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TREATMENT OF " BACTERIAL CYSTITIS " IN FULLY AUTOMATIC MECHANICAL MODELS SIMULATING CONDITIONS OF BACTERIAL GROWTH IN THE URINARY BLADDER

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Summary.—Two fully automatic models are described in which growing cultures can be continuously diluted and periodically discharged producing conditions of growth resembling those of the infected urinary bladder. Both models generate a continuous record of the opacity of the growing culture and the second model also generates a record of the Eh. The effect of adding ampicillin to a sensitive strain of *Escherichia coli* growing in these conditions is described and the relation of the results to human therapy is discussed.

DESPITE the availability of numerous agents active against the common infecting organisms, about 20% of patients with acute urinary tract infection fail to respond to treatment (Ormonde *et al.*, 1969). Various reasons for this failure have been postulated, mostly based on the ideas that the agent fails to reach the site of infection (generally supposed to be in the kidney) or that the organism survives in a form inaccessible to treatment—notably as spheroplasts.

Both these factors may play a part in the recrudescence of treated infection but they cannot provide a general explanation for the almost identical failure rates of many different agents. Experimental evidence shows that at least some agents reach the interstitial fluid of the kidneys in concentrations matching or exceeding those in the plasma (Chisholm, Calnan and Waterworth, 1968) and organisms are converted to spheroplasts only by that minority of agents used for the treatment of urinary tract infection which interfere with bacterial cell wall synthesis.

One possible explanation for the failure of many different antibiotics is that the performance of all antimicrobial agents against very large bacterial populations such as those found in infected urine is less, and sometimes very much less, than expected from conventional tests of inhibitory activity. Titrations of antibacterial agents against fully, or near fully, grown cultures show that markedly less activity is obtained (O'Grady and Cattell, 1966; O'Grady and Pennington, 1967) but as an explanation of the failure of therapy of urinary tract infection this is weakened by the fact that in the infected urinary tract conditions of growth and of interaction between organisms and agent are much more complex than those of conventional titrations.

As the culture grows in the urinary bladder, it is diluted continuously and supplied with fresh nutrients by the ureteric urine. On micturition, the greater part of the growing culture is discarded and the effect of the constant flow of ureteric urine on the diminished volume of urine which remains is correspondingly enhanced. The effect of antibacterial agents on a culture in which the concentra-

tion of organisms fluctuates profoundly as a result of these changes cannot readily be predicted. In order to examine directly the response of such cultures, we have added ampicillin to sensitive organisms growing in mechanical systems in which the conditions of medium supply, dilution and periodic displacement resemble those of the urinary bladder.

METHODS AND MATERIALS

Two new models based on those previously described (O'Grady and Pennington, 1966; O'Grady *et al.*, 1968) have been constructed. They overcome two outstanding difficulties of the earlier models: imperfect mixing (such that the incoming " urine " failed to dilute predictably the growing culture) and growth of organisms on the glass of the model's " bladder "—such that the light path of the photometer was progressively occluded by growth which was unaffected by dilution of the bladder contents.

Model 1.—The growth vessel and mounting frame of Model 1 is shown in Fig. 1. The " bladder " consists of an inverted 250 ml conical flask on to the end of which a 20 ml glass syringe barrel is welded. The vessel is sterilized with its stir bar in position. Immediately before use the plunger of a sterile 20 ml disposable plastic syringe is fitted aseptically into the glass syringe barrel. The vessel is then mounted in its rigid frame and clamped into a seating which contains the light source and sensory element of a simple photometer (Watson *et al.*, 1969). The free end of the syringe plunger is clamped on to the head of a pneumatic piston operating at 414 kPa. The piston is activated at 5-minute intervals *via* a motor-driven camshaft timer which causes the piston to rise and immediately fall, thereby sweeping the glass of the syringe barrel (which lies in the light path of the photometer and constitutes the opacity chamber) free from bacteria. Apart from the piston and its activating gear, the system is in principle the same as that previously described (O'Grady *et al.*, 1968). Fresh broth is delivered continuously to the bladder at a preset rate (usually 1 ml/min) by a Watson–Marlow pump and the bladder volume emptied down to a residual volume determined by the position of the drain tube by means of a second pump at preset intervals (usually one hour) by an automatically resetting clock. To maintain a constant residual volume during operation the piston camshaft timer is interlocked with the micturition pump control clock. Most traces from this machine have been recorded on a potentiometric recorder running at 3 cm/hour.

