

Effects of Some Antiparkinsonism Drugs and centrally Acting Muscle Relaxants on the Intercollicular Decerebrate Rigidity in Rats

HIDEOMI FUKUDA,¹⁾ TSUGUTAKA ITO,^{1a)} and MAKOTO KOKUBO¹⁾

Faculty of Pharmaceutical Sciences, Nagoya City University¹⁾

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The decerebrate rigidity in rats was prepared by means of transection between the inferior and superior colliculi. The strength of the rigidity was measured as electromyographic (EMG) activity of the rigid gastrocnemius muscle. Because of some similarity between Parkinsonism and intercollicular decerebrate rigidity, the effects of several antiparkinsonism drugs as well as centrally acting muscle relaxants on this type of the rigidity were studied. A small dose of chlorpromazine (125 μ g/kg, *i.v.*) markedly reduced the rigidity. The inhibitory effect of trihexyphenidyl, biperiden, orphenadrine and tigloidine injected intravenously was dose-related. L-Dopa exerted only a little effect on the EMG activity of the rigid muscle. Mephesisin, methocarbamol, tolperisone, phenprobamate and diazepam were all effective in reducing the rigidity. These results are discussed in relation to data on their effects upon the crossed extensor reflexes in spinal chicks.

Sherringtonian decerebrate rigidity due to an intercollicular transection has been used to quantify the effect of centrally acting muscle relaxants.²⁾ Rigidity of this type is thought to be dependent primarily on increased discharge from the muscle spindle due to the hyperactivity of gamma-efferents from the brain. It has been suggested that the rigidity in Parkinsonism is partly dependent upon disordered control of muscle spindle activity due to disintegration of the central gamma-efferent outflow.³⁾ Some similarity might exist between Parkinsonism and intercollicular decerebrate rigidity from an etiological aspect. For this reason, rigidity of this type may be useful for studying the efficacy of antiparkinsonism drugs. In the present study, the effects of some antiparkinsonism drugs as well as centrally acting muscle relaxants on intercollicular decerebrate rigidity were investigated in rats. The effects of drugs on polysynaptic reflexes in young chicks were also examined.

Materials and Methods

1) **Rigidity Due to Intercollicular Decerebration in Rats**—The arrangement was approximately the same as that described in a previous report.⁴⁾ The experiments were performed on male Wistar rats (Nippon Rat Co.) weighing from 270 to 460 g. Under ether anesthesia, the midbrain was sectioned by a spatula between the inferior and superior colliculi. The rat showing sustained rigidity was placed on its back and forelegs fixed. Electromyogram (EMG) was recorded by using a coaxial needle electrode inserted into the gastrocnemius muscle. A tension of 10 g was loaded, parallel to the tibial bone, to the ipsilateral hindleg toe in order to obtain a stable EMG frequency. EMG activities were amplified with an amplifier (Nihonkohden RB-2), transformed into square waves and fed into an integrator, the output of which was recorded by an inkwriting recorder (Nihonkohden WI-180). Exposed neural structures were covered with warm mineral oil, and body temperature was maintained constant using an infrared lamp. The location of the

- 1) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan*; a) Present address: *Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Suita, Osaka, 564, Japan*.
- 2) E.F. Domino, "Evaluation of Drug Activities: Pharmacometrics," Vol. 1, ed. by D.R. Laurence and A.L. Bacharach, Academic Press, London and New York, 1963, p. 313.
- 3) J. Stern and A. Ward, *Arch. Neurol.*, **3**, 193 (1960); G. Rushworth, *J. Neurol. Neurosurg. Psychiat.*, **23**, 99 (1960); H. Shimazu, T. Hongo, K. Kubota, and H. Narabayashi, *Arch. Neurol.*, **6**, 24 (1962).
- 4) H. Fukuda, K. Watanabe, and T. Ito, *Japan. J. Pharmacol.*, **22**, 457 (1972).

central nervous system (CNS) lesions was confirmed histologically.

2) **Polysynaptic Spinal Reflex in Young Chicks**—The arrangement was the same as that described previously.⁵⁾ Chicks ranging in age from 2 to 15 days after hatching were used. The chick was anesthetized with chloralose (50 mg/kg, *i.p.*), artificially ventilated and spinalized by transecting the cord at the level of C10. Crossed extensor reflexes of the leg were elicited by electrical stimulation of the contralateral peroneal nerve (supramaximal square wave pulses, 0.1 Hz, 1 msec in duration) and recorded on a kymograph using an isotonic lever.

