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Treatment of Chronic Cystitis by Instillation: Experiments Employing an Artificial Model

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Summary. An experimental model is described in which the conditions of chronic urinary bladder infections in man are simulated. The model was used to test the efficiency of mechanically controlled intermittent instillation therapy administered for more than twenty hours. Antiseptic drugs of the pre-antibiotic era, nephrotoxic and neurotoxic antibiotics as well as nitrofurantoin were instilled. Three species of bacteria which are common urinary pathogens were used as infecting agents. The results demonstrate that the model chosen effectively simulates chronic urinary tract infection in man. The results of prolonged automatic intermittent instillation therapy warrant the application of this technique to the treatment of chronic cystitis in man.

Key words: Model of urinary bladder, instillation therapy, chronic cystitis, antiseptic drugs, antibiotics.

The treatment of chronic urinary bladder infection may be extremely difficult under the following circumstances:
1. The infecting organism is resistant to all antibiotics.
2. The patient is allergic to the only appropriate antibiotics.
3. Debris in the bladder mitigates against effective therapy by harbouring bacteria. Unless these factors can be overcome eradication of the infection is unlikely. Local instillation of antibacterial drugs in adequate concentrations has been used for this purpose and has the advantage that nephrotoxic or neurotoxic drugs may be administered in this way.

Clinical observations do not allow an objective assessment of this type of therapy. Consequently an experimental model has been developed, so that reproducible results can be obtained under standard conditions.

Material and Methods

The experimental model is represented diagrammatically in Fig. 2. It simulates the clinical situation in which patients with chronic cystitis are treated by automatic instillation (Fig. 1). The bladder substitute is maintained at 37° C by means of a thermostat (Fig. 2). A blood clot which had been inoculated with bacteria was placed in the bladder substitute and nutrient broth (pH 7.0) was infused continuously at a rate of 2 ml/min to simulate urine flow. This solution, used as a minimal medium, contains 1% peptone, 0.5% beef extract, and 0.5% sodium choride.

The blood clots, designed as a substitute for a chronically infected bladder wall, were made by mixing 22 ml of fresh venous blood in a syringe with 1 ml of a broth culture containing 10^9 bacteria. This mixture was injected into a sterile glass bottle, the bottom of which was covered with four equal sized pieces of sterile metal gauze. When 2 ml thrombin are added a relatively solid coagulum develops. This coagulum is divided so that the four clots sticking to the metal gauze then contain 10^8 colony forming units/ml. One clot was used for each experiment.

The apparatus is assembled as indicated in Fig. 2. Both the influx of antibacterial solutions and the outlet for the "bladder" contents are regulated by an automatic switch[1]. 30 ml of antibacterial solution are infused in the first 5 min. Then, apart from the continuous inflow of substitute urine, the system remains closed for 40 minutes. During the next 15 min the contents of the "bladder" which will have reached 120 ml flows out. This cycle of events is repeated for 20 hrs.

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1 "Instillomat" Pfau - Wanfried Comp., 3508 Melsungen, F.R.G.

Samples of "bladder" content were taken for culture and bacterial counts 3 and 16 hrs after the infusion began. At the end of the experiment bacterial counts on the urine and coagulum were performed. Bacterial concentration in the coagulum after dissolution in 500 U. Varidase (R), a mixture of Streptokinase (R) and Streptodornase (R).

The blood clots were inoculated with E. coli, Pseudomonas aeruginosa, Proteus mirabilis, or a mixture of E. coli and Pseudomonas aeruginosa, all of which had been isolated from the urine of patients. The minimal inhibitory concentration (MIC) of the different drugs for the organisms tested are shown in Table 1. The following solutions were used for instillation: Phenylhydrargyriboras as a 0.1 ‰ Merfen (R) solution, the Acridine derivative Rivanol (R) as a 0.5 ‰ solution, Nitrofurantoin as "Furadantin pro instillatione" (R), Framycetin sulphate as a 0.25 ‰ solution or Kanamycin (R) as a 0.25 ‰ solution. The efficiency of these compounds had been tested on the selected bacteria under the conditions of the model.

