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EFFECTS OF ETHYL ALCOHOL ON EXCITABILITY AND ON NEUROMUSCULAR TRANSMISSION IN FROG SKELETAL MUSCLE

BY

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Ethyl alcohol is generally considered to be a central nervous system depressant having many properties in common with general anaesthetics. During the past decade, evidence has been accumulating that all anaesthetics, whether local or general anaesthetics, block excitability in nerve and in skeletal muscle by a common mechanism of action—namely, by suppressing the specific increase in sodium conductivity which normally follows an adequate stimulus (Thesleff, 1956; Shanes, Freygang, Grundfest & Amatniek, 1959; Taylor, 1959; Yamaguchi, 1961; Inoue & Frank, 1962 and 1965). Recently, evidence has been presented that ethyl alcohol depresses excitability in the squid giant axon by this same mechanism (Armstrong & Binstock, 1964; Moore, Ulbricht & Takata, 1964). On the other hand, earlier studies on frog nerve (Gallego, 1948) and on frog skeletal muscle (Knutsson, 1961) resulted in the suggestion that, in both of these tissues, ethyl alcohol depressed excitability by depolarizing the fibre membrane. This problem was considered further in the present study. The results obtained would indicate that so far as frog skeletal muscle is concerned, ethyl alcohol does not block by depolarization, but by the same mechanism found for anaesthetic agents in this tissue and for ethyl alcohol in the squid giant axon.

In contrast to ether and some other anaesthetic agents, ethyl alcohol does not depress neuromuscular transmission but rather tends to potentiate it. Thus, it has been reported that ethyl alcohol has a decurarizing effect (Feng & Li, 1941) and that it can potentiate the end plate potential recorded in the presence of curare (Okada & Adachi, 1962). Recently, Gage (1965) has shown that methyl, ethyl and n-propyl alcohol all potentiate neuromuscular transmission in the rat by both a presynaptic and a post-synaptic action. In the present study we have found a similar dual effect for ethyl alcohol on neuromuscular transmission in the frog.

METHODS

Sartorius muscle and sciatic nerve-sartorius muscle preparations isolated from the frog, *Rana pipiens*, were used in all experiments. The techniques used in the microelectrode studies were in no way unusual and the details of these techniques can be found elsewhere (Inoue & Frank, 1962, 1965). For recording the end plate potential, the nerve-muscle preparation was mounted horizontally in a bath consisting of three long compartments. There was a deep, narrow slit in the two partitions

separating these compartments near the centre of the bath. The muscle was mounted in one of the lateral compartments and the nerve was placed in the other two through the slits. The slits were then sealed with petroleum jelly. There was a silver wire in each of the two compartments containing lengths of the nerve only. These were used to stimulate the nerve with 0.1 msec square-wave pulses.

To observe the effects of ethyl alcohol on the neuromuscular junction while leaving the microelectrode implanted in a single fibre drops of ethyl alcohol from a syringe were put into the solution bathing the muscle close to the microelectrode without stirring the solution. This had the desired effect of producing rapid drug effects without disturbing the microelectrodes. The effects rapidly dissipated and, provided the amount of alcohol applied was not great, the experiment could be repeated without changing the bath solution. However, with this procedure the concentration of alcohol in the vicinity of the microelectrode was not constant and could not be estimated. In some experiments the alcohol was introduced into the bath by a perfusion method. In this way a known concentration of alcohol could be applied to the muscle but satisfactory electrode implantation in a single fibre could not be maintained long enough for the desired bath concentration of drug to be reached.

To determine the effect of ethyl alcohol on the sensitivity of the endplate, acetylcholine was applied iontophoretically by means of an external microelectrode containing 1 M acetylcholine, while recording with a second microelectrode implanted in the vicinity of an end plate (del Castillo & Katz, 1955). A "braking" current was continuously applied to the acetylcholine electrode to inhibit leaking out of the drug, and a 5 msec square wave pulse was used to release the acetylcholine when desired.

In the experiments on membrane excitability the Ringer solution contained d-tubocurarine, 10^{-4} g/ml., and in experiments in which the endplate potential was studied 5×10^{-6} g/ml. d-tubocurarine was employed. No d-tubocurarine was used in studies on miniature end-plate potentials (MEPP) or on the potentials produced by the iontophoretic application of acetylcholine.