3) **Chemicals**—Drugs used were chlorpromazine-HCl (Takeda), trihexyphenidyl-HCl (Takeda), biperiden lactate (Dainippon), orphenadrine-HCl (Dainippon), tigloidine-HBr (Boehringer), L-dopa (Nippon Kayaku), mephenesin (Chugai), methocarbamol (Grelan), carisoprodol, tolperisone-HCl (mydocalm-HCl, Nippon Kayaku), phenprobamate (Nihon-Chemiphar) and diazepam (Takeda). Drugs were dissolved in 0.9% saline for intravenous injection or suspended in 0.8% carboxymethyl cellulose (CMC) solution for intraperitoneal injection. Drug solutions were injected through a cannula either into a femoral vein or intraperitoneally.

Results

Effect on the Intercollicular Decerebrate Rigidity

Rigidity occurred in about half the number of rats which were sectioned between the inferior and superior colliculi of the midbrain. The frequency of spontaneous discharges in the rigid gastrocnemius muscle was usually 120–250 Hz. With an intravenous injection of 2.0 ml/kg of saline, a maximum decrease in the rate of discharge was 10%. An intraperitoneal injection of 1.0 ml/kg of CMC solution had no effect on the EMG activity of the rigid muscle.

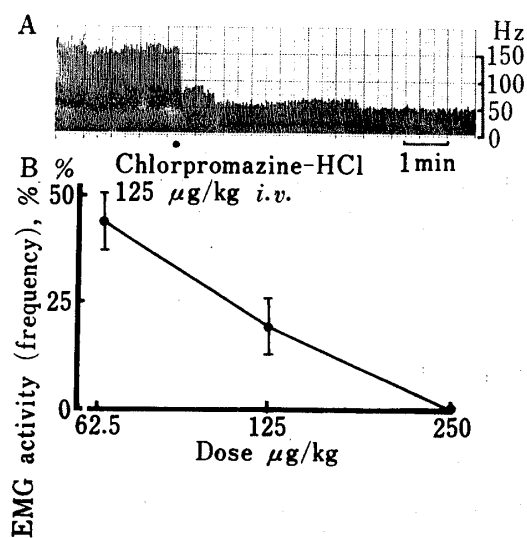


Fig. 1. Effect of Chlorpromazine on Intercollicular Decerebrate Rigidity in Rats

A: an example of the record of EMG frequency, B: dose-response relationship for chlorpromazine in the depression of the rigidity, abscissa: intravenous dose, on a logarithmic scale, ordinate: EMG activities 5 min after the injection, as expressed as percentage of the frequency of discharge to those obtained before the injection. Each point represents the mean of EMG activities in five different experiments, with the S.E. indicated.

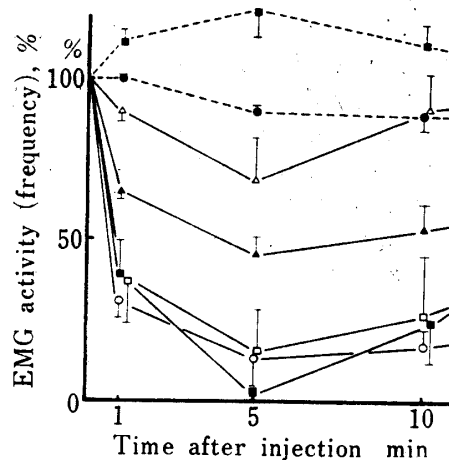


Fig. 2. Comparison of Discharge Patterns induced by Antiparkinsonism Drugs (*i.v.*) in Intercollicular Decerebrate Rigidity in Rats

abscissa: time after the injection, ordinate: EMG activities as expressed as percentage of the frequency of discharge to those obtained before the injection. Each point represents the mean of EMG activities in five different experiments, with the S.E. indicated.

