TREATMENT OF EXPERIMENTAL L-PHASE INFECTIONS OF THE URINARY TRACT

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L-PHASE organisms are increasingly isolated from clinical specimens, perhaps most often from the urinary tract. Some authors have found L-phase organisms in 20 per cent. of patients with suspected urinary-tract infections (Gutman *et al.*, 1965; Conner *et al.*, 1968). This might be due to the favourable conditions for survival of L-phase organisms within the urinary tract because kidney tissue and urine are very hypertonic compared with other organs and body fluids. Usually urine has a slightly acid pH due to the dietary intake of proteins; this was earlier (Gnarpe and Edebo, 1970) shown to be advantageous for the survival of osmotically fragile organisms.

Taubeneck (1962) and Guze and Kalmanson (1964) have shown that L-phase organisms may be eliminated from the urinary tract by treatment with erythromycin. Eastridge and Farrar (1968) have also shown that the elimination rate may be increased by osmotic diuresis.

This work was done to evaluate the effects of tetracycline treatment on penicillin-induced L-phase experimental infections of the urinary tract of rabbits with and without osmotic diuresis.

MATERIALS AND METHODS

Bacteria. A strain of *Proteus vulgaris* X19 (Type Culture Collection, the National Bacteriological Laboratory, Stockholm) was used as infecting organism, because the L-phase was easily induced with penicillins. Normal and osmotically stabilised media were used for the cultivation of bacteria and L-phase organisms as previously described (Gnarpe, 1970).

The conductivity of urine was measured as described earlier (Gnarpe, 1970) by means of a direct-reading conductivity bridge (Philips, Model PR9501).

Serum and urine antibiotic concentrations. Blood was drawn at intervals from the ear veins of the rabbits, allowed to stand at room temperature for 1 hr and then centrifuged. The serum and urine concentrations of penicillin and methacycline (6-methylene oxytetracycline) were then determined by means of a disk diffusion method (Ericsson, 1960) with test organisms that were resistant respectively to methacycline or penicillin.

Experimental procedure. Under Nembutal anaesthesia and additional local anaesthesia (Xylocain, 0.1 per cent., 2 ml) in skin and muscle layers, the left ureter was temporarily obstructed as described earlier (Gnarpe and Olding, 1970) for 24 hr before infection. The animals were then infected by injection of 5×10^{10} – 10^{11} *P. vulgaris* intravesically through an indwelling catheter. All received procaine penicillin in a dosage of 300,000 IU per kg daily for the whole experimental period (14 days).

After the 4th day of infection 84 rabbits treated in this manner were divided into four groups; all continued to receive penicillin together with: in group A, no further treatment; in group B, increased diuresis; in group C, methacycline; and in group D, methacycline plus increased diuresis. Methacycline was given intramuscularly in a dosage of 20 mg per kg

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daily, divided in two doses. Osmotic diuresis was established with 5.5 per cent. glucose as described by Andriole (1968). The animals were catheterised on the 4th day of infection. Urine cultures and determinations of the osmolalities were made. At least once more during the experimental period urine was cultured and its osmolality determined. During the experimental period the daily water intake was measured. Facilities for measurement of the urinary output were not available.

After 14 or 15 days of treatment the animals were killed and kidney and urine cultures made as described earlier (Gnarpe, 1970; Gnarpe and Olding, 1970).

RESULTS

Urine specimens obtained from the 84 rabbits before infection were all sterile except three with growth of 2000, 1000 and 500 untypable *Escherichia coli* per ml. Blood cultures obtained on different occasions from 67 animals were sterile.

The mean daily fluid intake in groups B and D was 145 ml during the first 4 days. During the period of osmotic diuresis the fluid intake increased to a mean value of 401 ml.

Urine cultures made on the 4th day of infection yielded only L-phase organisms in pure culture in 78 of the animals. The L-phase organisms reverted on all occasions to the bacterial phase within 1 wk. They were found identical with the infecting strain when tested by means of the Dienes' phenomenon. In four animals both L-phase and bacterial-phase organisms were found; in one bacteria only and from the final animal no micro-organisms were isolated.

The concentration of penicillin in the blood was above 50 IU per ml in all rabbits except seven. Among these were the four rabbits with growth of a mixed culture of bacteria and L-phase organisms. The penicillin concentrations were 12.5-25 IU per ml and the MIC for the infecting strain was 50 IU per ml.

Post-mortem findings

The results of post-mortem kidney and urine cultures taken on the 14th or 15th day are given in the table. In the 22 animals in group A, given no specific treatment for the elimination of L-phase organisms, bacteria in L-phase was isolated from kidney tissue of 16 and from urine of all these and from another three animals. A mixed culture of bacteria and L-phase organisms was isolated from the urine of one, but there was no growth of either from the kidney; and bacteria only were grown from the urine and kidney tissue of another. Kidney tissue from the four remaining rabbits and urine from one of them yielded no growth. The penicillin concentrations were above 50 IU per ml on all occasions except in the rabbit with mixed growth of L-phase organisms and bacteria, where the penicillin concentration was found to be 50 IU per ml. On all samples tested, the urinary osmolality was well above 400 mOsm per kg: the mean urinary osmolality in the samples obtained after death was 860 mOsm per kg.

The group B animals received 5.5 per cent. glucose solution instead of tap water and had a decrease of the urinary osmolality from a mean of 892 mOsm per kg to 340 mOsm per kg. L-phase organisms were isolated from the kidney

tissue and urine of three of these and from kidney tissue only of another four. Bacteria were not isolated from either urine or kidney tissue. The penicillin concentrations were above 50 IU per ml in all samples tested on the 8th day and after death.