RESULTS

Effects of ethyl alcohol on the electrical properties of the muscle fibre membrane

The effects of various concentrations of ethyl alcohol on some membrane properties are shown in Table 1. The concentrations employed produced only slight changes in the resting membrane potentials. It is obvious that ethyl alcohol does not depress excitability by depolarizing the muscle fibres. This is most clearly shown by the results

TABLE 1

EFFECTS OF ETHYL ALCOHOL ON SOME MEMBRANE ELECTRICAL PROPERTIES OF FROG SARTORIUS MUSCLE FIBRES

Ethanol concentration % (mol.)	Observations* (No.)	Resting potential (mV)†	Threshold depolarization (mV)†	Threshold current ($\times10^{-7}$ A)†	τ_m (msec)†	Effective resistance (K.Ω)†	Overshoot potential (mV)†
A. (5 muscles)							
0%	42	94 ±1·2	45 ±1·0	2·1±0·1	14±1·1	254±15	27±1·6
0·5% (0·08)	26	93 ±1·9	50 ±0·6	2·2±0·1	11±0·7	230±11	22±1·8
2·0% (0·33)	15	92 ±2·5	56 ±2·5	2·8±0·2	9±1·5	226±14	10±3·4
B. (6 muscles)							
0%	16	92·5±2·1	38·8±1·6	1·4±0·1			29·5±1·1
3·5% (0·57)	12	88·0±1·6	67·0±3·0	2·3±0·1			2·6±3·6
	17	87·0±1·2					

* All observations were obtained after the muscles had been exposed to the specified solution for at least 30 min. † Mean ± standard error of mean

obtained with 3.5% ethyl alcohol (Table IB). This concentration, which blocked action potential production in slightly more than half the fibres tested, produced only about a 4 to 5 mV depolarization of the fibres. A depolarization of this magnitude is insufficient by itself to cause inexcitability. Moreover, there was in effect no difference in the membrane resting potentials in those fibres which were blocked from the potentials in those which were not blocked in the presence of this concentration of ethyl alcohol.

In contrast to the trivial effects on the resting potential, all the concentrations employed produced effects indicating a depression of excitability. Thus with increasing concentrations there was an increase in the amount that the fibre had to be depolarized in order to initiate an action potential (Threshold Depolarization), an increase in the current of a 2 msec pulse required to initiate an action potential (Threshold Current), and with a sufficiently high concentration a complete block of action potential production. All the above effects are qualitatively similar to those previously reported for procaine (Inoue & Frank, 1962) and for ether (Inoue & Frank, 1965) and would indicate that ethyl alcohol depresses excitability by inhibiting the specific increase in sodium conductivity which normally follows an adequate stimulus. The depression in the overshoot potential (Table 1) and in the maximum rate of rise of the action potential (Fig. 1) also indicates that ethyl alcohol inhibits the specific increase in sodium conductivity. Increasing .the extracellular sodium concentration increased the maximum rate of rise of the action potential (Fig. 1).

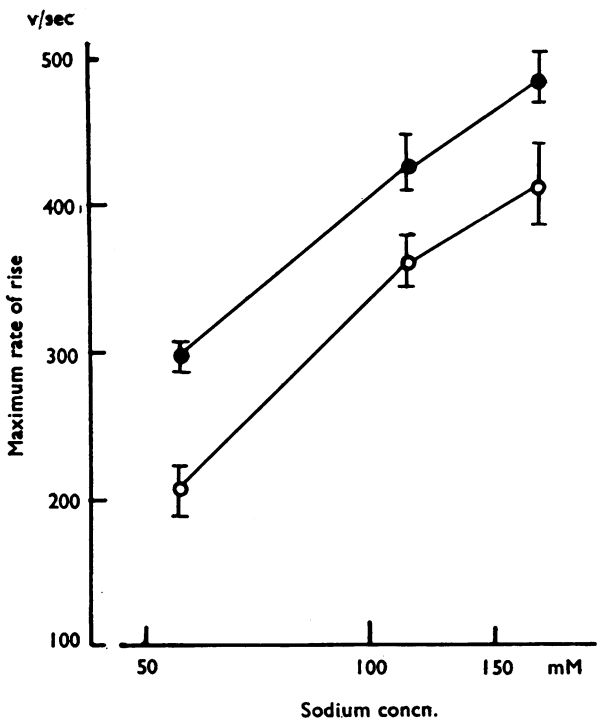

Fig. 1. Effects of ethyl alcohol and changes in extracellular sodium concentrations on rate of rise of action potentials in frog sartorius muscle fibres. (○) with and (●) without alcohol (1%). Osmolarity of the 1/2 Na-Ringer was adjusted with sucrose.