- : trihexyphenidyl-HCl 2.5 mg/kg
- △—: L-dopa 20 mg/kg
- : saline 2 ml/kg
- ▲—: tigloidine-HBr 5.0 mg/kg
- : biperiden lactate 2.5 mg/kg
- : trihexyphenidyl-HCl 10 mg/kg
- : orphenadrine-HCl 2.5 mg/kg

5) a) H. Fukuda, K. Watanabe, Y. Kudo, and T. Ohshima, *Yakugaku Zasshi*, **88**, 1338 (1968); b) Y. Kudo, T. Ohshima, S. Sato, K. Watanabe, and H. Fukuda, *Chem. Pharm. Bull.* (Tokyo), **18**, 591 (1970).

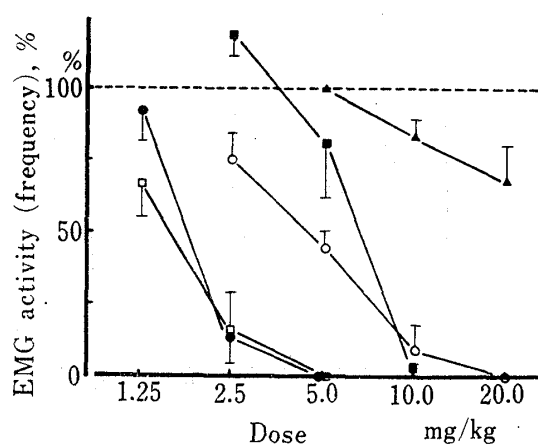


Fig. 3. Dose-response Relationships for Antiparkinsonism Drugs (*i.v.*) in the Depression of Intercollicular Decerebrate Rigidity in Rats

abscissa: intravenous dose, on a logarithmic scale, ordinate: EMG activities 5 min after the injection, as expressed as percentage of the frequency of discharge to those obtained before the injection. Each point represents the mean of EMG activities in five different experiments, with the S.E. indicated.

—□—: biperiden lactate
 —●—: orphenadrine-HCl
 —■—: trihexyphenidyl-HCl
 —○—: tigloidine-HBr
 —▲—: L-dopa

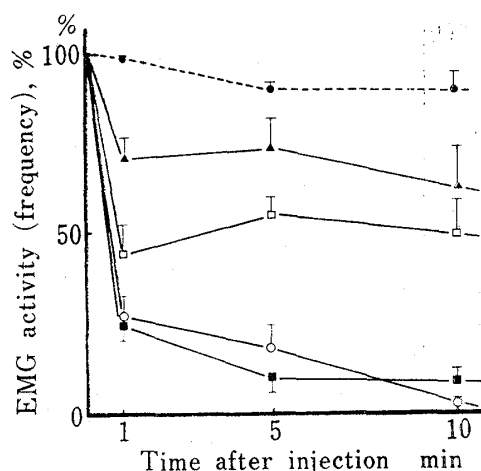


Fig. 4. Comparison of Discharge Patterns induced by centrally Acting Muscle Relaxants (*i.v.*) in Intercollicular Decerebrate Rigidity in Rats

Each point represents the mean of EMG activities in five different experiments, with the S.E. indicated.

—●—: saline 2 ml/kg
 —▲—: methocarbamol 50 mg/kg
 —□—: mephenesin 12.5 mg/kg
 —■—: tolperisone-HCl 2.5 mg/kg
 —○—: chlorpromazine-HCl 125 µg/kg

The effect of chlorpromazine, which in small doses abolished cat rigidity of this type,⁶⁾ was studied. The dose-dependent depression of discharge by chlorpromazine-HCl is depicted in Fig. 1. Chlorpromazine-HCl (125 µg/kg, *i.v.*) reduced the rate of discharge by about 74%, 83% and almost 100% in 1, 5 and 10 min respectively (Fig. 4).

As seen in Fig. 2, trihexyphenidyl-HCl (10 mg/kg, *i.v.*), biperiden lactate (2.5 mg/kg, *i.v.*), orphenadrine-HCl (2.5 mg/kg, *i.v.*) and tigloidine-HBr (5.0 mg/kg, *i.v.*) clearly reduced the rate of discharge. In contrast to chlorpromazine, the depressant effect of these drugs was not long lasting. For example, trihexyphenidyl-HCl (10 mg/kg, *i.v.*) reduced the rate of discharge by 62%, 99% and 77% in 1, 5 and 10 min respectively. The dose-response relationships for antiparkinsonism drugs used in this study are shown in Fig. 3. Biperiden lactate and orphenadrine-HCl significantly reduced the rate of discharge even at a low dose of 1.25 mg/kg. Unexpectedly a small dose of trihexyphenidyl-HCl (2.5 mg/kg, *i.v.*) caused an increase in the rate up to about 20%. The dose-response curve for tigloidine-HBr, a naturally occurring analogue of atropine, is gentler as compared with those for the other drugs; the range of effective doses was 2.5—20 mg/kg. L-Dopa (20 mg/kg, *i.v.*) exerted a weak effect on the rate of discharge.

