infections. When therapy was delayed as long as 5 days, the 3 antibiotics substantially reduced kidney damage and the number of positive cultures obtained if given in large doses. Sulfadiazine was not beneficial for either infection if treatment was delayed more than one day after inoculation. The same was true for polymyxin (Pseudomonas infection). When one treatment i.v. was given 1 hour after challenge, only those agents found active in the delayed therapy experiments gave good results. The other chemotherapeutics were ineffective by any test method. Gamma globulin as well as specific antisera reduced the incidence of gross kidney damage and infection when administered before or up to 24 hours after i.v. inoculation.

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The Estimated Size of *Pseudomonas aeruginosa* β-Galactosidase (32905)

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Variations in structure of the enzyme β-galactosidase provide an index for natural relationships among bacteria (1). Previous studies comparing *Bacillus megaterium* and *Escherichia coli* β-galactosidases suggest no relatedness (2); this is exemplified by the difference in size of their respective enzymes (mol. wt. 150,000 and 518,000). A similar comparison revealed the β-galactosidases of *E. coli* and *Aeromonas formicans* to be identical in size, but very different in amino acid composition (3). Because the *Escherichia* and *Aeromonas* enzymes alone are antigenically similar, size may be employed as a major distinction.

Members of the genera *Pseudomonas* and *Aeromonas* are taxonomically allied by similarities in morphology, i.e., polar flagellation. The natural relationship of *Aeromonas* species to pseudomonads, or to enteric organisms (e.g., *E. coli*), is unresolved despite the use of Adansonian analysis for overall similarity and methods for testing nucleic acid homologies (4). The isolation of β-galactosidase from *P. aeruginosa* provides new evidence for the reexamination of this controversy.

Materials and Methods. Ps. aeruginosa strain X was provided for this study by R. G. Doggett; it is one of 28 strains capable of hydrolyzing *o*-nitrophenyl-β-D-galactopyranoside (ONPG). The basal culture medium (Vogel and Bonner, medium E) was prepared by adding to 670 ml of distilled water 10 gm of $MgSO_4 \cdot 7H_2O$, 100 gm of citric acid $\cdot H_2O$, 500 gm of K_2HPO_4, and 175 gm of $Na(NH_4)$-$HPO_4 \cdot 4H_2O$; these ingredients diluted 50-fold will support growth of the organism and the addition of 0.2% (w/v) lactose insures enzyme production. Cells for extract preparation were grown in 2-liter Erlenmeyer flasks containing 1 liter of basal medium plus lactose. The flasks were incubated on a rotary shaker for 18 hours at 37°C. The final cell density was 150 units in a Klett-Summerson colorimeter (660-mμ filter). Cells were harvested by centrifugation and washed once with five times their wet weight of basal medium.

The extraction and enrichment procedures for *Ps. aeruginosa* β-galactosidase were performed at temperatures of 4–10°C. A 20% (w/v) suspension of cells in 0.1 *M* sodium phosphate buffer, pH 7.0, containing 10^{-3} *M* β-mercaptoethanol was sonified for 10 min; cellular debris was removed by centrifugation at 30,000*g* for 20 min. The assay of β-galactosidase employing ONPG has been described (3); the Lowry Folin–phenol method was used to determine total protein (5). The specific activity of the cell-free extract was 0.37 units/mg of protein. Fractionation

was accomplished with the addition of streptomycin sulfate (2.5%w/v) to precipitate nucleoprotein, ammonium sulfate (50%v/v) to precipitate β-galactosidase, and Sephadex G-100 filtration using previously reported methods (3). A single peak of enzyme activity was revealed in the filtration pattern. Selected fractions of high specific activity (9.28 units /mg of protein) were concentrated by ammonium sulfate precipitation for use in estimating the molecular weight of this enzyme. These preparations were stable during refrigeration for 1 week and yielded colorless solutions when dialyzed against extract buffer.

The molecular weight of *Pseudomonas* β-galactosidase was estimated by gel filtration on Sephadex G-100 using a Sephadex Laboratory Column (Pharmacia, Uppsala, Sweden). The column was 2.5 × 37 cm; the flow rate was 4.0 ml per cm^2 per hour. One ml fractions of eluate were automatically collected with a siphon which was siliconized by prior application of Desicote (Beckman Instruments, Inc., Fullerton, Calif.). The sample volume was 1.2 ml and the void volume was determined with Blue Dextran 2000 (Pharmacia). Andrews (6) has established the suitability of standards employed in this experiment. Bovine serum albumin (mol. wt. 67,000) and ovalbumin (mol. wt. 45,000) were determined as total protein. Cytochrome C (mol. wt. 12,400) was estimated spectrophotometrically at 412 mμ. A separate filtration for each standard located its relative position in the elution pattern. Standards combined with the enzyme were easily discernible and 1.0-ml fractions eliminated extrapolation of the solute peak to an apex (Fig. 1).