Effects of ethyl alcohol on neuromuscular transmission

The ability of ethyl alcohol to increase the amplitude of the end plate potential in the curare-blocked neuromuscular junction of the frog's skeletal muscle was previously demonstrated by Okada & Adachi (1962) using extracellular electrodes. The same effect is shown in Fig. 2 recorded with intracellular microelectrodes. In this experiment two stimuli were applied in rapid succession to the nerve and the drugs were applied dropwise into the bath close to the recording electrode. Ethyl alcohol produced increases in the endplate potentials without noticeably affecting the time course of these potentials (Fig. 2B). In contrast, ether (Fig. 2C) depressed the amplitude and decreased the rate of rise and the rate of fall of the endplate potential. The effects of ether could be overcome in part by the subsequent additon of more ethyl alcohol (Fig. 2D).

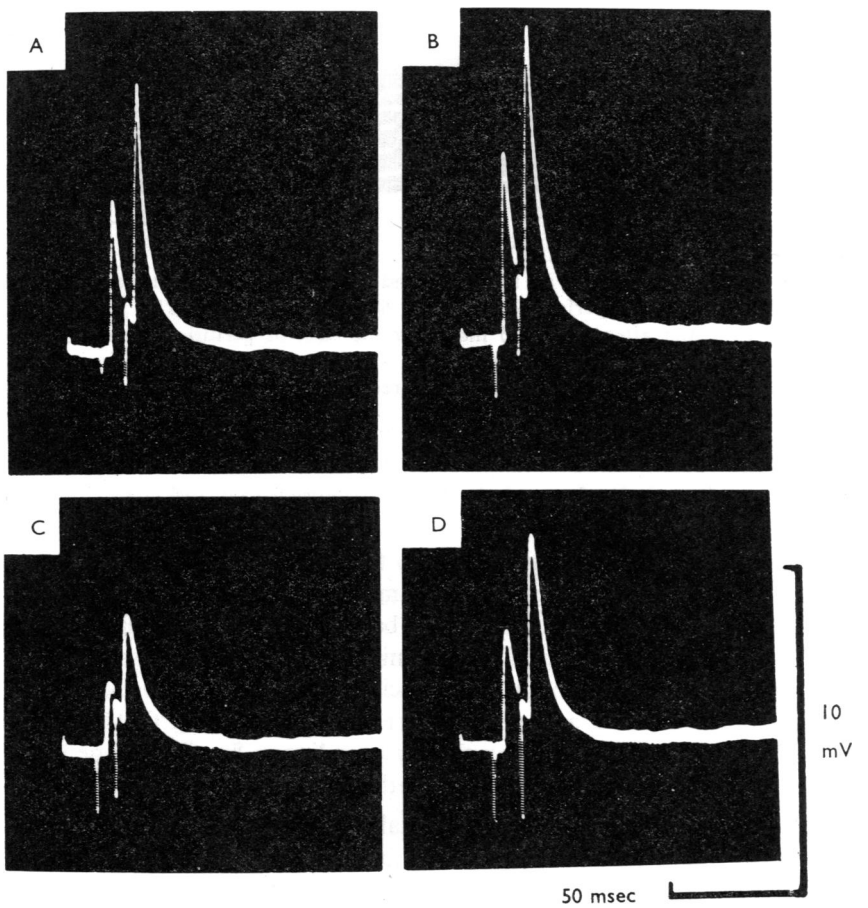

Fig. 2. Effects of ethyl alcohol and ether on the end plate potential recorded continuously from the same fibre treated with d-tubocurarine 5×10^{-6} g/ml. A, control; B, 3 min after the application of 5 drops of alcohol; C, less than 1 min after the application of 2 drops of ether; D, 3 min after the application of 6 drops of alcohol. Resting potential of the muscle fibre was 77 mV at the end of the experiment. Two stimuli were applied about 10 msec apart. Downward deflections on records are stimulus artifacts.