Mephenesin (12.5 mg/kg, *i.v.*, 25 mg/kg, *i.p.*), methocarbamol (50 mg/kg, *i.v.*), tolperisone-HCl (2.5 mg/kg, *i.v.*, 10 mg/kg, *i.p.*), phenprobamate (10 mg/kg, *i.p.*) and diazepam (5 mg/kg, *i.p.*) reduced the rate of discharge (Fig. 4 and 5). Tolperisone-HCl (*i.v.*) was more potent than mephenesin (*i.v.*) and methocarbamol (*i.v.*). In the intraperitoneal route, the order of potency of the drugs in reducing the intercollicular decerebrate rigidity was as follows: diazepam > tolperisone-HCl, phenprobamate, carisprodol > mephenesin. The dose-response relationships for the four centrally acting muscle relaxants and diazepam are shown in Fig. 6.

Because of the possibility that the reduction of the rigidity was partly or wholly due to

6) H.D. Henatsch and D.H. Ingvar, *Arch. Psychiat. Nervkr.*, 195, 77 (1956).

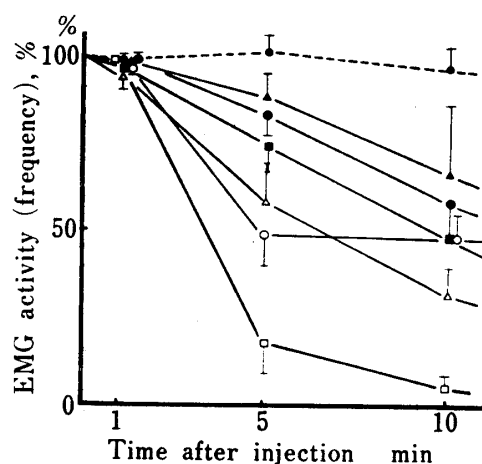


Fig. 5. Comparison of Discharge Patterns induced by centrally Acting Muscle Relaxants (*i.p.*) in Intercollicular Decerebrate Rigidity in Rats

Each point represents the mean of EMG activities in five different experiments, with the S.E. indicated.

- : 0.8% CMC 1 ml/kg
- ▲---: carisoprodol 10 mg/kg
- : phenprobamate 10 mg/kg
- : mephenesin 25 mg/kg
- : tolperisone-HCl 10 mg/kg
- △---: diazepam 5 mg/kg
- : mephenesin 50 mg/kg

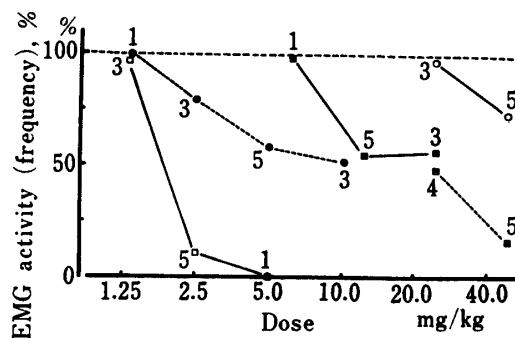


Fig. 6. Dose-response Relationships for centrally Acting Muscle Relaxants in the Depression of Intercollicular Decerebrate Rigidity in Rats

—: intravenous route, ----: intraperitoneal route, abscissa: dose, in a logarithmic scale; ordinate: EMG activities 5 min after the injection, as expressed as percentage of the frequency of discharge to those obtained before the injection. Each point represents the mean of EMG activities, with the S.E. indicated. The number of animals for each dose is given in parentheses.