Results and Discussion. There is a linear relationship between the logarithm of the molecular weight of the protein standards and the ratio of their elution volume, V to the void volume, V_0 of the column (Fig. 2). I found the elution profile of Pseudomonas β-galactosidase closely situated to that of ovalbumin in each determination; the two proteins cannot be distinguished by calculation of V/V_0.

Proteins offer a mosaic of properties which lends itself to detailed comparisons. Where

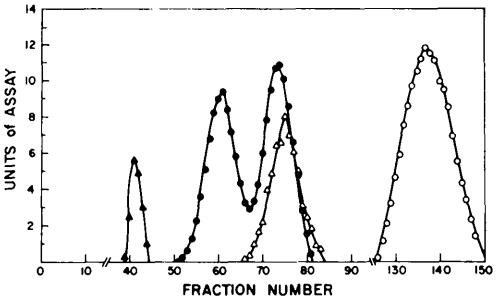

FIG. 1. The Sephadex G-100 filtration pattern of *P. aeruginosa* β-galactosidase (△) and standards: Blue Dextran 2000 (▲), bovine serum albumin and ovalbumin (●), cytochrome C (○). Each fraction (1.0 ml) was assayed according to the text; the respective assay values are represented on a scale of 1–12 units for profile comparison.

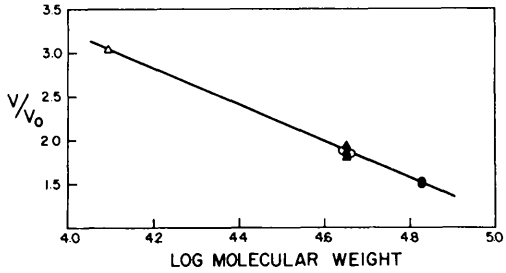

FIG. 2. Estimation of the molecular weight of *P. aeruginosa* β-galactosidase (○). The following proteins of known molecular weight (see text) were employed in the method of Andrews (6): bovine serum albumin (●), ovalbumin (▲), cytochrome c (△). Each symbol indicates a single value; the void volume (V_0) was separately determined for each of the three filtrations performed.

functionally analogous proteins derived from established taxonomic groups have been examined, results suggest that shared structural properties are an expression of the degree of relatedness (7,8). The estimated molecular weight of *Ps. aeruginosa* β-galactosidase (mol. wt. 45,000) contradicts the alliance of this organism and *A. formicans,* for aeromonads are known to closely resemble *E. coli* in terms of their β-galactosidases (mol. wt. 518,000). This suggests a natural separation between *Ps. aeruginosa* and the *Escherichia–Aeromonas* group. There is further evidence for this conclusion in the regulation of tryptophan biosynthesis (4).

The β-galactosidase is unique in the

amount of variation exhibited among bacteria. Size is a major distinction which may ultimately be related to the dimensions of a specific chromosomal segment in each organism. In certain cases, substrate specificity will be the primary concern; phosphorylation precedes hydrolysis in the catabolism of lactose by *Staphylococcus aureus* (9).

Summary. The β-galactosidase of *Pseudomonas aeruginosa* was isolated and enriched approximately 25-fold. Gel filtration experiments provide an estimated molecular weight of 45,000. The comparatively small size of this enzyme is evidence for the nonrelatedness of pseudomonads and previously characterized microorganisms (e.g., *Aeromonas formicans*).

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Anticonvulsant Activity of Antiparkinsonism Agents* (32906)

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Despite the progress in the treatment of epilepsy provided by the introduction of diphenylhydantoin and trimethadione, a large proportion of patients are only partially benefited and some seizure patterns are almost completely resistant to anticonvulsants currently available. Infantile myoclonic spasms and akinetic seizures in children are particularly resistant, and frequently are associated with a poor prognosis. A wider spectrum of anticonvulsant medications is urgently needed for the control of these and other refractory seizure patterns (1).

The choice of the synthetic, antiparkinsonism, atropine-like agents, procyclidine (Kemadrin) and trihexyphenidyl (Artane), for investigation as potential anticonvulsants was prompted by the evidence for a cholinergic factor in the propagation of the seizure discharge, the site of action of these drugs centrally at the level of the reticular system and centrencephalic structures, and their potency against nicotine-induced electroencephalographic abnormalities observed in experimental animals(2–4). Atropine is known to reduce spike-and-wave paroxysms in some patients with petit mal(2), but its chronic administration in anticonvulsant doses would be precluded by the occurrence of side effects. The newer, synthetic, antiparkinsonism agents are more specific in their central anticholinergic action and have fewer peripheral effects. The potential value of this class of compounds as anticonvulsants has been demonstrated in the present laboratory and preliminary clinical study.

Methods. Procyclidine and trihexyphenidyl were examined for their ability to protect mice from experimental seizures, and the anticonvulsant activity of procyclidine was also determined in children with frequently recurring seizures that had proved refractory to conventional antiepileptic compounds.

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