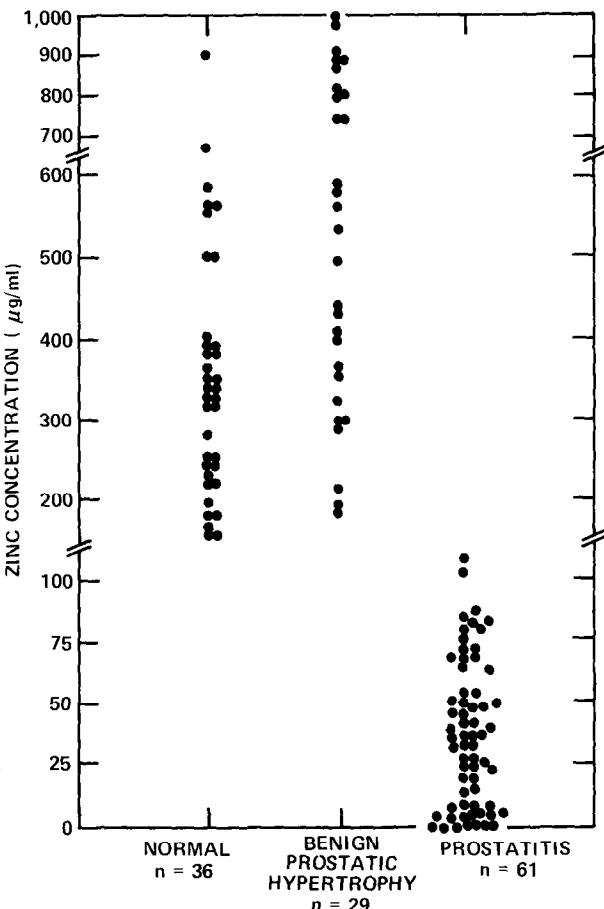


FIGURE 3. Zinc levels in expressed prostatic secretion obtained from men without prostatic disease (normal), those with benign prostatic hypertrophy but free from prostate infections, and men with documented bacterial prostatitis.

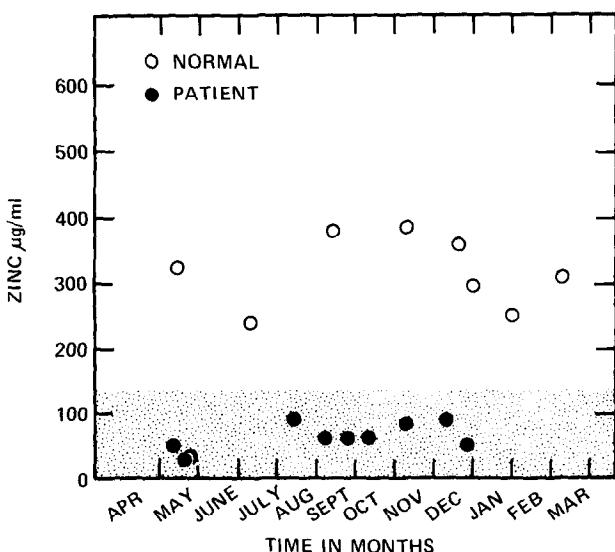


FIGURE 4. Serial zinc levels in expressed prostatic fluid of normal male and patient with bacterial prostatitis. Stippled area indicates range of EPS zinc levels found in patients with bacterial prostatitis.

TABLE I. Zinc concentration of human prostatic secretion

Study Group	No. of Samples	Zinc Concentration (μg . per ml.)	
		>150	<150
Normal men	36	36	0
Patients with benign prostatic hyper trophy	29	29	0
Patients with prostatitis	65	0	65
	p = <0.001		

method, and in no instance was a zinc level higher than 139 μg . per milliliter obtained. In the absence of such data in the literature, we chose to establish a value of 150 μg . per milliliter as the "lower limit of normal" for zinc in expressed prostatic secretions. Table I compares the prostatic fluid zinc levels of non-infected men with the same specimen obtained from patients with documented bacterial prostatitis with respect to this value.