- : tolperisone-HCl *i.v.*
- : diazepam *i.p.*
- : mephenesin *i.v.*
- : mephenesin *i.p.*
- : methocarbamol *i.v.*

the effect of drugs on blood pressure, the effect on arterial blood pressure was studied in the decerebrate rats. The mean blood pressure of the decerebrate rats was 80 to 140 mmHg. Chlorpromazine-HCl (125 μ g/kg, *i.v.*) produced a fall of blood pressure (70 mmHg).⁴⁾ Trihexyphenidyl-HCl (10 mg/kg, *i.v.*), orphena drine-HCl (5.0 mg/kg, *i.v.*), tigloidine-HBr (20 mg/kg, *i.v.*), methocarbamol (50 mg/kg, *i.v.*) and tolperisone-HCl (5 mg/kg, *i.v.*) produced a temporary fall of blood pressure (less than 40 mmHg); and L-dopa (10 mg/kg, 20 mg/kg, *i.v.*)

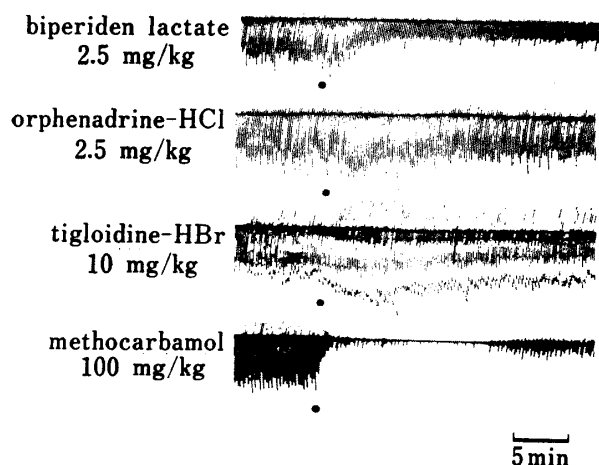


Fig. 7. Examples of Patterns of Polysynaptic Reflexes for Three Antiparkinsonism Drugs and One centrally Acting Muscle Relaxant in Spinal Chicks

Record of each response shows the crossed extensor reflex of the leg. Reflexes were elicited once every 10 sec by stimulation of the contralateral peroneal nerve.

produced a temporary rise of blood pressure (less than 30 mmHg). These changes, however, returned to control levels within several minutes. Biperiden lactate (2.5 mg/kg, *i.v.*), mephenesin (12.5 mg/kg, *i.v.*), carisoprodol (10 mg/kg, *i.p.*), phenprobamate (10 mg/kg, *i.p.*) and diazepam (5 mg/kg, *i.p.*) produced little or no change in blood pressure. Depression of discharges caused by the drugs was observed even after return of the blood pressure to control levels. Thus a direct relationship between the depressant effect on the rigidity and the change of blood pressure could not be recognized. A dose of 1.25 μ g/kg of acetylcholine chloride, sufficient to cause a fall of 50 mmHg or more, produced no significant effects on EMG activity in rigid rats.

Effect on the Polysynaptic Reflex

Our previous reports^{5a,7)} showed that 50% effective doses (*i.v.*) of mephenesin, carisoprodol, tolperisone-HCl and diazepam in causing a reduction in the crossed extensor reflexes in spinal chicks were about 16.5 mg/kg, >4.0 mg/kg, 8.4 mg/kg and 0.08 mg/kg respectively.

Trihexyphenidyl-HCl (10 mg/kg, *i.v.*) and biperiden lactate (2.5 mg/kg, *i.v.*, Fig. 7) strongly depressed the reflex. Methocarbamol caused 50% and 90% depression with 50 mg/kg and 100 mg/kg respectively (Fig. 7). In contrast, chlorpromazine-HCl (125 µg/kg, *i.v.*), tigloidine-HBr (10 mg/kg, *i.v.*), orphenadrine-HCl (2.5 mg/kg, *i.v.*), L-Dopa (20 mg/kg, *i.v.*) and phenprobamate (10 mg/kg, *i.p.*) did not depress the reflexes. Examples of kymographic records are shown in Fig. 7.

