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Urological Research

The Effects of Heparin on the Adherence of Five Species of Urinary Tract Pathogens to Urinary Bladder Mucosa

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Summary. Previous studies performed in our laboratory have indicated that the primary antibacterial defense mechanism of the rabbit urinary bladder is the antiadsorptive action of the surface mucopolysaccharide. Removal of this layer with an acid rinse increases bacterial adherence up to 100 fold. Exogenous mucopolysaccharide (heparin) has been shown to restore *Escherichia coli* adherence to control levels. To determine whether this antiadherence action of heparin is species specific, we compared the adherence of 5 common urinary tract pathogens (*Escherichia coli, Klebsiella ozonae, Proteus mirabilis, Pseudomonas aeruginosa*, and *Streptococcus fecalis*) to both mucin intact and mucin deficient rabbit bladders with and without prior heparin exposure. Bacteria were radiolabeled by addition of ^{3}H-adenine to the culture broth so that the number of bacteria adhering to the bladder could be determined using liquid scintillation spectrophometry. Results were as follows: 1) Acid removal of the mucin layer significantly increased the adherence approximately 10 fold for all 5 species tested. 2) Briefly exposing the mucin deficient bladders to heparin decreased the adherence of all species tested except *Pseudomonas* to mucin intact control levels. 3) Heparin treatment of mucin intact bladders slightly decreased adherence of all species except *Pseudomonas* below mucin intact controls, however, results were not statistically significant. 4) The magnitude of *Klebsiella* adherence was nearly 20 fold greater than all other species tested. While this non-species specific adherence inhibition of heparin may prove useful in the clinical setting, it appears to be less effective against *Pseudomonas*.

Key words: Heparin, Bladder mucosa, Bacterial adherence.

Introduction

One of the primary antibacterial defense mechanisms of the urinary bladder is the action of the luminal mucopolysaccharide layer. Acid removal of this sialomucin layer increases *Escherichia coli* (*E. coli*) adherence to rabbit urinary bladder up to 100 fold [1] and mucin resynthesis over time correlates with decreased *E. coli* adherence [2, 3]. The adherence of *Klebsiella pneumoniae, Staphylococcus aureus*, and dead or metabolically inhibited *E. coli* similarly increases upon removal of the mucin layer [4]. Chronic bacteriuria has been shown to have no deleterious effect on the antiadherence properties of the luminal sialomucin [5].

It has also been shown that the antiadherence activity of the endogenous mucin layer can be duplicated by brief exposure to small amounts of an exogenous mucopolysaccharide such as heparin [6–8] or sodium pentosanpolysulfate [9]. The antiadherence mechanism of action of heparin appears to be unrelated to its anticoagulant properties [10].

An exogenous glycosaminoglycan such as heparin with nonspecific antiabsorptive action may prove clinically useful in the prophylactic treatment of patients subject to bladder infection [7, 11]. In order to determine whether heparin's antiadherence effect is nonspecific regarding bacterial species as has been shown for the luminal mucopolysaccharide or species specific as some of the simple sugars are thought to be [12], we tested heparin against the adherence of 5 common urinary tract pathogens.

Methodology

Bacterial Strains

Lyophylized bacterial type or neotype strains were purchased from the American Type Culture Collection (ATCC, Bethesda, Maryland): *E. coli* 11775, *Proteus mirabilis* 29906, *Pseudomonas aeruginosa* 10145, and *Streptococcus fecalis* 19433. An in-house strain of *Klebsiella ozonae* was used.

