Isolation of an Antibiotic-Resistant, Lactose-Fermenting Strain of *Proteus rettgeri*

Within the past few months, a strain of *Proteus rettgeri* has been repeatedly isolated from the urinary tract, perineum, feces, facial cloths, sinks, and bed-pans, as well as the outside surface of catheter-urine collection bags of 20 patients, all from one ward. This strain was unusual in that it fermented lactose overnight and was resistant to all antibiotics tested.

The organism grew readily on blood, MacConkey, eosine-methylene blue (EMB) and Salmonella-Shigella agar. On EMB agar, colonies displayed a metallic sheen. The isolate produced a characteristic odor, comparable to that of 'cheese-crackers', on blood and MacConkey agar. The basic biochemical reactions[1] of this organism are listed in Table I. The antibiogram[2] and minimal inhibitory concentrations (MIC) of several antibiotics and chemotherapeutic agents versus this organism are presented in Table II. Mueller-Hinton broth (Difco) served as the growth medium and diluent for the tube dilution sensitivity studies; the bacterial inoculum was adjusted to result in 1.5×10^6 organisms per ml at zero time. The organism was found to be sensitive to methenamine mandelate only. It should be added that one patient received a standard regimen of gentamicin sulfate, following which this patient's strain had become resistant to gentamicin, in that there was no more zone of inhibition around the 10 µg disk and the MIC of this antibiotic exceeded 100 µg/ml. Antibiotic synergy studies, employing carbenicillin and gentamicin sulfate, failed to reveal any additive or synergic effect between these 2 antibiotics.

All attempts aimed at transferring the antibiotic resistance of this organism to recipient female strains of *E. coli* failed. In these experiments[3], *E. coli* strains 1485 lac$^+$ F$^-$ and CS100 lac$^-$ F$^-$ served as the recipients. Exponentially growing donor and recipient cells were mixed in the ratio of 1:10, by adding 14 ml of nutrient broth (Difco) (NB) containing 1.5×10^7 donor cells/ml to 10 ml of NB containing 1.5×10^8 recipient cells/ml in a 125 ml Erlenmeyer flask. For control purposes, the *P. rettgeri* isolates as well as *E. coli* strains 1485 and CS100 were grown in NB alone. As an additional control, *E. coli* strain RS-2 lac$^-$F$^+$, which carries a resistance factor containing markers for resistance to kanamycin sulfate, ampicillin, chloramphenicol, and sulfonamide, was conjugated with the 2 female recipient strains of *E. coli*, to prove the latter's competence in these experiments. The organisms were incubated overnight at 37 °C. Following stationary incubation, the flasks were shaken vigorously. Serial tenfold dilutions of the growth of each of the flasks were prepared in NB and 0.03 ml of each dilution were streaked with glass rods on MacConkey agar (Oxoid) (without crystal violet) plates, that contained 25 units/ml polymyxin B, 20 µg/ml kanamycin sulfate, 10 µg/ml chloramphenicol, 10 µg/ml tetracycline hydrochloride, and 10 µg/ml gentamicin sulfate, respectively. MacConkey agar plates, without added antibiotics, were inoculated with the donor and recipient control organisms. Following overnight incubation at 37 °C, the plates were examined.

Growth curve studies indicated that this organism multiplied in pooled fresh human urine as rapidly at room temperature as at 37 °C. This organism never gave rise to symptoms or signs of urinary tract infection in any of the 20 patients, from whom it had been isolated repeatedly over periods of several weeks and at numbers of greater than 10^5 organisms/ml. Serum bactericidal tests[4], employing fresh sera from patients and that of the author, indicated this organism to be exquisitely serum-sensitive. The indirect bacterial hemagglutination technique[5] failed to detect significant titers of circulating antibodies in these patients. One may conclude that this multiple-antibiotic resistant strain of *P. rettgeri* is avirulent and merely colonizes the urinary tract of these patients.

Table I. Biochemical characteristics of the lactose-fermenting strain of *P. rettgeri*

Test	Result
Motility 37 °C	—
22 °C	—
Indole	+
Methyl red	+
V–P	—
Citrate	+
Urea	+
Phenylalanine deamination	+
Lysine decarboxylase	—
Ornithine decarboxylase	—
Glucose	acid, no gas
Lactose	+
ONPG	+
Mannitol	+
Maltose	—
Arabinose	—
Inositol	—
Gelatin hydrolysis	—
Nitrate reduction (NO_2)	+
H_2S (Kligler iron agar)	—
O–F test (Hugh-Leifson)	fermentation

Table II. Antibiotic resistance pattern of the lactose-fermenting strain of *P. rettgeri*

Antibiotic	Disk content (µg)	Diameter of zone of inhibition (mm)	Minimal inhibitory concentration (µg/ml)
Ampicillin	10	6	> 100
Carbenicillin	100	6	> 500
Cephalothin	30	6	> 100
Chloramphenicol	30	6	> 100
Gentamicin sulfate	10	11	30
Kanamycin sulfate	30	6	> 100
Methenamine mandelate	1000 (1 mg)	20	
Neomycin	30	6	
Nitrofurantoin	30	6	
Polymyxin B	30	6	100
Streptomycin	10	6	
Tetracycline HCl	30	6	> 100
Triple sulfonamide	300	6	