The zinc level in the prostatic fluid of a given individual appears to be relatively constant, particularly in the patient with prostatitis. Figure 4 illustrates typical serial prostatic fluid zinc levels found in 2 men (1 patient and 1 normal) from whom we obtained multiple EPS collections, each of which was preceded by at least three days of sexual abstinence. Considering the value of 150 μg . per milliliter suggested previously as the lower limit of normal for zinc concentration in the EPS of normal men, it is apparent that the zinc level of the EPS in the normal man remained well above this value in multiple EPS specimens obtained during a twelve-month period. Similarly, the patient with a documented chronic bacterial prostatitis continued to have a low EPS zinc level over the course of follow-up. In the example shown in Figure 4, the patient was asymptomatic and had sterile urine while on prolonged suppressive therapy with 100 mg. minocycline hydrochloride twice daily, although direct cultures

of his EPS confirmed the presence of small numbers of *Pseudomonas* and the persistence of chronic bacterial prostatitis which was unaffected by the antibacterial therapy.

Table II lists the serum zinc levels found in 61 samples from control men and 42 samples from patients with bacterial prostatitis. As with the EPS levels, there was no appreciable difference between the "normal" males and those with benign prostatic hypertrophy with respect to serum zinc levels; both are included in the control group. The mean value of 0.89 μg . per milliliter in the controls was not significantly different from the mean value of 0.80 μg . per milliliter found in the patient population. Hence, we cannot classify a given individual as being in the control or patient groups solely on the basis of the serum zinc level.

We studied the effect of exogenously administered zinc salt on serum and prostatic fluid zinc levels in 7 patients with bacterial prostatitis. The patients received between 50 and 100 mg. of elemental zinc orally per day for periods of three to six months. Serum zinc levels increased from a mean value of 0.80 μg . to 1.08 μg . per milliliter, but prostatic fluid zinc levels did not undergo any appreciable change from the pretreatment levels while the patients were on this supplementation. More importantly, the additional oral zinc had no effect on the course of the infection, and the prostatic fluid cultures were still diagnostic of chronic bacterial prostatitis.

During the course of these studies 2 patients initially presenting with bacteriuria and subsequently diagnosed as having chronic bacterial prostatitis were observed to undergo complete clearing of the prostatic infection following antimicrobial therapy and remain uninfected for periods varying from six to twelve months. One of these patients will be discussed in detail (Fig. 5).

Case Report

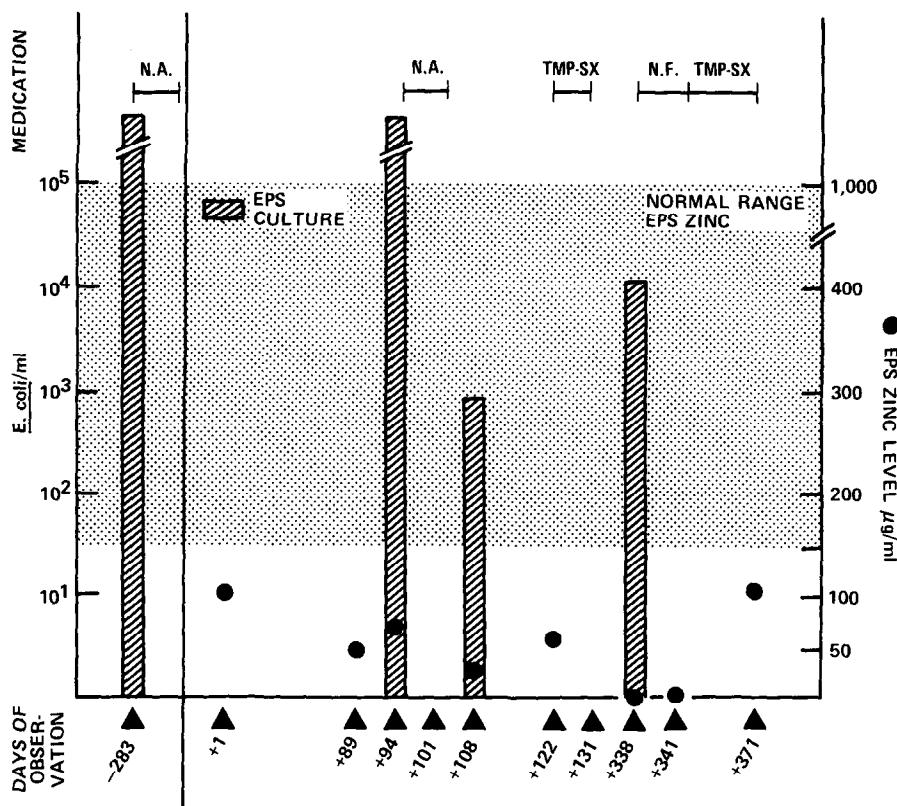
A forty-eight-year-old white male was first seen by one of us (W. R. F.) in July, 1972, with a history of recurrent urinary tract infections. Segmented

TABLE II. Serum zinc levels

Group	No. of Samples	Age (Years)	Concentration (μg . per ml. S.D.)	Range
Control	61	41 to 81 (62)*	0.89 \pm 0.17	0.60 to 1.28
Patients with prostatitis	42	27 to 70 (52)	0.80 \pm 0.14	0.58 to 1.16

*Figures in parentheses represent mean age.