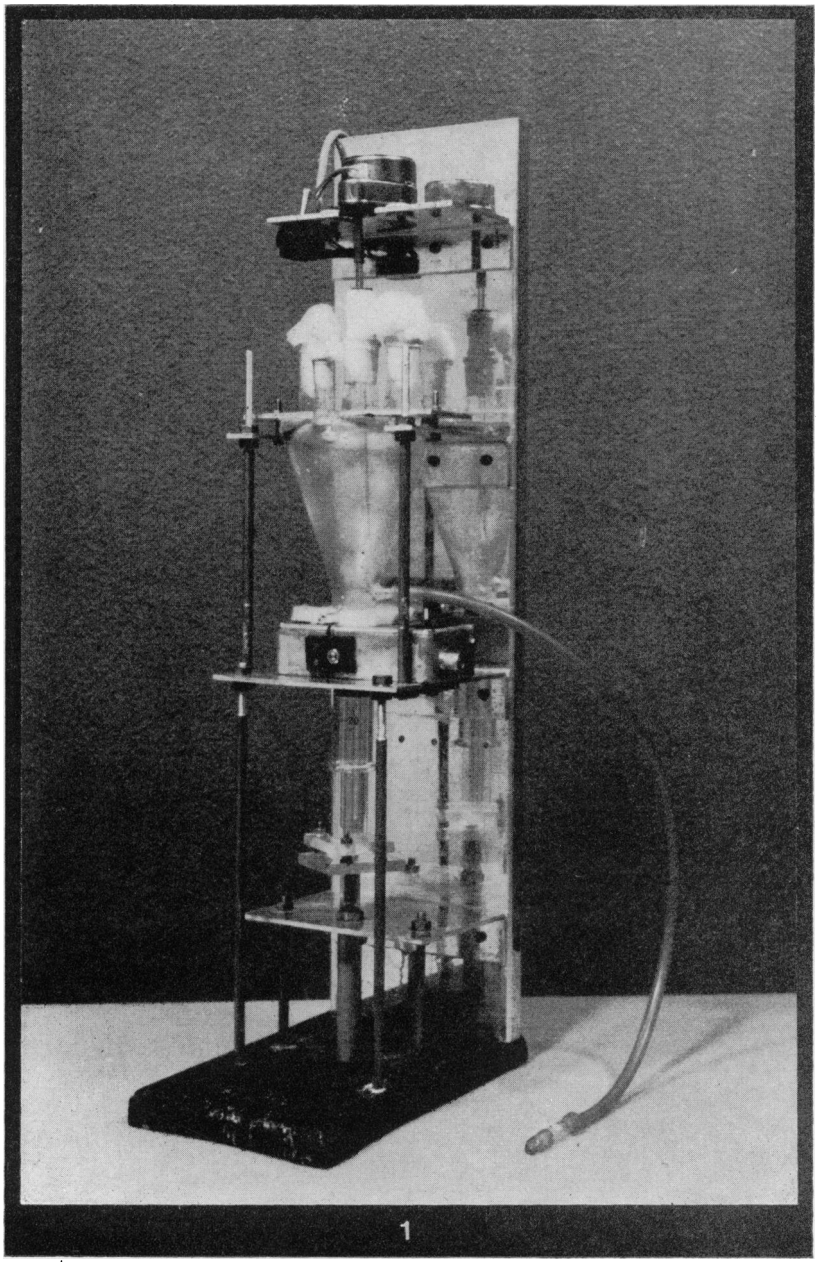
Model 2.—The second model is more complex. A disadvantage of relying exclusively on photometric measures of bacterial growth is that the opacity of a culture changes not only with the replication of the organism but with alteration of its shape such as may follow exposure to antimicrobial agents. A more elaborate model was therefore constructed which incorporates other measures of bacterial activity.