In two control series only normal saline was instilled. In one of these the normal rate of micturition was simulated and no infusion carried out but in the other normal saline was infused and the bladder emptied hourly.

Results

When the bladder substitute was infected with E. coli and a normal rate of micturition was simulated the bacterial counts rose to between 10^8 and 10^9 after five hours (Fig. 3, Table 2).

When normal saline was infused instead of a drug and the "bladder" was emptied hourly the bacterial count rose relatively rapidly inspite of the bladder irrigation (Fig. 3, Table 2). E. coli exhibited the most rapid growth rate, Proteus mirabilis the slowest. After 16 hrs the bacterial concentration remained constant at about 10^7/ml.

The effect of the five different antibacterial agents employed for infusion on the bacterial counts is summarized in Table 2. The reduction in

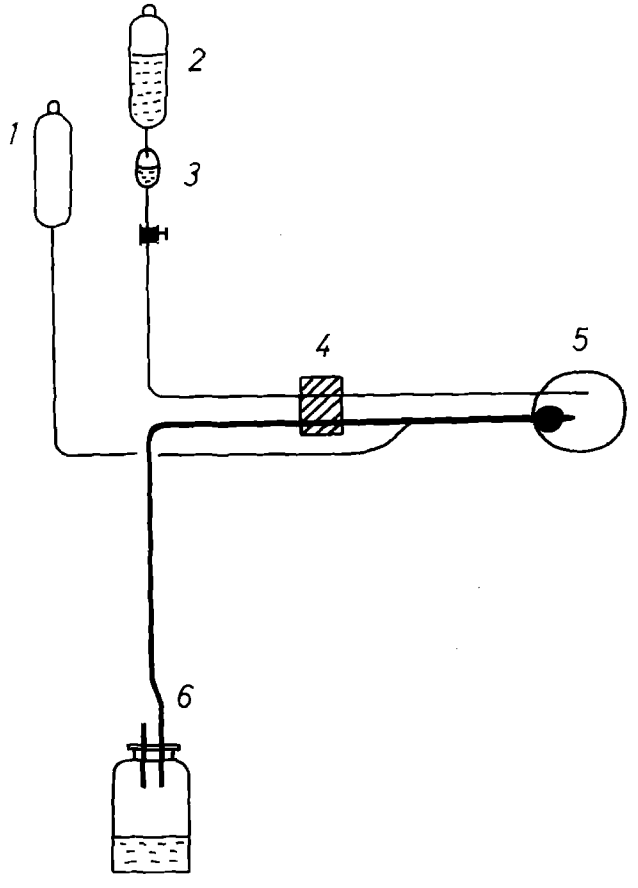

Fig. 1. Automatic instillation therapy of the bladder in man. 1. Alternative receptacle, 2. Solution for instillation, 3. Dropper, 4. Automatic switch, 5. Urinary bladder, 6. Receptacle for urine

