Induction of Cephalosporinase and Penicillinase in *Proteus* Species

Previous reports have shown that penicillinase (β-lactamase) is not an inducible enzyme in strains of *Escherichia coli*, *Klebsiella* and *Aerobacter*; but *Proteus* species have not been investigated[1–3]. There is also a species variation in penicillinase activity with different substrates[3]. Cephalosporinase activity has been described in many species of gram-negative bacilli[4] and is associated with cephalosporin resistance in *Proteus* species[5]. In the work recorded here, inducibility of cephalosporinase and penicillinase was investigated in strains of *Proteus morgani*, *P. vulgaris*, *P. rettgeri* and *P. mirabilis*. The strains were all resistant to ampicillin ((D(−)α-aminophenylacetamido)-penicillanic acid) and cephalothin ((7-thiophene-2-acetamido)-cephalosporanic acid), apart from *P. mirabilis* which was sensitive to cephalothin. Many strains of *P. mirabilis* are also sensitive to ampicillin, but these were not included in the present investigation as they either inactivate benzylpenicillin or cephalosporin slowly or not at all.

The organisms were shaken in nutrient broth containing 1 per cent glucose for 4 h at 37° C. Methicillin, benzylpenicillin or cephalothin was added after the first and second hour of incubation to a final concentration of 100 μg/ml. on each occasion. An exception was *P. mirabilis*, which was sensitive to cephalothin, and this was added to a final concentration of 5 μg/ml. at similar time-intervals, but methicillin and benzylpenicillin were added in the same concentration as with the other strains. 1 ml. of the 4 h shaken broth culture of approximately the same optical density was used for each assay and chloramphenicol (50 μg/ml.) was added before assaying, to prevent further enzyme formation. Cephalosporinase activity was estimated manometrically[6,7] with cephalothin as the substrate in a final concentration of 4 mg/ml. at *p*H 7·0 and incubated at 37° C. This method was not sufficiently accurate for estimating low levels of penicillinase, and for this enzyme assays were made by an iodometric method with benzylpenicillin as the substrate[2,8].

Typical results are shown in Tables 1 and 2.

Table 1. Cephalosporinase Activity
(μmole cephalosporanic acid/ml. of broth culture/h 37° C)

Inducing agent	*Proteus morgani*	*P. vulgaris*	*P. mirabilis*	*P. rettgeri*
Nil	< 5	< 5	—	13·9
Benzylpenicillin	42·4	33·7	—	63·2
Cephalothin	34·5	31·5	—	56·8
Methicillin	< 5	15·6	—	53·3

−, Nil detected.

Table 2. Penicillinase Activity
(μmole penicilloic acid/ml. of broth culture/h 37° C)

Inducing agent	*Proteus morgani*	*P. vulgaris*	*P. mirabilis*	*P. rettgeri*
Nil	0·4	0·2	3·4	0·4
Benzylpenicillin	7·4	3·4	4·0	1·4
Cephalothin	5·2	2·5	8·0	1·3
Methicillin	1·4	1·6	3·0	1·5

Results of a similar order have been obtained with other strains of these species. Samples of the same broth culture were used for estimating the activity of both enzymes, but the results are not strictly comparable as the two assay techniques were different. However, the tables show that both cephalosporinase and penicillinase activity was inducible with the strains of *P. morgani*, *P. vulgaris* and *P. rettgeri*, and that cephalosporinase activity was greater than that of penicillinase. The enzymes were mainly cell-bound and the increased activity in the presence of the antibiotic was therefore not due only to the release of enzyme into the medium. Cephalosporinase activity was not detected in the culture of *P. mirabilis*, and penicillinase in this organism appears to be constitutive. Methicillin appears to be less effective as an inducing agent than benzylpenicillin or cephalothin, and experiments have shown it to be inactivated, very slowly by these organisms. Although cephalosporinase and penicillinase are referred to as separate enzymes, it is possible, in the strains of *P. morgani*, *P. vulgaris* and *P. rettgeri* examined, that there is one enzyme which mainly inactivates cephalosporin and has some associated activity against benzylpenicillin.

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Note added in proof. Since this communication was submitted, J. M. T. Hamilton-Miller[9] has reported induction of penicillinase in *Proteus morgani*.

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PHYSIOLOGY

A Technique for Urine Collection in the Female Rat for Investigating Iodine Metabolism

In examining iodine metabolism in the rat, the greatest source of error lies in the technique of urine collection. The main obstacle has been contamination of the urine by exogenous iodide from sources such as fæces, foodstuffs, glassware, etc. Many methods have been used in an attempt to overcome this difficulty[1–10], but none of them has been wholly successful. Another source of error is loss of urine by evaporation and absorption by fæces. In methods where radioactive iodine is used, another disadvantage lies in the washing[5] necessary to avoid loss of radioiodine, with consequent loss of counting efficiency.

The present experiments are an attempt to devise a technique which will overcome these difficulties and to compare the results obtained with the standard method of Isler *et al.*[2]. The basis of the technique which we have used is to tie the urethra of the rat and to obtain the required urine within the bladder itself and in this way to avoid contamination. The experiments have also been designed to investigate the effect of the procedure on renal function, particularly the ability of the kidney to maintain urinary specific gravity under constant conditions, and the possible production of dilatation of the ureters has also been studied.

Exp. 1. The effect of tying the urethra on renal function.

Day 1. Six adult female rats (Norwegian Hooded Lister strain), weighing 200–230 g, were given 5 ml. water/100 g body-weight orally, using the method of Reinecke *et al.*[11]. The animals were caged separately and the urine collected over a 5 h period, using the method of Isler *et al.*[2] except that, during this period of urine collection, no food or water was given to the animals.

The volume of urine was measured for each rat and the rate of urine flow per hour was estimated. Two ml. of urine was weighed using a Stanton Unimatic Balance, model *CL*.1, and the specific gravity of each sample calculated.

Day 2. The six rats were anæsthetized by intraperitoneal injection of 1·5 ml. of avertin solution in normal saline (2·5 g/100 ml.), the bladder was emptied by suprapubic pressure[12] and the urethra ligatured in the following way:

The clitoris was tied with Ethicon Surgical linen thread, Code *LR*.35, size 1 U.S.P., 3–5 mm from the distal end in