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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*Laboratory studies.* Major tonic–clonic seizures were elicited in male albino mice, 4–6 weeks old, by a 60-cycle alternating electric current applied by corneal electrodes after instillation of a local anesthetic to the conjunctival sac.(1) The animal was restrained only by hand and was released at the moment of stimulation in order to permit observation of the seizure throughout its entire course. A current of 50 mA for 0.2 sec, five times the threshold level, was sufficient to induce maximal tonic extensor seizures in all control animals. Protection from the extensor component of the toxic seizure was required for an anticonvulsant effect to be recorded. Pentylenetetrazol was employed for the induction of seizures that were principally clonic in pattern. A dose of 85 mg/kg by subcutaneous injection caused seizures in more than 95% of control mice within a 20-min period of observation, and the ability of the drug to protect the animal from a generalized clonic seizure was the end point of the test.

*Times of peak anticonvulsant effect.* In order that the relative potencies of procyclidine and trihexyphenidyl could be compared, it was necessary to determine for each drug the time required after administration for peak activity to be exhibited. The approximate dose necessary to protect 50% of the animals ($ED_{50}$) was first determined by means of the maximal electroshock test. Varied doses between 10 and 500 mg/kg, in 0.1–0.3 ml of acacia suspension, were injected intraperitoneally in groups of mice each containing five animals. The anticonvulsant effect was determined at intervals of 30 min after administration until the time of peak effect had passed, as indicated by fewer animals being protected than at an earlier time. Since frequent repetition of the electroshock seizure will itself modify the pattern of the seizure response, each group of animals was tested only once.

*Anticonvulsant $ED_{50}$.* Groups of at least five animals were given various doses of procyclidine and trihexyphenidyl, between 10 and 30 mg/kg and 30 and 45 mg/kg, respectively. Individual animals were challenged with the electroshock stimulus at the time of peak anticonvulsant effect. At least three doses were established in the range between complete protection and no protection. Results were plotted on logarithmic probability paper and a regression line drawn. The $ED_{50}$ and 95% confidence limits for each drug were calculated and the significance of the differences determined by the methods of Litchfield and Wilcoxon.(5)

The $ED_{50}$ of procyclidine against pentylenetetrazol seizures was established by employing dose levels between 50 and 100 mg/kg in five groups of animals.

*Toxic dose and protective index.* Acute toxicity was evaluated in animals receiving the drug in preliminary tests for anticonvulsant potency, and subsequently in experiments designed to establish the degree of toxicity more accurately. The posture, gait, muscle tone, and righting reflexes of each animal were examined before and at 30 min after administration of procyclidine, at six dose levels between 40 and 100 mg/kg. The dose that induced neurotoxicity in 50% of mice ($TD_{50}$) was determined by the same method employed for calculation of the $ED_{50}$. The ratio of the $TD_{50}$ to the $ED_{50}$, the protective index, is a measure of the potential therapeutic value of the drug.

*Clinical studies.* The anticonvulsant efficacy and toxicity of procyclidine were examined in a short-term, preliminary study of 16 children whose seizures had been found completely resistant to conventional and various new investigational compounds. Fifteen patients had generalized myoclonic spasms that were complicated by akinetic seizures in 4, and 1 patient had petit mal. The electroencephalogram was abnormal, showing seizure discharges in all patients; polyspike or 2/sec spike-and-wave patterns were present in 11 patients, hypsarhythmia in 4 patients, and bi-frontal sharp waves in 1 patient. Procyclidine was prescribed in 5 mg tablets, and each child received an initial dose of 1.25 mg, or one-quarter tablet, 2–4 times daily, according to the age and weight. Anticonvulsants given before the trial were continued in the same dosages during treatment with procyclidine. The parent received a printed form on which to record the number of seizures each day and any unusual symptoms or signs. The daily

incidence of seizures during the period of treatment with procyclidine was compared with the incidence recorded in a period of equal length before the trial.

*Results. Animal studies.* In the laboratory animals, the times of peak anticonvulsant effect of procyclidine and trihexyphenidyl were approximately 30 and 45 min, respectively.

The dose ($ED_{50}$) of procyclidine necessary to protect 50% of mice from the tonic component of the maximal electroshock seizure was 16 mg/kg, with 95% confidence limits of 14–17 mg/kg (Fig. 1). The $ED_{50}$ of trihexyphenidyl against electroshock seizures was 37 (32–40) mg/kg. The anticonvulsant potency of procyclidine in mice was significantly greater than that of trihexyphenidyl ($p<0.05$).

Procyclidine was also effective against clonic seizures induced by pentylenetetrazol; the $ED_{50}$ was 83 (69–100) mg/kg. Mild neurotoxicity was observed in 15% of mice after 50 mg/kg doses, and a dose of 500 mg/kg was lethal in all animals tested. Ataxia was the principal side effect. The dose of procyclidine necessary to induce ataxia in 50% of mice ($TD_{50}$) was 78 (64–94) mg/kg. The protective or therapeutic indices of procyclidine in mice for tonic electroshock and clonic pentylenetetrazol seizures were 5.0 and 0.9, respectively.

*Clinical studies.* A reduction in the incidence of generalized myoclonic spasms was obtained in 10 of 15 patients treated with procyclidine, and the degree of seizure control was 60% or greater in 5 patients (Table I). Two patients had an increased incidence of seizures, only 1 of 4 patients with akinetic seizures was benefited, and a single patient with petit mal was unaffected by the drug. The duration of therapy ranged from 7 to 46 days. The initial dose of procyclidine was 0.1–0.5 mg/kg daily, and the maximum daily dose was 0.3–0.7 mg/kg. The side effects reported in 3 patients were transient skin rash, infrequent urination, constipation, and drowsiness.