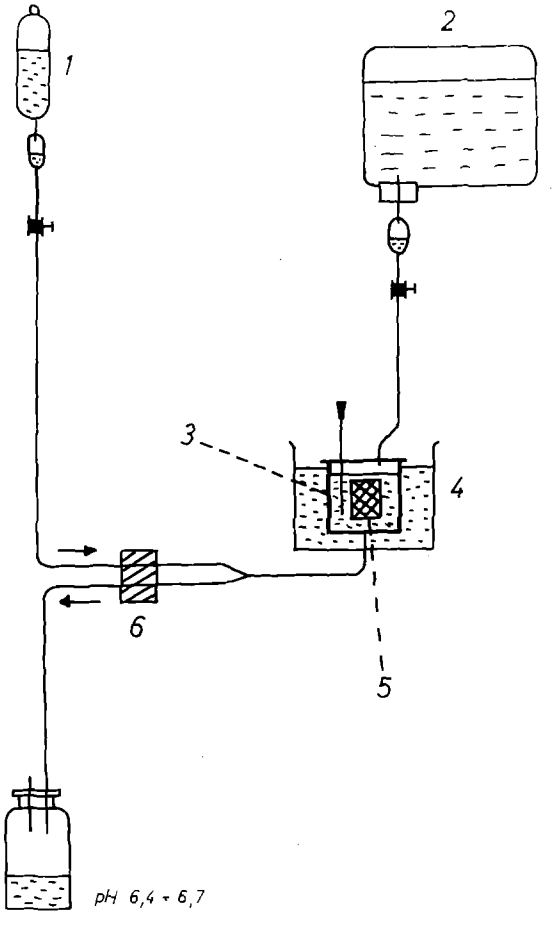

Fig. 2. Automatic instillation of the chronically infected bladder model. 1. Solution for instillation, 2. Nutrient broth, 3. Bladder substitute, 4. Thermostat, 5. Infected coagulum

Table 1. Minimal inhibitory concentrations of Rivanol (R), Merfen (R), Furadantin (R), Framycetin sulphate, and Kanamycin (R) for E. coli, Pseudomonas aeruginosa, and Proteus mirabilis

Microorganisms	Strain No	MIC (µg/ml)	Compound
E. coli	16933	62,0	Rivanol
Ps. aeruginosa	231171	500,0	"
Proteus mirabilis	2075	375,0	"
E. coli	16933	31,0	Merfen
Ps. aeruginosa	231171	1,0	"
Proteus mirabilis	2075	4,0	"
E. coli	16933	4,0	Furadantin
Ps. aeruginosa	231171	—	—
Proteus mirabilis	2075	—	—
E. coli	16933	1,6	Kanamycin
Ps. aeruginosa	231171	8,0	"
Proteus mirabilis	2075	1,6	"
E. coli	16933	1,6	Framycetin-sulphate
Ps. aeruginosa	231171	1,6	"
Proteus mirabilis	2075	6,2	"

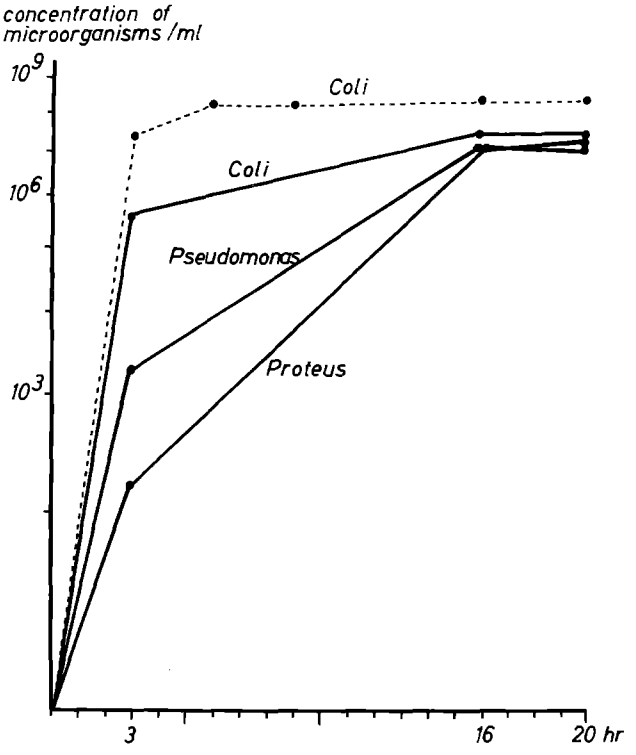

Fig. 3. Logarithmic representation of the growth of E. coli in the infected bladder model during simulated normal micturition(dotted line) and of E. coli, Pseudomonas aeruginosa and Proteus mirabilis during automatic instillation therapy with normal saline