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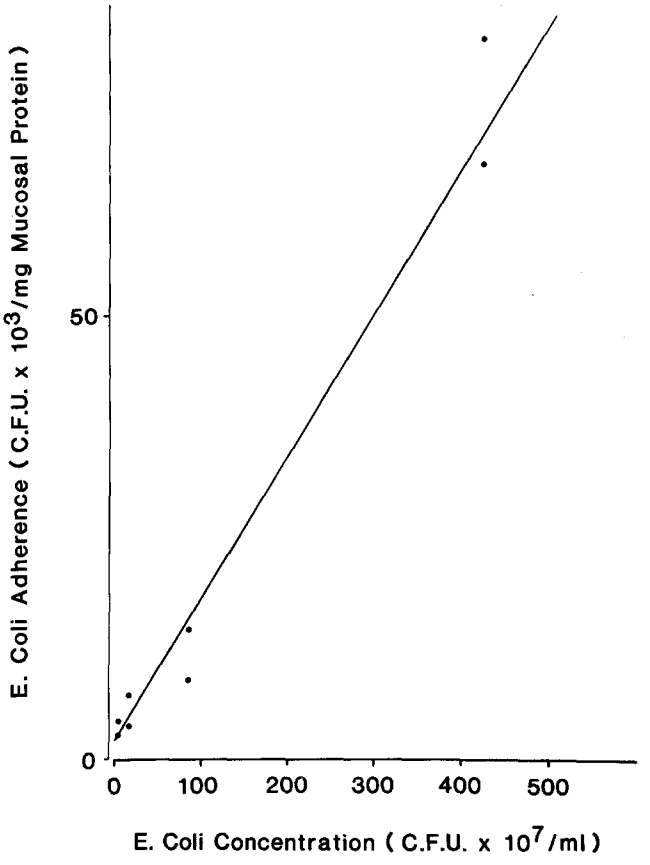

Fig. 1. Effect of *E. coli* concentration on adherence. Each *point* represents the mean of triplicate determinations from an individual mucin deficient bladder as described in Methods

Preparation of Bacteria

Bacterial strains were rehydrated in sterile 0.9% NaCl and serially passed repeatedly in brain-heart infusion broth (BHI; Difco Laboratories, Detroit, Michigan) at 37 °C. Species identification was verified periodically using the API20E for gram negative organisms and the API20S for *Streptococcus fecalis* (Analytab Products, Plainview, New York). To label the organisms with ^{3}H, a 0.4% inoculum (v/v) was added to 4 ml of fresh BHI containing 5 μCi ^{3}H-Adenine (New England Nuclear, Boston, Massachusetts) per ml and incubated at 37 °C for 18–24 h. The bacteria were sedimented at 3,000 x g for 20 min and resuspended in their original volume of 0.9% NaCl. Colony forming units (CFU) were determined by making serial 10 fold dilutions in 0.05% Tween 80. A 0.025 ml volume of each dilution was placed on tripticase soy agar supplimented with 5% (v/v) yeast extract and incubated at 37 °C overnight. After incubation, the numbers of colonies of the various bacterial species were counted on plates containing 10 to 30 colonies per dilution and the colony forming units per ml of the original suspension was calculated.

Basic Model

Male New Zealand white rabbits weighing 4–4.5 Kg were anesthetized with 1.5 ml Ketamine/Zylazine (7 : 5 v/v) I.M. followed by 1 ml Nembutal (50 mg/ml) I.V. Rabbits were secured, the urinary bladders were catheterized with a #8 catheter and the bladders were emptied. Prior to introduction of bacteria the bladder was flushed with 3, 10 ml aliquots of 0.9% NaCl introduced through the catheter. Approximately 8 x 10^8 CFU ^{3}H labeled bacteria were introduced into the bladder and flushed in with 5 ml of 0.9% NaCl. After 20 min exposure to the labeled bacteria the bladder was emptied and flushed with 3, 10 ml aliquots of 0.9% NaCl. The animal was then sacrificed, the bladder removed and the mucosa was dissected free from the underlying muscle layer and assayed for ^{3}H activity.

Control experiments were performed as follows: adenine-labelled bacteria were removed by filtration through a 0.45 μ milipore filter and the filtrate (which contained unincorporated adenine) was introduced into an acid-treated bladder. The bladder was then treated as described above. No significant labelling of the mucosa occurred in the absence of the bacteria.

Acid Treatment of Bladder

Prior to introduction of bacteria into the bladder 10 ml of 0.4 N HCl was infused through the catheter, left in the bladder for 1 min and removed. The bladder was then flushed with 3, 10 ml aliquots if 0.5 M dibasic potassium phosphate followed by 3, 10 ml aloquots of 0.9% NaCl. Labeled bacteria were then introduced.

Heparin Treatment of Bladder

Immediately after the acid treatment or after flushing the bladder with 3, 10 ml aliquots of 0.9% NaCl; 0.25 ml of sodium heparin (Pan Heparin 20,000 U.I./ml, Abbott Laboratories, Chicago, Illinois) suspended in 5 ml of 0.9% NaCl was infused through the catheter and left in the bladder for 15 min. The bladder was emptied and labeled bacteria were then introduced.