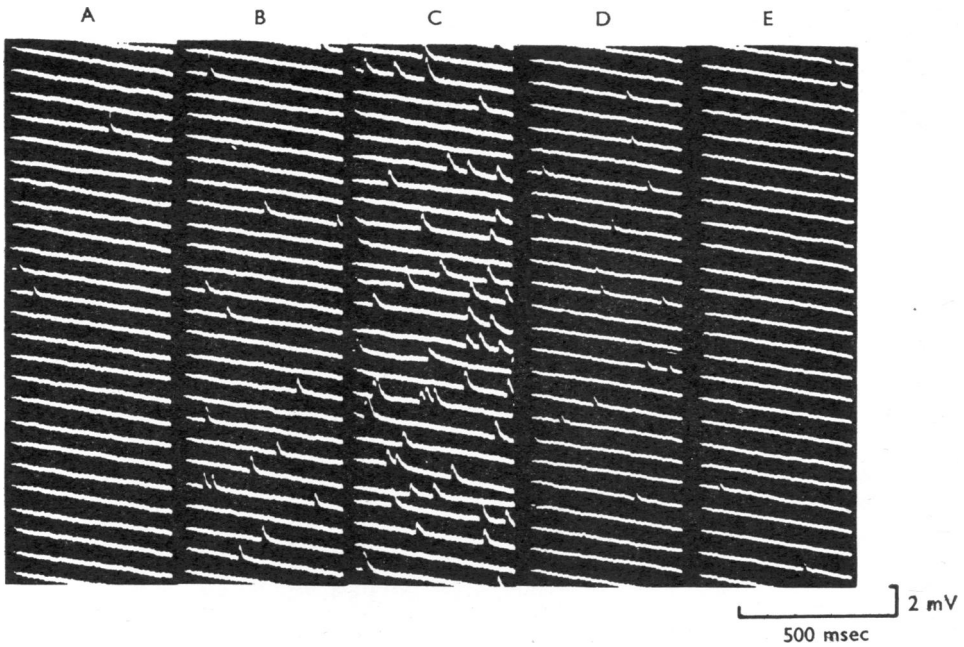

Fig. 3. Effects of 4% ethyl alcohol on miniature end plate potentials. Consecutive recordings from the same fibre. The bath perfused with about 60 ml. of solution. The perfusion took 4 min. A, control; B, 1 min after, and C, 5 min after the start of perfusion with 4% alcohol. D, 2 min and E, 5 min after the start of perfusion with Ringer solution. Resting potential 92 mV at the beginning and 85 mV at the end of the record taken.

Recently Gage (1965) has shown that ethyl alcohol enhanced neuromuscular transmission in mammalian skeletal muscle by both a prejunctional and a postjunctional effect. We have observed a similar dual effect for ethyl alcohol on neuromuscular transmission in the frog. When ethyl alcohol was added to the solution bathing the muscle there was an increase in the frequency of occurrence of miniature end plate potentials (Fig. 3). In this experiment the alcohol was added to the bath by perfusing the bath with a solution containing 4% alcohol at the rate of 15 ml./min into the 13 ml. of solution in the bath. This concentration of ethyl alcohol was sufficient completely to block action potential formation by both the nerve and muscle fibres, clearly showing that the increase rate of MEPP firing was due to an effect on the nerve endings. Smaller but definite increases in MEPP firing rates were produced by lower ethyl alcohol concentrations.

When acetylcholine was applied to individual endplates by iontophoresis it was found that ethyl alcohol increased the acetylcholine induced potentials thereby produced (Fig. 4). Since there was no d-tubocurarine in the bath solutions in these experiments it was not practical to introduce the ethyl alcohol by a perfusion technique because, with concentrations above 2%, occasional spontaneous muscle twitches occurred which dislodged the intracellular electrode. Topical application of ethyl alcohol (Figs. 4B and C) greatly increased the height of the potential produced by the application of a fixed dose of acetylcholine to a single end plate. The rate of rise and the rate of decline of the