*Discussion.* The antiparkinsonism agents, procyclidine and trihexyphenidyl, have been found effective in the control or modification of experimental seizures in animals. Procyclidine was a more potent anticonvulsant than trihexyphenidyl; its protective or therapeutic

TABLE I. Anticonvulsant Activity of Procyclidine (Kemadrin) in Children with Refractory Myoclonic Seizure Disorders.

| Patient | Age (year) | Sex | Seizure incidence | | Seizure control (%) | Daily dose of procyclidine (mg/kg) | Duration of trial (days) |
|---|---|---|---|---|---|---|---|
| | | | Pretrial (daily) | Trial (daily) | | | |
| 1. | 7 | M | 4 | 0.25 | 95 | 0.2[b] | 23 |
| 2. | 12 | F | 6[a] | 2 | 66 | 0.1–0.7 | 36 |
| 3. | 2 | F | 6 | 2 | 66 | 0.4 | 30 |
| 4. | 2 | M | 2 | 0.7 | 65 | 0.5 | 14 |
| 5. | 2.75 | F | 1.4 | 0.6 | 60 | 0.3–0.4 | 30 |
| 6. | 1 | F | 1.2 | 0.7 | 42 | 0.2–0.4 | 7 |
| 7. | 2 | F | 4 | 2.4 | 40 | 0.3–0.6 | 46 |
| 8. | 4 | F | 1.9 | 1.2 | 36 | 0.3–0.5[b] | 10 |
| 9. | 2 | F | 0.3 | 0.2 | 33 | 0.3–0.5 | 15 |
| 10. | 5 | M | 0.4 | 0.3 | 25 | 0.2–0.4 | 30 |
| 11. | 1 | M | 1.5[a] | 1.5 | 0 | 0.4–0.7 | 18 |
| 12. | 7 | M | 2.5[a] | 2.5 | 0 | 0.1[b] | 7 |
| 13. | 0.5 | M | — | — | 0 | 0.4–0.6 | 6 |
| 14. | 1.5 | M | 4 | 11 | 0 | 0.2–0.4 | 8 |
| 15. | 8 | F | 3[a] | 6 | 0 | 0.2–0.4 | 21 |

[a] Akinetic and myoclonic seizures.
[b] Side effects noted.

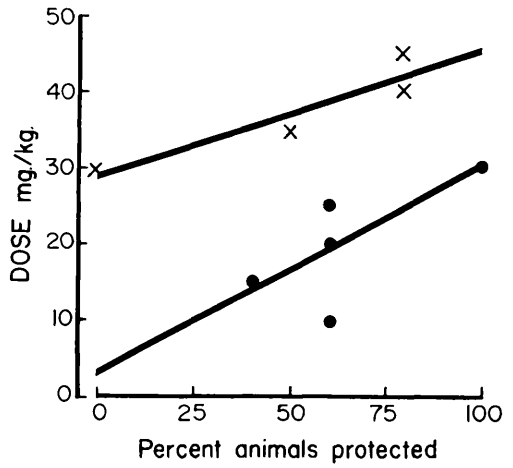


FIG. 1. Comparison of the anticonvulsant potencies and $ED_{50}$'s of procyclidine (●; Kemadrin, $ED_{50}$ = 16.5 mg/kg) and trihexyphenidyl (×; Artane, $ED_{50}$ = 37 mg/kg) against maximal electroshock seizures in mice.

index against electroshock seizures was relatively high and approximately double the value reported for phenobarbital.(1)

A preliminary clinical trial of procyclidine in infants and children with generalized myoclonic spasms has confirmed the findings in animal studies, demonstrating a degree of anticonvulsant activity against a seizure pattern usually refractory to conventional medications(6). The number of patients in the trial was small and the duration of treatment short, but the seizures were sufficiently frequent before the introduction of procyclidine to allow the recognition of an immediate anticonvulsant effect.

In the patients selected for this initial investigation seizures had been found resistant or tolerance had developed to several treatments, including corticotropin (ACTH), nitrazepam (Mogadon), acetazolamide, primidone, or oxazolidinediones. The seizure control obtained with procyclidine was not complete, and the continuing recurrence of seizures necessitated the substitution of alternative medications after periods of treatment varying from 1 to 7 weeks. A study of patients with seizure patterns less resistant to conventional medications would be required for the determination of the relative anticonvulsant efficacy of procyclidine.

The majority of anticonvulsant drugs in current use have been developed by minor modifications of the molecular structure of barbiturates, hydantoins, and oxazolidinediones. The need for anticonvulsants with different chemical formulas is evident from the poor response to existing medications reported in 25% of epileptic patients and particularly in children with myoclonic spasms and akinetic seizures. The demonstration that procyclidine, a synthetic atropine-like agent, has anticonvulsant activity should encourage further investigations of this class of compounds. The determination of structure–activity relationships and the site of action of these drugs might elucidate the mechanism of seizures and facilitate the development of new and more effective therapies.

*Summary.* The anticonvulsant activity of synthetic antiparkinsonism agents has been studied in mice with experimental seizures and in children with myoclonic spasms refractory to conventional medications. Procyclidine and trihexyphenidyl protected mice from maximal electroshock seizures. Procyclidine was the more potent compound and was also effective against pentylenetetrazol seizures. In a preliminary, short-term clinical trial, procyclidine caused a reduction in the incidence of seizures in 10 of 16 patients treated. The antiparkinsonism agents are free of severe chronic toxicity and are contraindicated only in adults with glaucoma or urethral obstruction. Further laboratory and clinical investigation of the anticonvulsant activity of this class of compounds is recommended.

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