As shown in Fig. 2 the " bladder " in this model consists of a 250 ml glass cylinder on to the base of which a 20 ml glass syringe barrel is welded. A stainless steel plate is attached to the top of the flask and is used to clamp the flask into its frame. The plate also provides location for the inlet and outlet tubes as well as electrodes for the measurement of pH, Eh and temperature. The vessel is sterilized with the plate and electrodes in position. The piston system is so arranged that the syringe plunger is held flush with the floor of the bladder cylinder and is withdrawn only for sufficient time to allow the opacity reading to be taken.

A specially designed electronic clock selects in turn the signals from each electrode and, having caused the plunger to be withdrawn, from the photometer. The same clock arranges for micturition to occur at preset intervals, ensuring that at the same time the syringe plunger is in the correct position and that digital readings are not taken during the turbulent conditions which exist while the bladder is being emptied. The voltages generated by each of the electrodes are fed to a voltmeter and paper tape pump which are programmed to generate a digital multiplexed record of the opacity, pH, Redox and temperature signals on paper tape.

EXPLANATION OF PLATE

FIG. 1.—The " bladder " of the first model consisting of an inverted conical flask with tubulures set into the base, a drainage tube at the side and a glass syringe welded to the neck. The stirrer motor above, the photometer box at the waist and the piston arrangement at the base are all mounted on a rigid metal frame.



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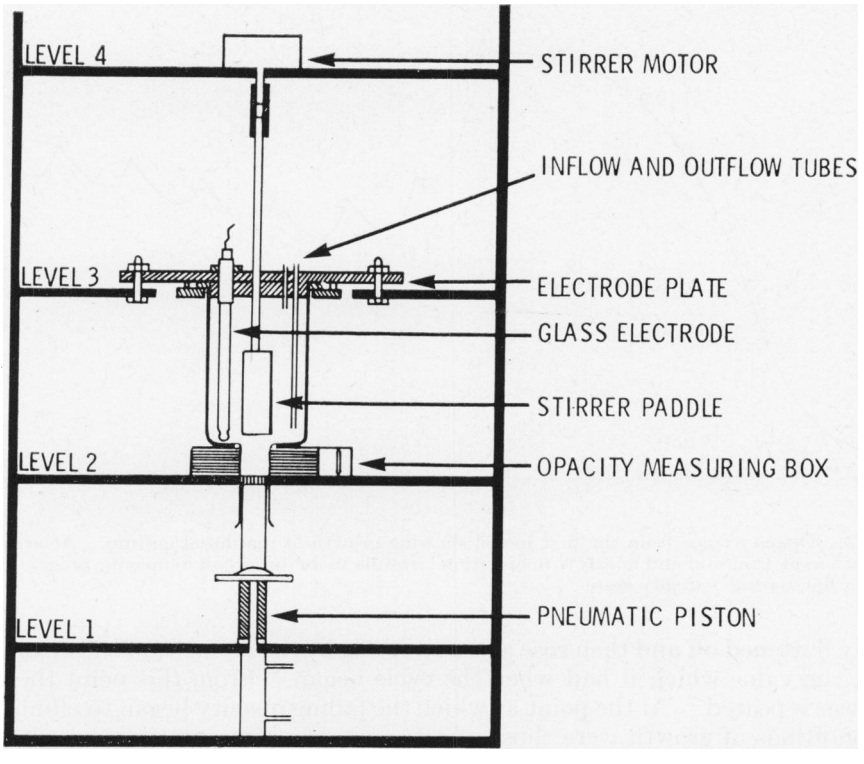

FIG. 2.—The modified cylindrical flask arrangement of the second model which permits more rigid mounting and accommodates other electrodes.

A complete set of readings are taken within each 5-minute piston cycle. The output from the pH electrode in 2 calibrated buffers is obtained at the end of each experiment (as the E_0 value changes during steam sterilization of the pH electrode).