Table 2. Bacterial counts in urine and coagulum of E. coli, Pseudomonas aeruginosa, and Proteus mirabilis after simulated micturition, instillation of normal saline solution, Merfen (R), Rivanol (R), Furadantin (R), Framycetin sulphate or Kanamycin (R)

	urine									coagulum		
	3 hr			16 hr			20 hr			20 hr		
	Coli	Pseudom.	Proteus	Coli	Pseudom.	Proteus	Coli	Pseudom	Proteus	Coli	Pseudom.	Proteus
simulated micturition	$3,0 \cdot 10^7$	$2,7 \cdot 10^5$	$1,9 \cdot 10^5$	$2,4 \cdot 10^8$	$3,3 \cdot 10^8$	$2,4 \cdot 10^8$	$2,3 \cdot 10^8$	$2,5 \cdot 10^8$	$2,2 \cdot 10^8$	$1,2 \cdot 10^9$	$9,1 \cdot 10^8$	$8,3 \cdot 10^8$
NaCl 0,9 %	$5,7 \cdot 10^5$	$2,5 \cdot 10^3$	$1,9 \cdot 10^2$	$4,2 \cdot 10^7$	$1,3 \cdot 10^7$	$1,1 \cdot 10^7$	$3,9 \cdot 10^7$	$1,3 \cdot 10^7$	$1,9 \cdot 10^7$	$5,1 \cdot 10^9$	$1,6 \cdot 10^9$	$1,7 \cdot 10^9$
Merfen 0,1 ‰	$4,1 \cdot 10^3$	0	0	$1,6 \cdot 10^4$	0	0	$2,7 \cdot 10^4$	0	$5,6 \cdot 10^3$	$1,7 \cdot 10^8$	$8,6 \cdot 10^7$	$1,2 \cdot 10^8$
Rivanol 0,5 ‰	0	0	0	0	$1,0 \cdot 10^5$	$1,4 \cdot 10^5$	0	$5,1 \cdot 10^5$	$6,4 \cdot 10^3$	$3,4 \cdot 10^6$	$2,5 \cdot 10^8$	$1,7 \cdot 10^8$
Furadantin 0,4 ‰	$6,5 \cdot 10^3$			$1,8 \cdot 10^4$			$2,2 \cdot 10^5$			$1,6 \cdot 10^8$		
Framycetinsulph. 0,25 ‰	0	0	0	0	0	0	0	0	0	$5,6 \cdot 10^3$	$5,0 \cdot 10^2$	$1,3 \cdot 10^3$
Kanamycin 0,25 ‰	0	0	0	0	0	0	0	0	0	$3,6 \cdot 10^4$	$4,1 \cdot 10^2$	$4,9 \cdot 10^3$
Rivanol 0,5‰ + Merfen 0,1‰	0	0		0	0		0	0		$7,1 \cdot 10^7$	$7,1 \cdot 10^7$	