In the animals in group C, treated with methacycline and penicillin, kidney and urine cultures yielded growth of L-phase organisms from kidney tissue of six animals and the urine of five of these. In all other cases neither L-phase organisms nor bacteria were isolated, but in two cases round bodies and rod forms were observed on direct examination in the phase-contrast microscope. Because subcultures of these samples failed, the samples were considered to

TABLE

Results of urine (U) and kidney (K) cultures after continuous treatment with (A) penicillin only (22 animals); (B) penicillin and osmotic diuresis (17 animals); (C) penicillin and methacycline (25 animals); (D) penicillin, methacycline and osmotic diuresis (20 animals)

	Number of animals with growth of the stated organisms							
	Group A		Group B		Group C		Group D	
	K	U	K	U	K	U	K	U
L-phase only	16	19	7	3	6	5	2	0
L-phase and bacteria	0	1	0	0	0	0	0	0
Bacteria only	1	1	0	0	0	0	0	0
Neither	5	1	10	14	19	20	18	20

be negative. The penicillin concentrations were at least 50 IU per ml in 19 of the 25 animals; in two animals the penicillin concentration was found to be 25 IU per ml and in three animals no determinations were made after death because of technical failures. These rabbits had earlier had penicillin concentrations respectively of 50, 50 and 100 IU per ml of serum. In all animals in group C except three, in which no determinations were made because of technical failure, the methacycline concentration in blood was found to be between 2 μ g and 4 μ g per ml.

Of the 20 animals in group D treated with a combination of increased diuresis and methacycline, L-phase organisms were not isolated from the kidney tissue or urine in 18. In two rabbits, L-phase organisms were found in kidney tissue but not in urine. Round forms and a few rod forms were seen in the urine of the two rabbits with growth of L-phase organisms from kidney tissue and in a urine sample from another rabbit. No growth was obtained on repeated subculture and the samples were recorded as negative. Determinations of the penicillin concentrations in 15 rabbits after death yielded values of 50 IU per ml (three cases) or above. The highest concentration found was 100 IU per ml (two cases). The methacycline concentration was found to be between 1.5 μ g per ml (two cases) and 6.0 μ g per ml (seven cases). The urinary osmolality decreased from a mean value of 880 mOsm per kg on the 4th day to a mean value of 350 mOsm per kg at necropsy.

DISCUSSION

When bacteria are subjected to the action of antibiotics that inhibit cell-wall synthesis they are often converted to L-phase organisms. Earlier experiments by Taubeneck (1962), Guze and Kalmanson (1964) and others have shown that L-phase organisms may persist in the urinary tract. This is due to the hypertonic environment, which protects the L-phase organisms from osmotic lysis, as was demonstrated by Alderman and Freedman (1963) and Braude *et al.* (1968). Gnarpe and Edebo (1970) demonstrated that L-phase organisms were stabilised and viable in hypertonic environments provided that the pH value was below 5.5; this may explain their survival within the urinary tract. The present work confirms that L-phase organisms persist in the urinary tract of proteus-infected animals receiving penicillin treatment, since they were absent from the urine of only 2.5 per cent. (2 of 84) after 4 days' treatment and 9 per cent. (2 of 22) after 14 days' treatment.

Increased diuresis has been shown to diminish the renal damage in experimental pyelonephritis (Shapiro *et al.*, 1969) and should be highly effective against osmotically fragile L-phase organisms. In the present study, glucose-induced diuresis eliminated L-phase organisms from the urine of up to 82 per cent. of the animals but from only 59 per cent. of the kidneys. Methacycline eliminated L-phase organisms from nearly 80 per cent. kidneys, and there was no striking difference between the findings in kidney tissue and urine (80 and 76 per cent., respectively).

The different findings in urine and kidney tissue between the groups treated with diuresis and with methacycline might be due to preservation of L-phase organisms from osmotic lysis by the higher osmolality of the kidney tissue and the more acid environment of the renal papillae. In animals treated by both increased diuresis and methacycline, L-phase organisms were eliminated from all 20 urine samples but only from 90 per cent. of the kidneys. Similar findings in human infections have been reported by Kalmanson and Guze (1968). Apart from indicating the superior effect of combined treatment with diuresis, the clinical importance of these findings is that L-phase infections may not be detected by ordinary methods. As reported earlier (Sanford *et al.*, 1956; Gnarpe and Edebo, 1965; Gnarpe, 1970; Gnarpe and Olding, 1970) leucocytes may be absent from the urine especially when *Proteus* spp. are the causative organisms. Several cultures both for L-phase and bacillary form should be made especially in patients with chronic infections or those treated with antibiotics that inhibit cell-wall synthesis before urinary tract infection can be considered successfully treated.

SUMMARY

Ascending urinary-tract infection with *Proteus vulgaris* was established in rabbits. The bacteria were converted to L-phase organisms *in vivo* by treatment with penicillin. L-phase organisms were eliminated without further treatment from 9 per cent. In animals in which osmotic diuresis was established, L-phase organisms were eliminated from 59 per cent. In those treated with methacycline

the elimination rate was 79 per cent. without and 90 per cent. with diuresis. A greater proportion of L-phase organisms survived in the kidney tissue than in the urine of animals treated by osmotic diuresis.

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