The multiplexed data are converted to a continuous record using a Honeywell DOP 516 computer and associated graph plotting facilities. The programme uses the output of the pH electrode in calibrated buffers to scale the pH curve and automatically spreads each curve across the full width of chart scale. The programme prints out calibration marks on both the Y- and time axes.

RESULTS

A typical trace obtained from Model 1 is shown in Fig. 3. The characteristic effect of diluting at 1 ml/min a fully grown broth culture with a residual volume of approximately 20 ml and hourly micturition is seen. Initially there was a very rapid fall in the opacity of the slowly growing culture but as the organisms were provided with fresh nutrient and emerged from the lag-phase they entered after 6–7 hours a " fluctuating steady-state " in which the same cycle of opacity changes occurred between each micturition. Immediately after " micturition" when the volume in the " bladder " was smallest and the dilution effect of the incoming " ureteric urine " greatest, the opacity fell rapidly. As the volume in the bladder increased and the effect of dilution correspondingly decreased, the

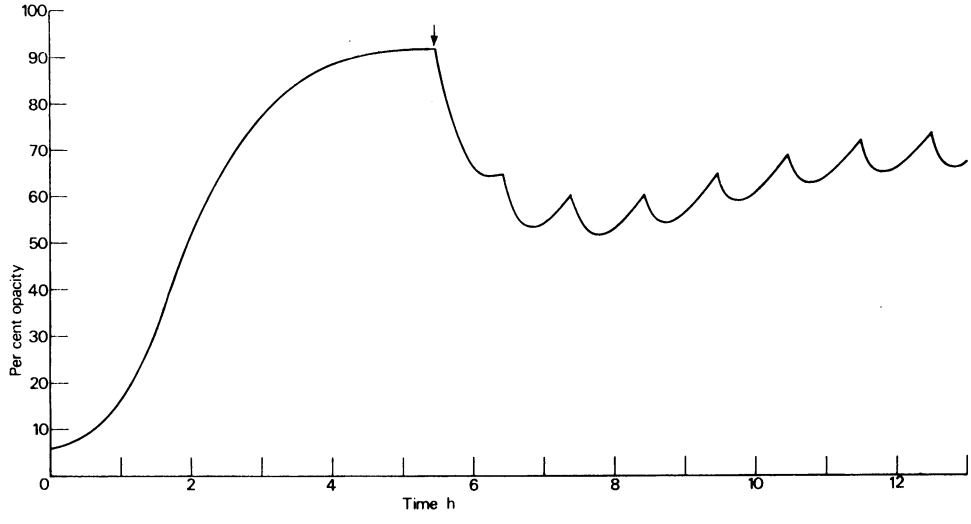

FIG. 3.—Opacity trace from the first model showing growth of undiluted culture. At arrow, dilution at 1 ml/min and hourly " micturition " results in an initial fall in opacity progressing to a fluctuating " steady state ".

opacity flattened off and then rose again to reach, at the moment of the next micturition, the value which it had when the cycle began. From this point the whole cycle was repeated. At the point at which the falling opacity began to climb again, the conditions of growth were those of a true steady-state continuous-cultivation system in which the rate of bacterial growth equalled the rate of dilution by fresh medium. In such conditions, where the relationship of residual bladder volume and ureteric urine flow rate lead to a" fluctuating steady-state ", infection cannot be spontaneously eradicated.

The effect on such growth conditions of adding an antibiotic to which the organism was sensitive is shown in Fig. 4. Infection was with a strain of *Escherichia coli* sensitive to 4 μg of ampicillin per ml. At Point A, sufficient ampicillin was added to the " bladder " to give a concentration of 50 μg/ml. Almost immediately there was a profound fall in opacity corresponding with that seen in static cultures (Greenwood and O'Grady, 1969, 1970*a*). On subsequent voidings, further but lesser falls occurred until the opacity of the culture fell below the level of sensitivity of the recording system. As in treatment of human infection, the concentration of ampicillin in the system declined as a result of dilution by " ureteric urine " and discharge from the bladder as samples were voided. At the point marked " MIC " in Fig. 4, the concentration of ampicillin was calculated to have fallen below the inhibitory level for the infecting organisms. Within 2 hours the opacity of the culture began to climb and after 7–8 hours reached a new steady state.