Recording of Radioactivity

Bladder mucosa were homogenized overnight in 1 ml of 1.0 N NaOH at 37 °C. The volume was then brought to 5 ml with 0.9% NaCl and 1.5 ml triplicates were placed in 20 ml glass scintillation vials and acidified with 0.1 ml glacial acetic acid. The remaining 0.5 ml of mucosal homogenate was used for determination of protein content by the method of Lowry [13]. Eighteen ml of Hydroflour (National Diagnostics, Somerville, New Jersey) was added and the vials were vortexed. 0.1 ml triplicates of the bacterial suspensions were also suspended in 20 ml of Hydroflour. The radioactivities of the tissue and the bacterial viable count (CFU) to ^{3}H uptake per ml of bacterial suspension were used to convert counts per min to the actual number of bacteria (in colony forming units) attached to the mucosa per milligram mucosal protein.

Results

Preliminary studies showed that *E. coli* adherence to the mucin deficient bladder was unaffected by the specific radioactivity of the bacteria and was linear with bacterial concentration well over the range utilized (Fig. 1). These results are important since they allow comparison of data collected on different days with different bacterial concentrations and specific activities.

Figure 2 displays the comparative adherence of the five species of bacteria tested to mucin intact bladders. As can be seen, the adherence of *Klebsiella ozonae* was the greatest followed by *Pseudomonas aeruginosa* while *E. coli* and *Streptococcus fecalis* displayed the least adherence.

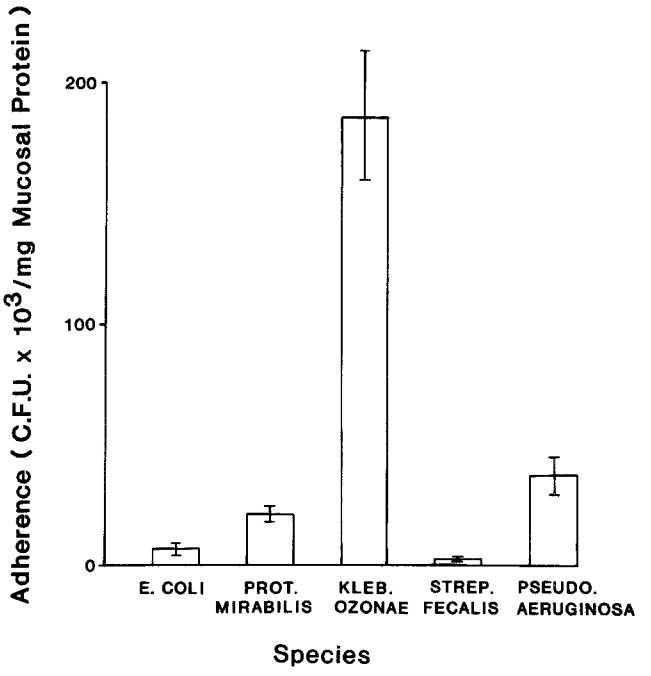

Fig. 2. Comparative adherence of 5 urinary tract pathogens to mucin intact bladders. Each *block* represents the mean of triplicate determinations performed on 5 to 8 rabbits, *bars* represent standard error of the mean