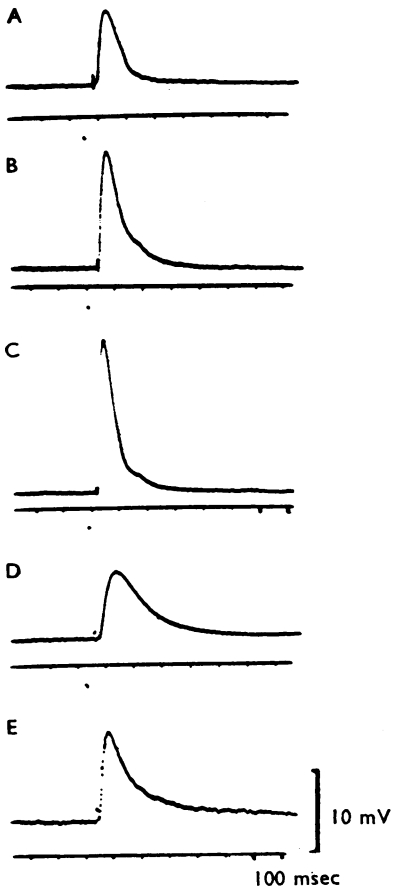

Fig. 4. Effects of ethyl alcohol and ether on the end plate potential produced by the iontophoretic application of acetylcholine. Consecutive recordings from a single fibre. Resting potential 92 mV at start and 86 mV at end of the experiment. A, control; B, 3 min and C, 6 min after the application of 2 drops of alcohol. D, 2 min after the application of 2 drops of ether near recording electrode; E, 3 min after 3 drops of alcohol.

potential were both accelerated. Topical application of ether (Fig. 4D) had the opposite effects and the effects of ether could be partially overcome by the further topical application of ethyl alcohol (Fig. 4E).

DISCUSSION

The results obtained in the present study clearly show that ethyl alcohol does not block action potential production in frog skeletal muscle by depolarization but by inhibition of the specific increase in the sodium conductivity which normally follows an adequate stimulus, just as has been previously shown for the squid giant axon (Armstrong & Binstock, 1964; Moore *et al.*, 1964). Interestingly Knutsson (1961), who did not directly study excitability, found that only with high concentration (1–1.2 M), prolonged exposures, and under unusual conditions (exposure to 100% O_2) could ethyl alcohol produce

depolarizations which would ordinarily be considered to be of sufficient magnitude to block action potential production. He also published current-voltage curves in which the earliest change produced by ethyl alcohol was the suppression of the deflection in the depolarization portion of the curves thought to be due to the specific increase in sodium conductivity.

The effects of ethyl alcohol on neuromuscular transmission in the frog are in essence the same as previously reported for the rat diaphragm (Gage, 1965). Ethyl alcohol potentiates neuromuscular transmission by both a prejunctional and a post-junctional effect. In the rat diaphragm, Gage (1965) found that ethyl alcohol increased the " input " resistance (=effective resistance, Table 1) and he suggested that this accounted in part for the post-junctional effect of ethyl alcohol. In contrast, in the frog sartorius we have only observed a decrease in the " effective " resistance (Table 1). Thus, in the frog sartorius changes in " effective " resistance can in no way account for the increase in the end plate sensitivity to acetylcholine produced by ethyl alcohol.

Gage (1965) provided some evidence that the effects of ethyl alcohol on neuromuscular transmission were not due to a possible anticholinesterase action. This possibility was not directly investigated in the present study. However, the increases in the end plate potential (Fig. 2) and in the acetylcholine potential (Fig. 4) were produced by ethyl alcohol without a noticeable prolongation of these potentials making it highly unlikely that these increases resulted even in part from an inhibition of cholinesterase. Only the MEPPs in the presence of 4% alcohol showed any sign of prolongation (Fig. 3C) but even in this case the prolongation was not great and may well have been due to an increased sensitivity of the end plate receptors to minute doses of acetylcholine. Lower ethyl alcohol concentrations increases MEPP frequency and amplitudes without any indication of MEPP prolongation.