FIGURE 5. Results of serial prostatic fluid zinc levels and cultures in patient in Case Report. Each culture is indicated by black triangle. Where no vertical striped bar is shown on graph, EPS culture was sterile. Black circles indicate EPS zinc levels. Days of observations are expressed as minus or plus the day of the first EPS zinc determination. N.A. = nalidixic acid; TMP-SX = trimethoprim-sulfamethoxazole; N.F. = nitrofurantoin.



urine and prostatic fluid cultures done elsewhere confirmed the diagnosis of chronic bacterial prostatitis due to an *Escherichia coli* infection. From January, 1971, to July, 1972, he had multiple episodes of urinary tract infection characterized by frequency, dysuria, nocturia, perineal pain, and occasional fever and chills. He had no nausea, vomiting, or flank pain. An intravenous urogram was normal, the prostate was not grossly enlarged, and no prostatic calculi were seen. The bladder appeared to empty normally with no significant residual. Physical examination was normal, and the prostate was minimally enlarged and firm but without nodules or tenderness. Initial urine and prostatic fluid cultures (day 283) contained greater than 10^5 *E. coli* per milliliter. The patient was treated with nalidixic acid 500 mg. four times daily for ten days; his symptoms subsided, and urine cultures became sterile.

He had no further difficulty for more than one year; during this time he received no antibiotics. In this one-year interval EPS zinc concentration was measured on two occasions and found to be 100 μg. per milliliter (day one) and 49 μg. per milliliter (day 89). Both values are well below normal levels. Urine and EPS cultures were sterile both times. Five days later (day 94) or 377 days since his last urinary tract infection, lower

tract symptoms developed. Urine and EPS cultures grew $> 10^5$ *E. coli* per milliliter. He was again treated with nalidixic acid and his symptoms disappeared. Urine and EPS cultures were sterile one week later while the patient was still taking antibiotics. He returned for a follow-up visit four days after stopping the medication (day 108); a mid-stream urine culture contained only 90 *E. coli* per milliliter but the EPS grew out 1,000 *E. coli* per milliliter confirming the prostatic source of his infection.

Twelve days later the patient again became symptomatic. He was seen by another physician who prescribed trimethoprim-sulfamethoxazole (TMP-SX) two tablets three times daily but did not obtain a urine or prostatic fluid culture. His symptoms promptly cleared; and when seen in our clinic (day 122), his cultures were sterile. An EPS zinc at this time was again markedly depressed at 60 μg. per milliliter. The patient returned nine days later (day 131); at this time he was asymptomatic and had discontinued the medication. He was seen three more times during the next six and one-half months; the urine cultures remained sterile and his EPS zinc levels were below 100 μg. per milliliter.

Almost eight months after his last positive EPS culture he again became symptomatic; urine and

TABLE III. *In vitro antibacterial activity of zinc sulfate*

Organism	No. of Strains Tested	Sensitivity (μg . per ml.)
E. coli "common"	19	20.4
E. coli "uncommon"	21	10.2
Pseudomonas species	5	40.8
P. mirabilis	6	81.6
Klebsiella species	5	5.1
S. faecalis	5	>650.0
S. albus	5	20.4

EPS cultures confirmed another urinary tract infection (day 338), and he was treated with nitrofurantoin to clear the bacteriuria and placed on suppressive antibacterial medication (TMP-SX). Two urine and prostatic fluid cultures have been sterile since that time. The EPS zinc levels, however, remain depressed.