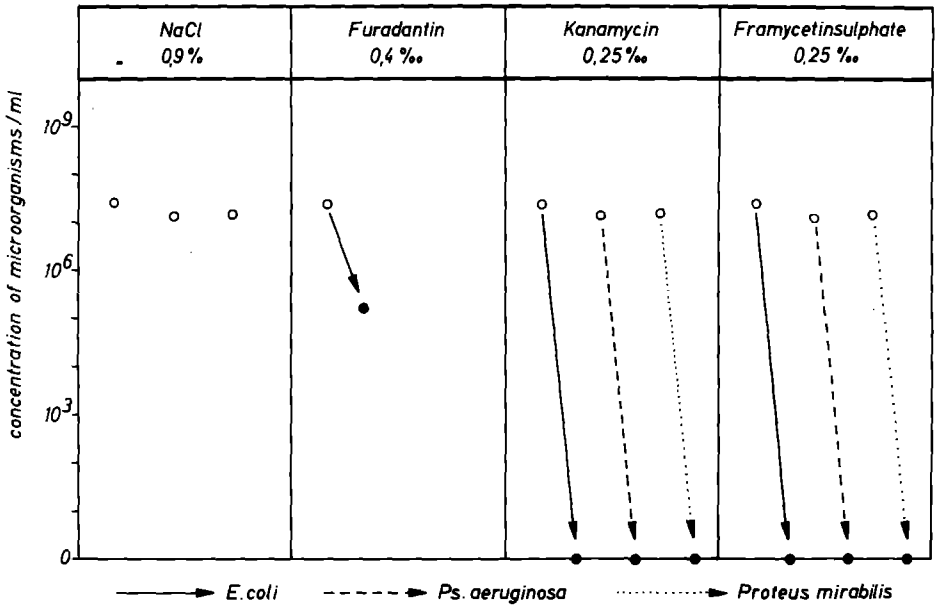

Fig. 4. Inhibition of the growth of E. coli, Pseudomonas aeruginosa, and Proteus mirabilis in the urine by normal saline, Furadantin (R), Kanamycin (R), or Framycetin sulphate. Bacterial counts 20 hrs after the experiment started in normal saline ○ and in nutrient broth combined with antibiotics ●.

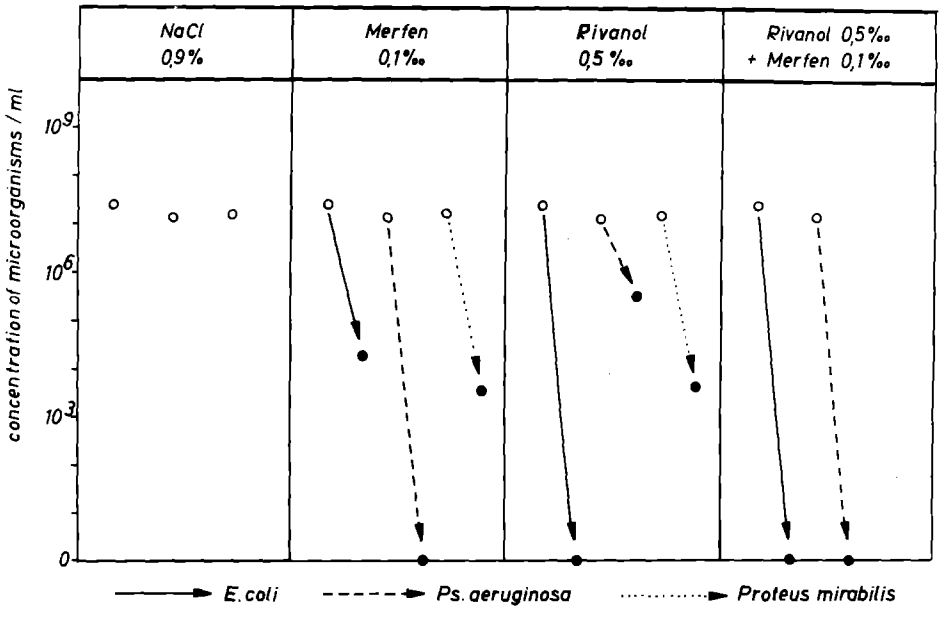

Fig. 5. Inhibition of E. coli, Pseudomonas aeruginosa, and Proteus mirabilis in the urine by normal saline solution, Merfen (R), Rivanol (R), or a mixture of Merfen (R) and Rivanol (R). Bacterial counts 20 hrs after the experiment started in normal saline ○ and in nutrient broth combined with antiseptic drugs ●.