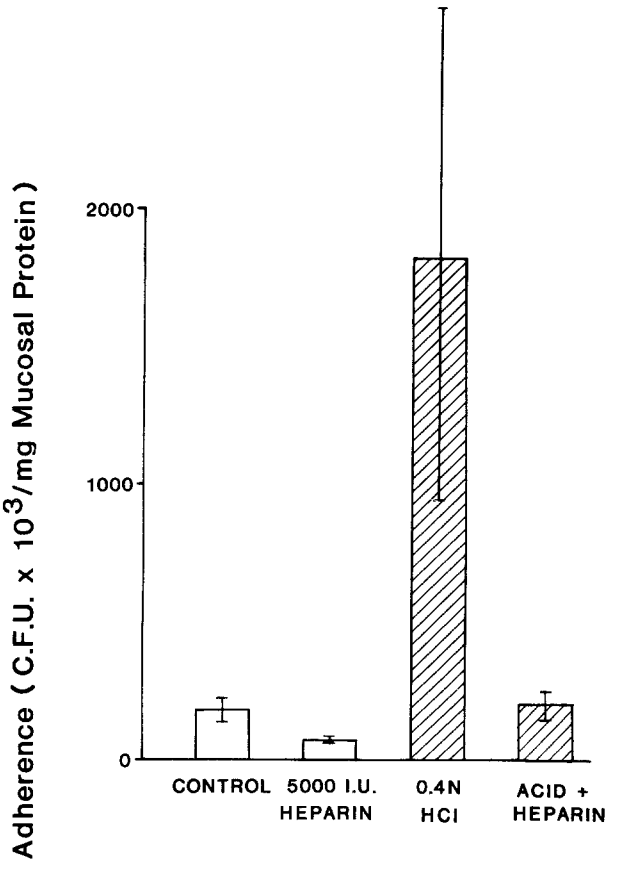

Fig. 4. Heparin inhibition of *Klebsiella ozonae* adherence. Each *block* represents the mean of triplicate determinations performed on 5 to 8 rabbits, *bars* represent standard error of the mean

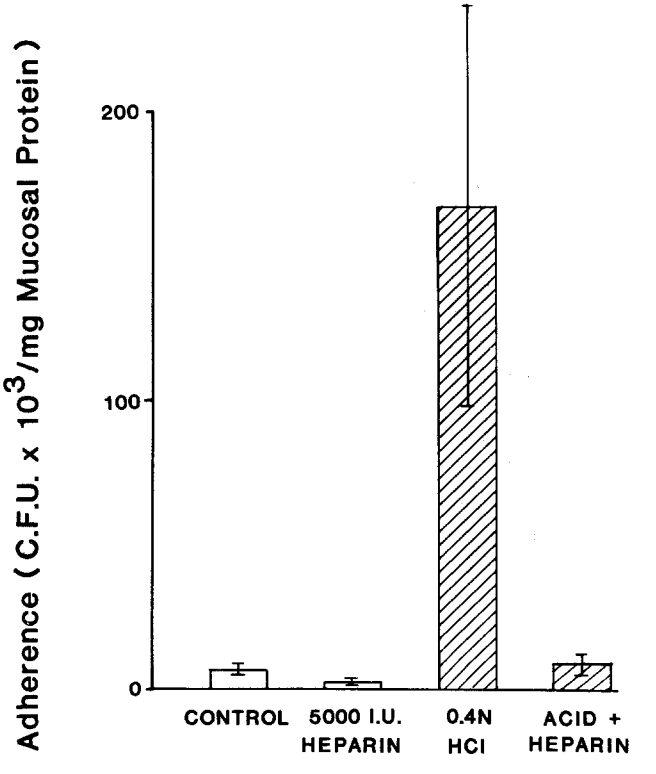

Fig. 3. Heparin inhibition of *E. coli* adherence. Each *block* represents the mean of triplicate determinations performed on 5 to 8 rabbits, *bars* represent standard error of the mean

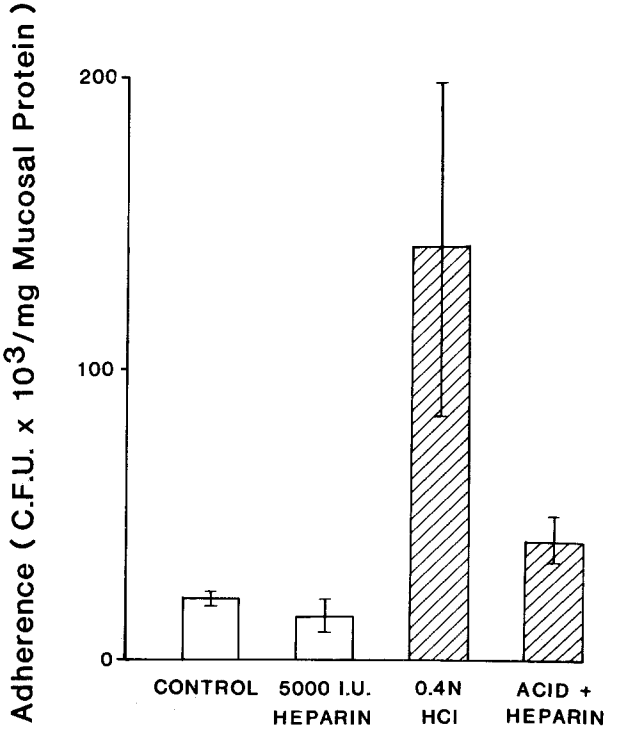

Fig. 5. Heparin inhibition of adherence of *Proteus mirabilis* adherence. Each *block* represents the mean of triplicate determinations performed on 5 to 8 rabbits, *bars* represent standard error of the mean