This patient had periods of more than seven and twelve months during which the EPS cultures were sterile, he was asymptomatic and received no antibiotic therapy. However, multiple EPS zinc determinations during these periods revealed that the zinc concentration in the EPS was markedly depressed. It appears that the decrease in prostatic fluid zinc levels is not due simply to bacteria in the prostatic secretions. Rather, it appears as if the marked drop in EPS zinc concentration preceded the bacterial invasion and did not return to normal levels even when the prostatic fluid was sterile and the patient had discontinued antibiotic therapy. Unfortunately, serotyping of the organism was not done so that it is not possible to state whether or not a new organism was responsible for each infection, but the sterile urine and prostatic fluid cultures and the lack of symptoms for more than one year in the absence of antibiotic ingestion would suggest that relapse from a chronic prostatic infection was unlikely.

The antibacterial activity of various concentrations of a zinc sulfate solution was tested against a variety of bacteria isolated from the urine of patients with urinary tract infections. The organisms were "sensitive" to a particular level of zinc, expressed as micrograms per milliliter, only if all the strains tested at that level had a 50 per cent or greater decrease in the number of bacteria within six hours after the addition of the zinc salt to a culture of the organism in MOPS medium.

As shown in Table III the sensitivity of the organisms varied widely, but with the exception of *Proteus mirabilis* and *Streptococcus faecalis* all of the organisms were sensitive to levels of zinc

that are easily attained in the prostatic fluid of most human males, even those with chronic bacterial prostatitis.

The 66 strains tested varied in sensitivity to zinc from 5.1 μg . for *Klebsiella* to > 650 μg . per milliliter for *S. faecalis*. Despite the relative resistance of the *S. faecalis*, *S. albus*, the other gram-positive organism tested, was very sensitive at the level of 20.4 μg . per milliliter. No attempt was made to study the effect of protein binding or the possibility of a "in vivo" zinc inhibitor in this study.

To determine if a differential response to zinc could be of any significance in the observation that relatively few strains of *E. coli* are responsible for the majority of human urinary tract infections, we also quantified the response of the strains commonly found as urinary tract pathogens, that is "common *E. coli*" (01, 04, 06, 07, 075) versus the sensitivity of strains rarely found to be uropathogens (034, 043, 053, 063, 0103). The "uncommon" *E. coli* were slightly more sensitive to the effect of the zinc salt, but the differences between the two groups were not significant.

Comment

In 1967 we reported on the antibacterial activity of prostatic fluid.³ Unknown to us at that time, similar observations were made almost thirty years previously by Youmans, Liebling, and Lyman.² In both these reports the experiments were done on canine prostatic fluid, and it appeared that the antibacterial activity described was due to a similar substance. Since that time additional experiments in our laboratory confirmed antibacterial action in the fluid of the dorsolateral lobe of the rat prostate⁶ and in the seminal plasma or prostatic fluid of human males as reported here. Furthermore, the EPS or seminal plasma obtained from the normal human male quantitatively had a much greater antibacterial activity than that found in men with proved bacterial prostatitis and raised the possibility that the PAF might serve as a defense mechanism against prostatic and urinary infections in the male.

Purification and crystallization of this material led to identification of the antibacterial activity of prostatic fluid as being related to its concentration of zinc. The high zinc content of prostatic tissue was first observed by Bertrand and Vladescu in 1921.⁹ Later other investigators reported that the prostate contained more zinc than any other organ in the body.^{10,11} Only about 20 per cent of the zinc in the prostate can be accounted for by carbonic

anhydrase and other enzyme systems.¹⁰ In 1962 McKenzie, Hall, and Whitmore¹² measured the zinc level in expressed human prostatic fluid and reported that the fluid contained more zinc on a dry weight basis than any human tissue or any other human secretion. The reason for the high level of zinc in the prostate and its secretion is not known.

Tissue levels of zinc have been reported as being altered secondary to various prostatic disease states.¹³ Mawson and Fischer¹⁴ noted that the amount of zinc present in the prostate was directly proportional to the amount of glandular (alveolar) tissue present. They reported a decrease in the tissue zinc in one specimen with a histologic diagnosis of chronic prostatitis. Hoare, Delory, and Penner in 1956¹⁵ also documented decreased tissue levels of zinc in some patients diagnosed as chronic bacterial prostatitis on the basis of histology.