The results with ethyl alcohol clearly demonstrate the fundamental differences in the membrane processes which underlie excitability, from those which are responsible for transmitter release at the nerve endings and those which determine the response of the endplate to the chemical transmitter. In contrast, ether, like procaine and some other anaesthetics, depresses both excitability and neuromuscular transmission. The ability of ethyl alcohol to inhibit excitability, whereas it potentiates neuromuscular transmission, would argue against a mechanism of action for general anaesthetics involving synaptic transmission. On the other hand the present findings would support previous suggestions that general anaesthesia results from a depression of excitability of neurones in the central nervous system due to an inhibition by all anaesthetic drugs of the specific increase in sodium conductivity which normally follows an adequate stimulus (Thesleff, 1956 ; Inoue & Frank ; 1962, 1965 ; Frank & Sanders, 1963 ; Frank & Pinsky, 1966).

SUMMARY

1. The effects of ethyl alcohol on excitability and on neuromuscular transmission in frog skeletal muscle were investigated.

2. It was demonstrated that ethyl alcohol depressed excitability by inhibiting the increase in sodium conductivity which normally follows an adequate stimulus.

3. In contrast, ethyl alcohol potentiated neuromuscular transmission. The end-plate potential in the presence of d-tubocurarine was increased by ethyl alcohol.

4. The frequency of miniature end-plate potentials was increased, even in the presence of ethyl alcohol concentrations sufficient to block action potential production.

5. The end-plate potential change produced by the iontophoretic application of acetylcholine was increased showing that ethyl alcohol potentiates neuromuscular transmission by both a presynaptic and a post-synaptic effect.

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REFERENCES

ARMSTRONG, C. M. & BINSTOCK, L. (1964). The effects of several alcohols on the properties of the squid giant axon. *J. gen. Physiol.*, **48**, 265–277.

DEL CASTILLO, J. & KATZ, B. (1955). On the localization of acetylcholine receptors. *J. Physiol.*, **128**, 157–181.

FENG, T. P. & LI, T. H. (1941). Studies on neuromuscular junction XXV. Eserinelike actions of aliphatic alcohols and ketones. *Chin. J. Physiol.*, **16**, 317–340.

FRANK, G. B. & SANDERS, H. D. (1963). A proposed common mechanism of action for general and local anaesthetic in the central nervous system. *Br. J. Pharmac. Chemother.*, **21**, 1–9.

FRANK, G. B. & PINSKY, C. (1966). Tetrodotoxin-induced central nervous system depression. *Br. J. Pharmac. Chemother.*, **26**, 435–443.

GAGE, P. W. (1965). The effect of methyl, ethyl and n-propyl alcohol on neuromuscular transmission in the rat. *J. Pharmac. exp. Ther.*, **150**, 236–243.

GALLEGO, A. (1948). On the effect of ethyl alcohol upon frog nerve. *J. cell. comp. Physiol.*, **31**, 97–106.

INOUE, F. & FRANK, G. B. (1962). Action of procaine on frog skeletal muscle. *J. Pharmac. exp. Ther.*, **136**, 190–196.

INOUE, F. & FRANK, G. B. (1965). Action of ether on frog skeletal muscle. *Can. J. Physiol. Pharmac.*, **43**, 751–761.

KNUTSSON, E. (1961). Effects of ethanol on the membrane potential and membrane resistance of frog muscle fibres. *Acta physiol. scand.*, **52**, 242–253.

MOORE, J. W., ULBRICHT, W. & TAKATA, M. (1964). Effect of ethanol on the sodium and potassium conductances of the squid axon membrane. *J. gen. Physiol.*, **48**, 279–295.

OKADA, K. & ADACHI, M. (1962). Effect of ethylalcohol on the end-plate potential. *J. physiol. Soc. Japan*, **23**, 655–664.

SHANES, A. M., FREYGANG, W. H., GRUNDFEST, H. & AMATNIEK, E. (1959). Anesthetic and calcium action in the voltage-clamped squid giant axon. *J. gen. Physiol.*, **42**, 793–802.

TAYLOR, R. E. (1959). Effect of procaine on electrical properties of squid axon membrane. *Am. J. Physiol.*, **196**, 1071–1078.

THESLEFF, S. (1956). The effect of anesthetic agents on skeletal muscle membrane. *Acta physiol. scand.*, **37**, 335–349.

YAMAGUCHI, T. (1961). Electrophysiological studies on the mechanism of effects of anaesthetics on the isolated frog muscle fibre. *J. Fac. Sci.*, Hokkaido Univ. (Ser. IV, Zool.), **14**, 522–535.