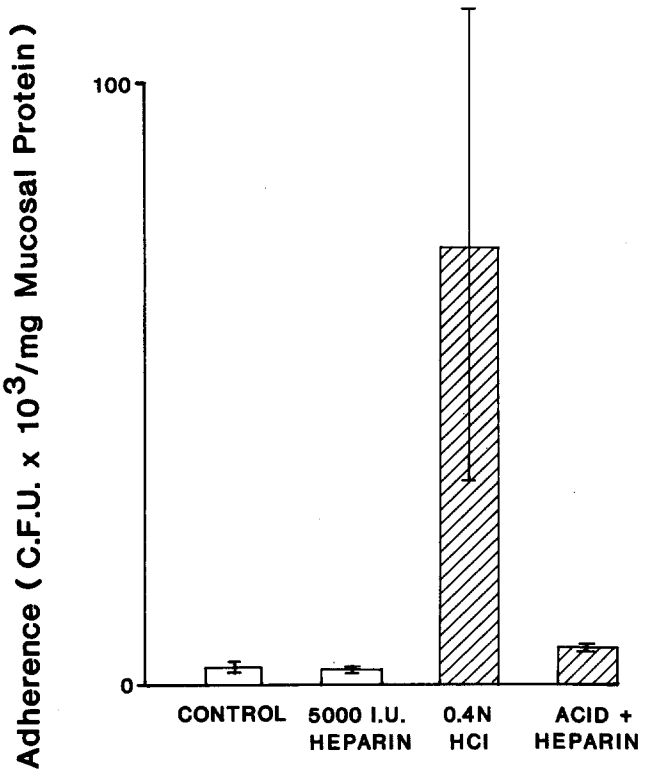

Fig. 6. Heparin inhibition of *Streptococcus fecalis* adherence. Each *block* represents the mean of triplicate determinations performed on 5 to 8 rabbits, *bars* represent standard error of the mean

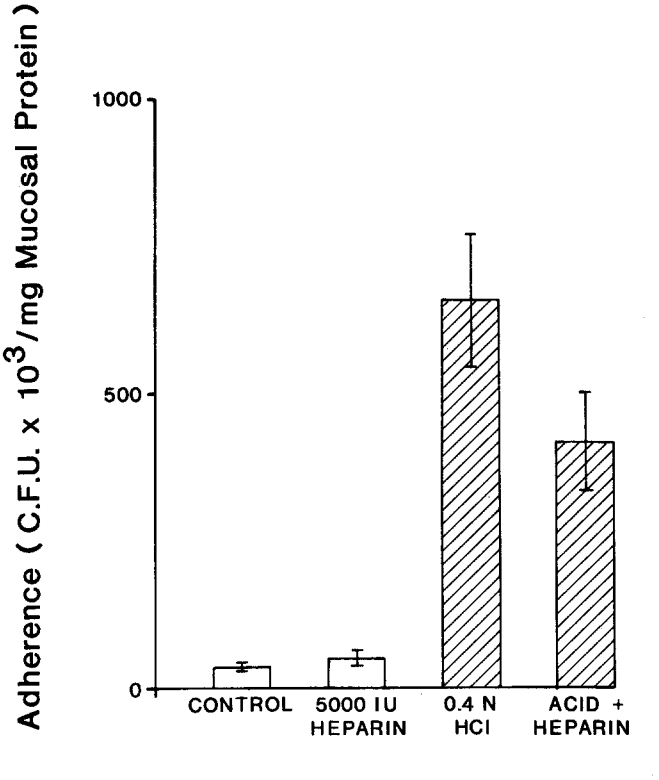

Fig. 7. Effect of Heparin on *Pseudomonas aeruginosa* adherence. Each *block* represents the mean of triplicate determinations performed on 5 to 8 rabbits, *bars* represent standard error of the mean

The effect of heparin on the adherence of the 5 bacterial species tested to mucin intact and mucin deficient bladders is displayed in Figs. 3–7. Acid treatment increased the adherence of all species tested between 10 to 20 fold. Heparin treatment of mucin deficient bladders did not significantly decrease *Pseudomonas aeruginosa* adherence but did lower adherence for all of the other 4 species to levels not significantly different from mucin intact controls (analysis of variance).