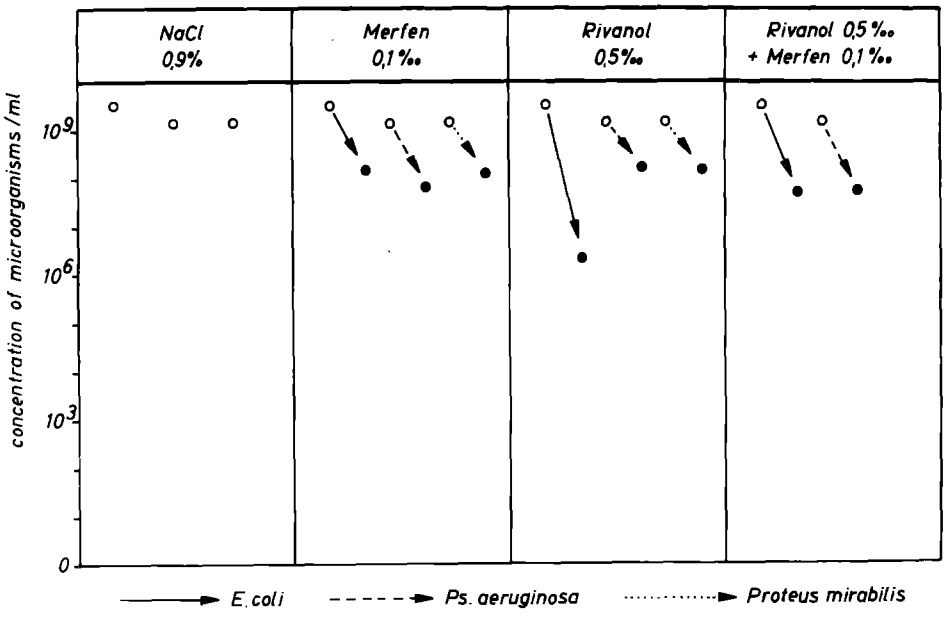

Fig. 6. Inhibition of E. coli, Pseudomonas aeruginosa, and Proteus mirabilis in the coagulum by normal saline solution, Merfen (R), Rivanol (R), or a mixture of Merfen (R) and Rivanol (R). Bacterial counts 20 hrs after the experiment started. Saline ○. Nutrient broth combined with antiseptic drugs ●.

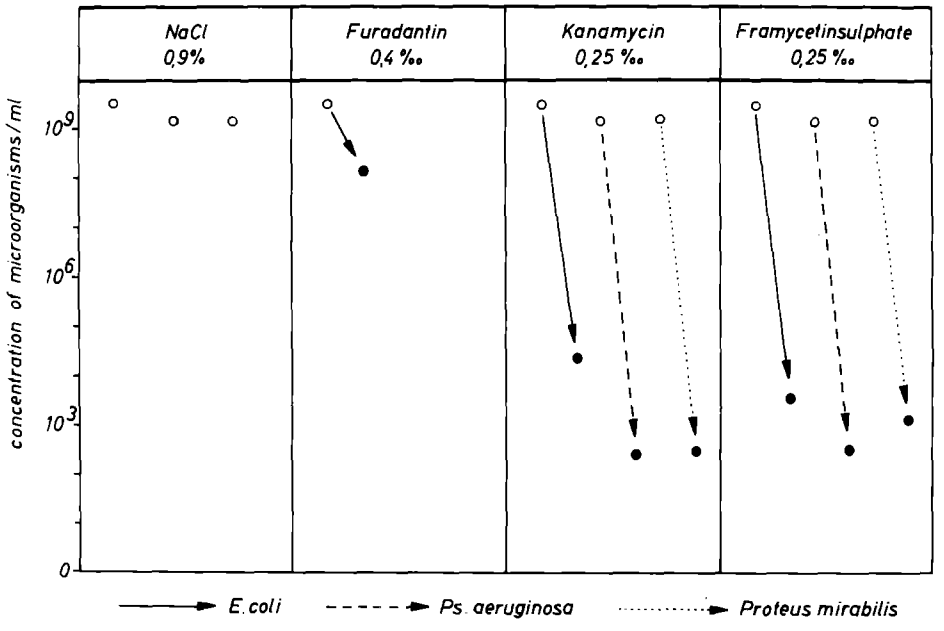

Fig. 7. Inhibition of E. coli, Pseudomonas aeruginosa, and Proteus mirabilis in the coagulum by normal saline solution, Furadantin (R), Kanamycin (R), and Framycetin sulphate. Bacterial counts 20 hrs after the experiment started. Saline ○. Nutrient broth combined with antibiotics ●.

bacterial count achieved by the antibacterial agents compared with the normal saline controls at 20 hrs is displayed in Figs. 4, 5, 6 and 7.