Evidence that such escape from therapy cannot be overcome simply by increasing the dose of ampicillin is provided by Fig. 5. The addition of sufficient ampicillin to produce an initial concentration of 500 μg/ml delayed but did not prevent regrowth, which again occurred within 2 hours of the concentration of the agent falling below the inhibitory level.

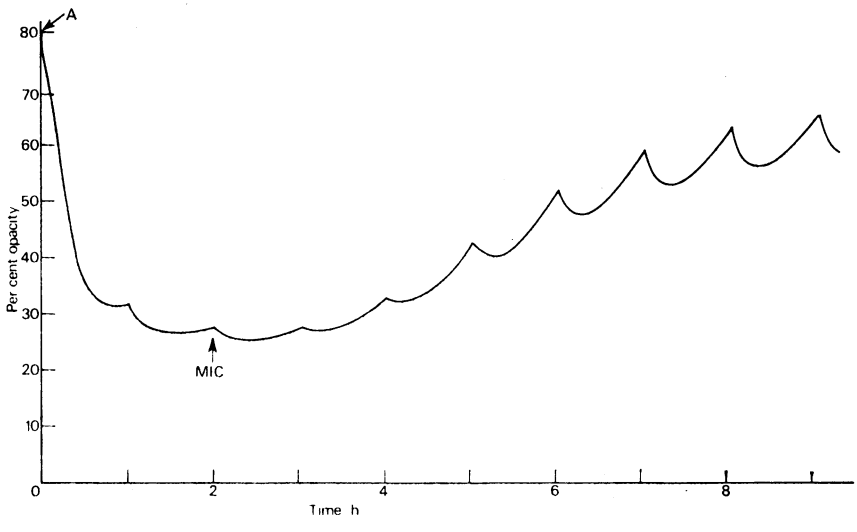

FIG. 4.—Effect on " steady state " culture of adding at " A " sufficient ampicillin to produce an initial concentration of 50 μg/ml. At " MIC " dilution and micturition have caused the concentration of ampicillin to fall to the MIC for the organism (4 μg/ml).

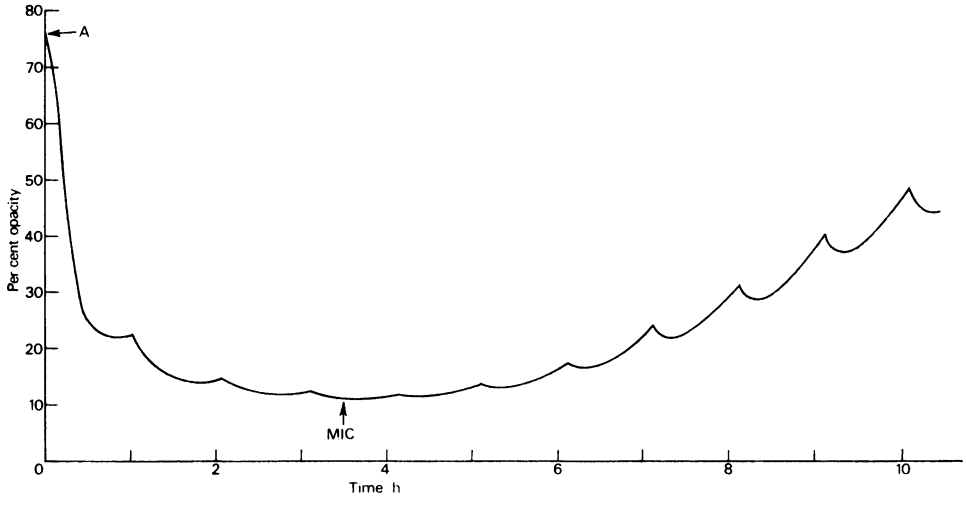

FIG. 5.—Similar experiment to that shown in Fig. 4, but with the addition of sufficient ampicillin to produce an initial concentration of 500 μg/ml.