Discussion

Previous work on the antiadherence activity of heparin in the mucin deficient bladder utilized *Escherichia coli* as the bacterial species and indicated that heparin duplicated the antiadherence activity of the bladder surface mucin. The purpose of the present study was to determine whether these properties of heparin are nonspecific with regard to bacterial species as has been shown for the endogenous bladder surface mucin [4]. The results indicate that the antiadherence properties of mucin can indeed be duplicated by heparin for all species except *Pseudomonas*. Thus one could postulate that heparin's mode of action may be somewhat similar to that of the luminal mucopolysaccharide rather than blocking simple sugar residue sites on the mucosal transitional epithelium glycocalyx – the proposed mechanism by which mannose presumably blocks *E. coli* attachment [12]. Heparin is the strongest naturally occurring organic acid in the body [14] and it may be that its antiadherence property is charge related, possibly through its hydrophylic properties, placing water between the epithelial membrane and the luminal environment.

The fact that increased *Pseudomonas* adherence in the mucin deficient bladder was not prevented by heparin indicates that the mechanism of action of the endogenous antiadherence factor in mucin may be somehow different from that of heparin and that the mechanism of attachment of *Pseudomonas* may be different from that of the other species tested. *Pseudomonas* is known to be particularly virulent in experimental bladder infections, manifested by ulceration of the bladder mucosa, submucosal edema and migration of polymorphonuclear leucocytes [15]. Further study of this difference in response of *Pseudomonas* to heparin may help delineate heparin's specific antiadherence mechanism of action.

References

1. Parsons CL, Greenspan C, Mulholland SG (1975) The primary antibacterial defense mechanism of the bladder. Invest Urol 13:72–76

2. Parsons CL, Greenspan C, Moore SW, Mulholland SG (1977) Role of surface mucin in primary antibacterial defense of bladder. Urology 9:48–52
3. Shrom SH, Parsons CL, Mulholland SG (1977) Role of urothelial surface mucoprotein in intrinsic bladder defense. Urology 9: 526–533
4. Parsons CL, Mulholland SG (1978) Bladder surface mucin: its antibacterial effect against various bacterial species. Am J Pathol 93:423–432
5. Hanno PM, Fritz RW, Wein AJ, Mulholland SG (1981) The effect of pre-existing bacteriuria on bladder resistance to superinfection in the rabbit. Urol Res 9:99–103
6. Hanno PM, Fritz R, Wein AJ, Mulholland SG (1978) Heparin as antibacterial agent in rabbit bladder. Urology 12:411
7. Hanno PM, Parsons CL, Shrom SH, Fritz R, Mulholland SG (1978) The protective effect of intravesical heparin in experimental bladder infection. J Surg Res 25:324
8. Parsons CL, Mulholland SG (1979) Antibacterial activity of bladder surface mucin duplicated by exogenous glycosaminoglycan (Heparin). Infect Immunol 24:552–557
9. Parsons CL, Pollen JJ, Anwar H, Stauffer C, Schmidt JD (1980) Antibacterial activity of bladder surface mucin duplicated in the rabbit bladder by exogenous glycosaminoglycan (sodium pentosanpolysulfate). Infect Immunol 27:876–881
0. Hanno PM, Fritz RW, Mulholland SG, Wein AJ (1981) Heparin – examination of its antibacterial adsorption properties. Invest Urol 18:273–276
11. Parsons CL (1982) Prevention of urinary tract infection by the exogenous glycosaminoglycan sodium pentosanpolysulfate. J Urol 127:167–169
12. Ofek I, Mirelman D, Sharon N (1977) Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. Nature 265:623–625
13. Lowry OH, Rosenbrough NJ, Farri AL, Randall RJ (1951) Protein measurement using the Folin phenol reagent. J Biol Chem 193:265–275
14. Levine WG (1975) Anticoagulant, antithrombotic and thrombolytic drugs. In: Goodman LS, Gilman A (eds) The pharmacological basis of therapeutics, 5th ed. The Macmillan Publishing Company Inc, New York, pp 1350–1368
15. Montgomerie JZ, Morrow JW, Howard EB (1981) Urinary tract infection after injection of Pseudomonas into bladder. Urology 17:454–456

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