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This organism was found to display certain characteristics that are typical for *E. coli*, such as, the prompt fermentation of lactose and the production of a metallic sheen on EMB agar. On the other hand, the isolate was shown to have the following features consistent with *P. rettgeri*: citrate utilization, deamination of phenylalanine, hydrolysis of urea, absence of gas formation during glucose fermentation, and lack of fermentation of arabinose and maltose. This is why we tentatively chose to designate this organism a promptly lactose-fermenting strain of *P. rettgeri*. It is conceivable that this organism is a 'hybrid' that might have arisen as a result of an as yet unknown genetic interaction between a strain of *E. coli* and a strain of *P. rettgeri*. To the best of our knowledge, this is the first strain of *P. rettgeri* isolated that ferments lactose overnight[6-11].

This organism can be retrieved without difficulty from clinical material and all of the isolated strains consistently ferment lactose, constantly display identical antibiograms and produce the typical, unmistakable odor. A detailed summary of the outbreak due to this organism will be reported elsewhere (C. E. McCALL, in preparation)[12].

Zusammenfassung. Ein avirulenter, prompt Laktosefermentierender, Antibiotika-resistenter Stamm von *Proteus rettgeri* wurde aus klinischem Material isoliert.

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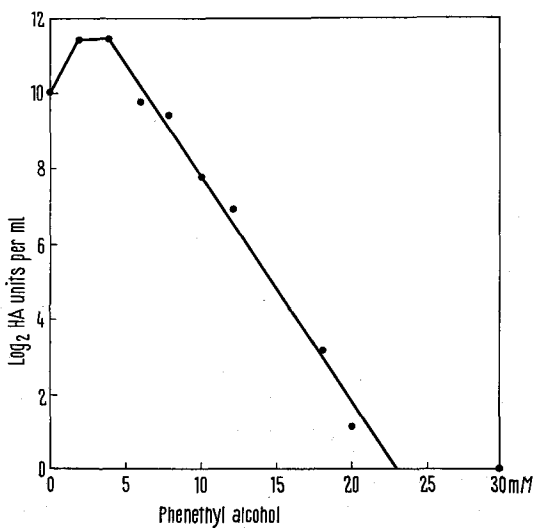
Effects of Phenethyl Alcohol on Influenza Virus Growth and Macromolecular Synthesis in Chick Embryo Cells

The bacteriostatic drug phenethyl alcohol has previously been shown to inhibit the replication of several DNA viruses[1-3], and the growth of small RNA viruses in bacteria[4] and animal cells in suspension culture[5]. In all cases the effect was reversible, and the drug had to be present during the time of viral replication in order to cause inhibition. The site of action of phenethyl alcohol is not known, although there are reports of alterations in cell membrane function in treated cells[6-8]. This results in inhibition of cell growth, and of the synthesis of RNA, DNA, and protein in both bacteria[9,10] and animal cells in suspension culture[11-13]. These effects on macromolecular synthesis and cell growth have been shown to be reversible following removal of the drug even after treatment for 12 days[11].

The replication of influenza viruses differs from that of small RNA viruses in a number of respects, and in particular displays a high degree of dependence on host cell metabolism[14]. It was therefore of interest to study the effect of phenethyl alcohol on influenza virus growth in monolayer cultures of chick embryo fibroblast cells. Since there are no previous reports of the action of phenethyl alcohol on cells in monolayer culture, as opposed to suspension cultures which are dividing rapidly[13], we also measured RNA and protein synthesis in treated cells.

Cell cultures were prepared and infected with influenza virus (fowl plague virus, Rostock strain) as detailed elsewhere[14]. Following virus adsorption, the cells were washed in phosphate-buffered saline, then incubated at 37 °C in growth medium supplemented with various concentrations of phenethyl alcohol[15]. After 24 h, the yield of virus released into the medium was assayed by haemagglutination titration (Figure). Each point represents the average haemagglutinin yield from 2 Petri dish cultures. It can be seen that very small concentrations of phenethyl alcohol, up to 5 m*M*, caused some increase in virus yield, but higher concentrations resulted in a marked inhibition which was complete at about 20 m*M*.

The inhibitory effect of phenethyl alcohol on influenza virus replication was not due to irreversible cell damage. Cells were incubated with 10 m*M* phenethyl alcohol for 2 h, then washed free of the drug before infection. Virus yields 24 h later did not differ significantly from those obtained from untreated cells. In further experiments phenethyl alcohol (10 m*M*) was added at intervals after infection and the haemagglutinin yield determined 24 h post-infection. Inhibition was observed when the drug was added at any time up to 3 h post-infection: thereafter the effect diminished and if added later than 10 h post-infection the virus yield at 24 h was normal. Since virus



Effect of various concentrations of phenethyl alcohol on the yield of influenza virus from chick embryo fibroblast cell monolayers.