Further evidence that growth was resumed quickly once the concentration of antibiotic fell below the MIC is provided by Fig. 6 which shows the opacity and Eh traces given by the second instrument. As growth of the initial overnight culture developed, the Eh fell progressively and subsequently rose and fell periodically with the growth cycles of the fluctuating steady-state. On the addition of ampicillin, as the opacity fell so the Eh rose. When dilution and discharge

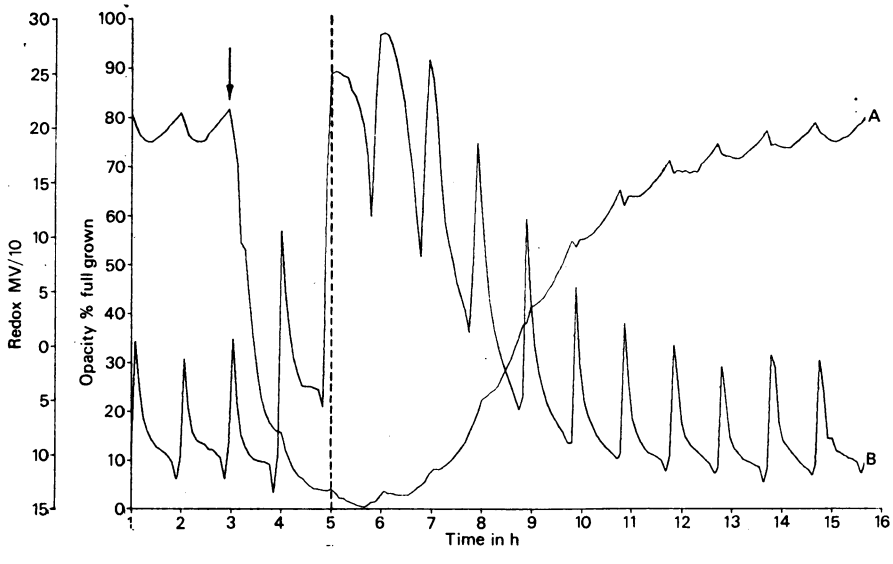

FIG. 6.—Computer-drawn trace from data generated by the second model. At the beginning, the upper trace is of the opacity and the lower the Eh of the culture. In the initial " steady state " period, the Eh falls with growth in the inter-micturition periods and rises steeply as the " bladder " empties. On the second micturition after the addition (at arrow) of sufficient ampicillin to produce a concentration of 50 μg/ml, the Eh rises rapidly to levels indicating very low metabolic activity. As soon as the concentration of ampicillin falls below the MIC (at the dotted line) metabolic activity and inter-micturition cycles of growth are resumed.

caused the concentration of ampicillin to fall below the MIC, the Eh began almost at once to decline and soon resumed the periodic pattern typical of fluctuating steady-state growth.

One weakness of all these systems as models of conditions inside the urinary bladder is that the chamber above the level of the " urine " contains air. When the " bladder " is emptied, a thin film of culture remains on the wall—as it doubtless does on the wall of the bladder—but in the model this film is exposed to air. It was thought possible that the growth characteristics of bacteria in this film and the conditions of their exposure to antibacterial agent could be sufficiently peculiar for them to constitute the main source of re-growth. Calculations suggested that it was very unlikely that such a surface film could contribute the numbers of organisms required to account for the rapidity of re-growth, but to exclude the possibility that exposure of the film to air (the outstanding difference from the bladder) could cause it to generate unexpectedly large numbers of survivors, the experiment was repeated with the whole system flushed continuously with nitrogen. Anaerobic indicators suspended in the system showed that oxygenation of the film could not have occurred, but the response to therapy and the course of re-growth was unaffected.