Discussion

It has been demonstrated by Henatsch, *et al.*⁶⁾ that chlorpromazine-HCl (0.5—2.5 mg/kg, *i.v.*) abolishes intercollicular decerebrate rigidity in cats. On the other hand, Maxwell, *et al.*⁸⁾ reported that larger doses were required to depress ischemic decerebrate rigidity in cats. It has been generally said that chlorpromazine exerts a selective depressant effect on the gamma-motoneurons and consequently counteracts the intercollicular decerebrate rigidity which depends primarily upon hyperactivity of gamma-efferents. In the present study, small doses of chlorpromazine (62.5—125 µg/kg) also depressed intercollicular decerebrate rigidity in rats (Fig. 1). Thus it can be considered that the intercollicular decerebrate rigidity of rats may also include hyperactivity of the gamma-system as in the case of the cat rigidity of this type.

The mode of drug action in reducing the decerebrate rigidity is not uniform, and, besides the gamma-system, possible sites of drug action should include the following: neuromuscular junction, muscle, muscle spindle, spinal interneurons, motoneurons, and lower brain stem.

Trihexyphenidyl, biperiden, orphenadrine and tigloidine were dose-dependently effective in reducing the intercollicular decerebrate rigidity in rats (Fig. 3). It thus appears that antiparkinsonism drugs of both antihistaminic and antispasmodic types were effective in reducing the intercollicular rigidity. L-Dopa (20 mg/kg, *i.v.*) exerted only a weak effect on the EMG activity of the rigid muscle, although it has been reported that the drug abolishes reserpine rigidity.⁹⁾

The centrally acting muscle relaxants studied here efficiently reduced the rigidity of rats, being consistent with the studies using rats¹⁰⁾ and cats.¹¹⁾ Diazepam was four times more potent in reducing the rigidity and 200 times more potent in reducing the polysynaptic reflex than mephenesin. Therefore, it seems unlikely that depression of the polysynaptic reflex by diazepam predominantly contributes to the abolition of the rigidity. Among the centrally acting muscle relaxants studied, tolperisone was the most effective in reducing the rigidity, being seven times more potent than mephenesin. In the depression of polysynaptic reflex in chicks, however, the ratio of the effect of tolperisone to that of mephenesin was only approximately 2.0. Our previous study⁷⁾ showed that tolperisone reduced the neuromuscular transmission in skeletal muscle and also the activity of muscle spindle afferents. These effects might contribute to the strong depressant effect on the intercollicular rigidity in rats. Or-

7) H. Fukuda, K. Watanabe, Y. Kudo, T. Ohshima, and T. Ito, *Pharmacometrics*, **4**, 125 (1970).

8) D.R. Maxwell and M.A. Read, *Neuropharmacology*, **11**, 849 (1972).

9) I. Jurna, *Arch. Pharmakol. exp. Path.*, **259**, 181 (1968).

10) A. Matsushita and C.M. Smith, *Brain Research*, **19**, 411 (1970).

11) E.M. Keary and D.R. Maxwell, *Brit. J. Pharmac. Chemother.*, **30**, 400 (1967); J.H. Chin and C.M. Smith, *J. Pharmac. exp. Ther.*, **136**, 276 (1962); R.D. Hudson and M.K. Wolpert, *Neuropharmacology*, **9**, 481 (1970).

phenadrine has been reported in cats to exert a muscle relaxant activity without association of the polysynaptic reflex pathways¹²⁾ and to cause a decrease in the intercollicular decerebrate rigidity in cats.¹³⁾ Orphenadrine reduced this type of the rigidity in rats (Fig. 2 and 3), and enhanced the crossed extensor reflexes in chicks (Fig. 7). Tigloidine, which structurally resembles atropine, has been found to be helpful in the treatment of Parkinsonism, and was found to cause potentiation of mono- and poly-synaptic reflexes in cats probably due to a weak depressant effect on the inhibitory neurones.¹⁴⁾ In the present study also, tigloidine reduced the intercollicular decerebrate rigidity of rats (Fig. 2 and 3) and caused an increase in the crossed extensor reflexes of chicks (Fig. 7).

Further studies involving the direct measurement of gamma-activity and a comparison of the intercollicular decerebrate rigidity with the ischemic decerebrate rigidity are required to delineate the mechanisms of the intercollicular decerebrate rigidity of rats and to elucidate the effects of drugs on the rigidity.

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13) C.M. Smith, *Proc. Soc. Exp. Biol. Med.*, **116**, 75 (1964).

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