Several authors have commented on the decreased levels of zinc in the seminal plasma of patients with prostatitis.^{16,17} However, the zinc level of the expressed prostatic secretion was not measured in these studies. More importantly, the diagnosis of chronic prostatitis was made by the microscopic finding of more than 10 white blood cells per high-powered field, and bacteriologic studies were not done. Microscopic examination of EPS is highly inaccurate in diagnosing chronic bacterial prostatitis,¹⁸ a diagnosis that can be made only by actually culturing the organisms from the prostatic secretion.⁸ Many patients with symptoms of prostatitis do not have a bacterial infection; the term "prostatosis" has been suggested for this uninfected symptomatic condition.¹⁹ As a result of their own investigations and a review of the literature, Broström and Andersson¹⁶ concluded "that the values noted in the group with chronic prostatitis overlap those without demonstrable inflammation. Although a low value suggests prostatic inflammation, such a condition is not excluded by a high one. This limits the diagnostic value of these determinations." In our study no overlap was noted; we believe that this is due to the fact that only those patients with unequivocally documented prostatic infections were included in the patient group.

Of particular significance are a few patients that we have been able to follow between episodes of infection. The patient in the case reported here was asymptomatic and free of prostatic infection as determined by sterile EPS cultures for more than one year, yet persisted with low EPS zinc levels and later another prostatic infection de-

veloped. This pattern of repeated episodes of infection or relapse is characteristic of chronic bacterial prostatitis and may be due to a decrease in normal defense mechanisms. The prostatic fluid and seminal plasma of patients with chronic bacterial prostatitis showed a marked decrease in antibacterial activity normally present in these secretions. The antibacterial activity of the EPS specimens appeared to be directly related to the zinc content of the fluids. Hence, a decreased zinc content in prostatic fluid may be a necessary prelude to bacterial growth and multiplication in prostatic fluid. It is thus possible that the zinc content of prostatic secretion serves a role as a defense mechanism in the normal male, rather than a decreased zinc level being simply a secondary effect of bacterial growth. This may also help to explain the much lower incidence of urinary tract infections in the male as compared with the female.

The *in vitro* study included here confirms the marked sensitivity of the usual urinary tract pathogens to a zinc salt at concentrations far below those normally present in the prostatic fluid. In these experiments, 55 of the 66 organisms tested were sensitive to levels of zinc that were much below normal levels and similar to those found only in patients with chronic bacterial prostatitis. However, no attempt was made to study the effect of anionic binding agents or the effect of other *in vivo* zinc inhibitors in this study. The antibacterial activity of zinc is well documented.^{20,21}

Recently, attempts have been made to elucidate the nature of the vehicle for zinc secretion in the prostate. Johnson, Wickström, and Nylander²² characterized a zinc containing polypeptide which on hydrolysis yielded arginine, aspartic acid, serine, glutamic acid, proline, glycine, alanine, and valine. Reed and Stitche²³ in a study of benign hypertrophic human prostatic tissue also identified at least one zinc-binding protein. This protein on analysis was characterized by large quantities of histidine and alanine,²⁴ although subsequent experiments from the same laboratory have shown that the high histidine level is a variable finding. Our studies indicate that the PAF fraction contained in addition to zinc, a peptide which yielded 11 amino acids when hydrolyzed, including all those described by Johnson and associates.²² The role the zinc-binding protein in prostatic secretion plays in determining the over-all level of zinc in the same fluid is not known.

While a slight decrease was observed in the mean serum zinc level in the prostatitis patients,

the magnitude of the decrease in the EPS levels in the patient group far exceeded the serum changes, that is, the difference in prostatic fluid zinc levels was not simply a reflection of a similar change in serum values. In addition, serum zinc levels have been shown to be decreased in a variety of bacterial infections^{25,26} secondary to release of an endogenous mediator from polymorphonuclear leukocytes.²⁷ The decrease in serum zinc observed in the patient group may be a response to a bacterial infection and have no direct relationship to the lower EPS zinc level.

In summary, we have identified the "prostatic antibacterial factor," responsible for the antibacterial activity of normal prostatic fluid, as a zinc compound. It appears as if the bactericidal activity of the EPS is related to the amount of zinc present in the fluid and may play a role in the natural resistance of the male urinary tract to infection. In addition, the determination of the zinc content of the expressed prostatic secretion may be a useful test in diagnosing patients with chronic bacterial prostatitis or those who are likely to be susceptible to prostatitis. The factors responsible for the marked decrease of zinc in the prostatic fluid of patients with bacterial prostatitis and methods of altering the zinc level in the fluid as a possible means of eradicating chronic bacterial prostatitis or increasing the resistance of the patient to the disease, are important questions requiring further study.

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