In therapeutic practice more than one dose of antibiotic is given, and during the overnight period the rate of secretion of urine falls to about a quarter of the day time value and micturition ceases. Fig. 7 shows the result of a schedule of " treatment " in which three doses of ampicillin were given, each producing an

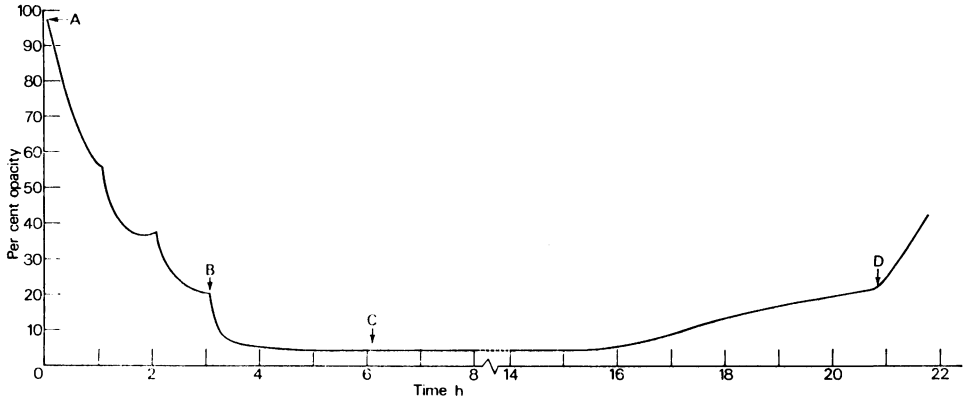

FIG. 7.—Effects of a " therapeutic regimen ". At " A " an overnight broth culture was diluted at 1 ml/min, sufficient ampicillin to produce a concentration of 50 μg/ml added and hourly micturition initiated. At " B " a second similar dose of ampicillin was added. At " C " a third similar dose of ampicillin was added, micturition was stopped and the dilution rate reduced to 0·25 ml/min, thus simulating the " overnight " period. At " D " on " waking " dilution at 1 ml/min and micturition were resumed.

initial concentration of 50 μg/ml. The first pulse was added to the fully-grown overnight broth culture and little initial effect was obtained on the stationary phase culture. The other doses were given at 3-hour intervals and after the third dose—at " bed time "—the rate of addition of fresh broth was reduced to 0·25 ml/min and voiding stopped. At this dilution rate and without micturition the concentration of ampicillin did not fall below the MIC for about 10 hours and re-growth of the organism was correspondingly delayed.

DISCUSSION

Experience with these models strongly supports the view previously put forward (Greenwood and O'Grady, 1970*b*) that potent bactericidal agents, even in high concentrations, will not by themselves extinguish large bacterial populations of the kind commonly encountered in infected tissue or urine. Even in the complex conditions of growth resembling those of the urinary bladder, where the constant supply of fresh medium and discharge of old culture might be expected to ensure a total population of actively metabolizing, and thus susceptible, cells, sufficient organisms remained unaffected by the presence of high concentrations of ampicillin for re-growth to occur soon after dispersal of the agent by dilution. It appears that in these conditions—as in the simpler conditions we have examined previously—the majority of the cells in large populations of sensitive *Esch. coli* rapidly lyse on exposure to ampicillin, but significant numbers are affected only in a bacteristatic manner such that they are able quickly to resume growth when the agent is removed.

Several therapeutic lessons follow from these findings. If a rapidly excreted, actively bactericidal agent is given infrequently, re-growth of the bacteristatically affected minority of bacterial cells can occur. Once the initial rapid destructive effect of the bactericidal agent has been achieved, and with β-lactam antibiotics this is dependent on a relatively low osmolality (Greenwood and O'Grady, 1972),

nothing is gained by persisting with high dose therapy. Beyond the early and hopefully massive killing, further exposure to the agent serves only to prevent the organisms from re-growing—just as a bacteristatic agent would—while dilution and displacement (together, in the *in vivo* situation, with humoral and cellular clearance mechanisms) achieve final elimination of infection. This behaviour may well explain the very similar results obtained from treatment over relatively prolonged periods with bacteristatic or bactericidal agents. It also suggests that initial intensive treatment need only be brief since continuation of therapy with doses calculated to provide no more than inhibitory levels of the drug is likely to be just as effective.

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