Nitrofurantoin, which is especially potent against E. coli was a poor inhibitor of bacterial growth when infused (Fig. 4). It was not assessed against the other test organisms because of its known low potency against them.

Framycetin sulphate and Kanamycin(R) infusions resulted in 100% inhibition of all three test organisms (Fig. 4).

Rivanol(R) and Merfen(R) were weak inhibitors of Proteus mirabilis (Fig. 5) and there was considerable variation in the bacterial counts when Merfen(R) was infused. These two antiseptic compounds were much more effective against Pseudomonas aeruginosa and E. coli. Merfen (R) inhibited the growth of Pseudomonas completely but was less effective against E. coli, the reverse was true of Rivanol (R). When used in combination in a proportion of 1 : 1 the growth of both species was inhibited completely (Fig. 5).

Culture of the blood clots 20 hrs after the infusions commenced revealed a less significant reduction in bacterial counts than in the urine (Table 2). This was particularly true when Furadantin (R) (Fig. 7) Merfen (R), Rivanol (R), or the Rivanol (R) Merfen (R) (Fig. 6) mixture had been infused. Framycetin sulphate and Kanamycin (R) were more effective (Fig. 7).

Discussion

These experiments demonstrate that it is possible to produce standard conditions in a bladder model and compare the effect of different antimicrobial agents. Nevertheless the model is open to criticism. In the first place the experimental conditions are quite different from those in chronic human bladder infection. Secondly nutrient broth was used instead of urine. This compromise was necessary because it is difficult to sterilize large enough quantities of urine by filtration. Moreover if a mixture of urine samples from several people had been used the results would have been unreproducible. There are several reports in the literature suggesting that nutrient broth is a satisfactory substitute for urine in experimental models.

Some samples of substitute urine were stored at +4° C for several hours whilst awaiting completion of the experiment. In spite of the inhibitory effect on metabolism at this temperature it is likely that the instilled drugs still exerted an antibacterial effect during storage. As all experiments were conducted in the same manner it can be considered that this has no effect on the overall conclusions.

The coagulum is not an ideal substitute for the chronically infected bladder but it fulfills the requirement of a source of bacteria capable of constantly infecting the "urine". A constant observation in all experiments was the decrease in size of the coagulum during irrigation. However, the quantity left at the end of each experiment did not vary greatly.

Despite the shortcomings mentioned the experiments enabled us to make comparable investigations into the antibacterial effect of different compounds under conditions similar to those of chronic bladder infection in man.

Evaluation of the results allows certain conclusions to be drawn.

1. In the treatment of chronic cystitis automatic instillation of an antibacterial substance for a long period is more effective than sporadic instillation.
2. The period the drug remains in the bladder must exceed the generation time of the bacteria.
3. If, as in chronic cystitis, there is a source of continuous reinfection of the urine the therapeutic effect of frequent voiding is insignificant.
4. Antiseptic drugs of the preantibiotic era such as Rivanol (R) and Merfen (R) are able to keep the bladder urine sterile. Their capacity to act on bacteria in debris, however, is insignificant and reinfection occurs.
5. A mixed infection of the urine with E. coli and Pseudomonas aeruginosa can be erradicated by a mixture of Merfen (R) and Rivanol (R). The effectiveness of this combination is poor in the presence of debris.
6. Under the chosen experimental conditions Nitrofurantoin is remarkably ineffective against E. coli even when administered by automatic instillation. Merfen (R) and Rivanol (R) are more effective. In the presence of debris Nitrofurantoin is less effective than Rivanol (R) and no better than Merfen (R).
7. Framycetin sulphate and Kanamycin (R) inhibit the growth of all three bacterial strains completely even in the presence of debris when both agents are much more effective than Nitrofurantoin, Merfen (R) or Rivanol (R).

As a result of these experiments mechanically controlled instillation of Framycetin sulphate and Kanamycin (R) has been employed in the treatment of chronic cystitis of varying aetiology in patients. The results of this series of experiments explain the very impressive and long lasting remissions of infection that have been observed.

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