LETTERS TO THE EDITOR

The Effects of Tubocurarine, Decamethonium, Suxamethonium, Edrophonium and Neostigmine upon Flux of Calcium-47 in Frog Skeletal Muscle

SIR,—The importance of calcium ions (Ca^{++}) in maintaining muscle and nerve in a state of normal excitability is well established (Hodgkin, 1951; Brink, 1954; Frankenhaeuser and Hodgkin, 1957; Frankenhaeuser, 1957; Shanes, 1958).

We have investigated the effects of tubocurarine, decamethonium, suxamethonium, edrophonium and neostigmine upon $^{47}Ca^{++}$-uptake and release in paired resting frog sartorius muscles and have compared this with their effects upon uptake and release of $^{42}K^{+}$ and $^{24}Na^{+}$ uptake.

Uptake experiments were carried out by suspending the isolated sartorius muscles in oxygenated frog Ringer's solution (NaCl, 0·65; KCl, 0·014; $CaCl_2 6H_2O$, 0·012; $NaHCO_3$, 0·02; $NaH_2PO_4 2H_2O$, 0·001, and glucose 0·2 per cent) in which part of the stable Ca^{++}, K^{+} or Na^{+} was replaced by $^{47}Ca^{++}$, $^{42}K^{+}$ or $^{24}Na^{+}$. At intervals of 30 min. the muscles were removed, washed, blotted dry and counted in a thallium-activated, sodium iodide scintillation

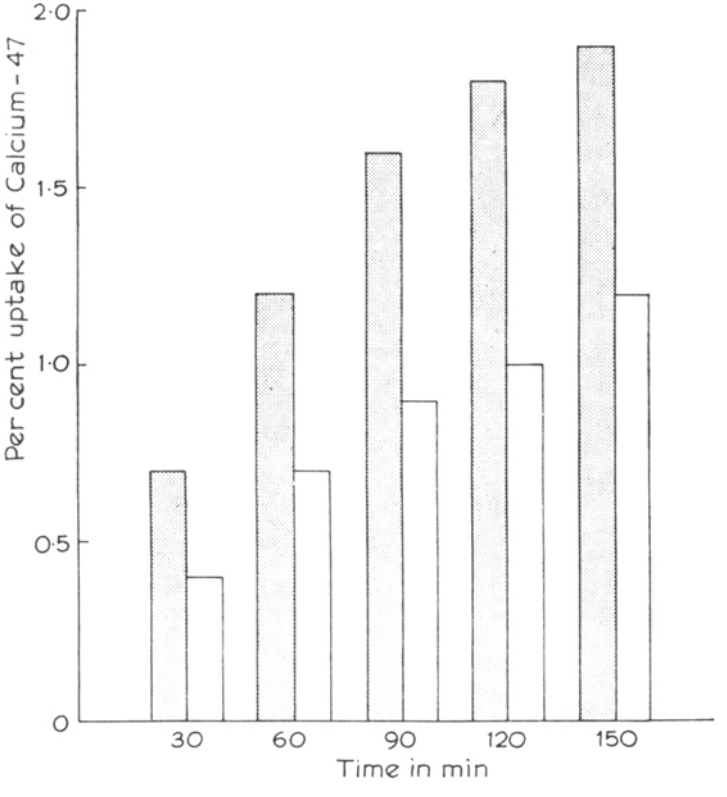

FIG. 1. Effect of suxamethonium chloride dihydrate (5 mg./ml.) (stippled areas) on the uptake of $^{47}Ca^{++}$ by resting frog sartorius muscle. Unstippled area = control muscle.

crystal (Ekco type N597) connected through a photomultiplier to an automatic scaler (Ekco type N530D).

For studying $^{47}Ca^{++}$-release muscles were soaked for a period of from 4 to 6 hr. in oxygenated frog Ringer's solution in which part of the stable Ca^{++} was replaced by $^{47}Ca^{++}$. The muscles were then passed along two parallel series of 7 tubes each containing 10 ml. of non-radioactive, Ca^{++}-free, oxygenated frog Ringer's solution, one series being used for control purposes only. The muscle

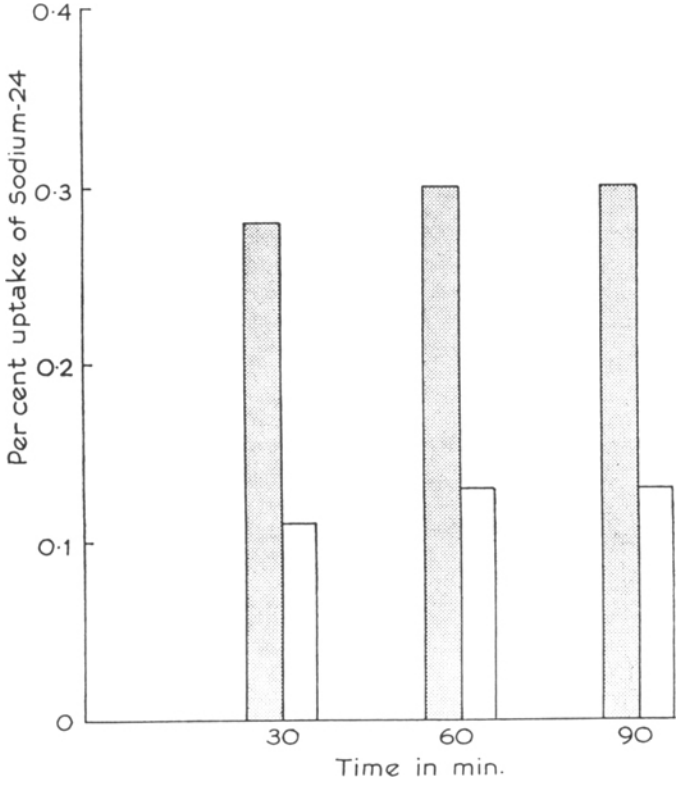

FIG. 2. Effect of neostigmine methyl sulphate (100 μg./ml.) (stippled areas) on the uptake of $^{24}Na^{+}$ by resting frog sartorius muscle. Unstippled areas = control muscle.

was kept in each tube for 10 min., and the drug added to the fourth tube in the test series. After the muscles were transferred to the next tube in the series, the fluid remaining was counted, as before.

For estimation of $^{42}K^{+}$-release, the procedure was similar, but the muscles were labelled by injecting the frogs with ^{42}KCl into the dorsal lymph sac. They were killed and the sartorius muscles removed after an equilibration period of 2 hr. The muscles were passed through two series of tubes each containing non-radioactive, K^{+}-free, oxygenated frog Ringer's solution in the manner used for estimating $^{47}Ca^{++}$-release. The fluid remaining was counted by means of a Geiger-Müller liquid counter (type M6) connected through a probe unit to an automatic scaler (Ekco type N530D).

Decamethonium (50 μg./ml. to 0·5 mg./ml.) and suxamethonium (1 to 10 mg./ml., Fig. 1) caused increased uptake of $^{47}Ca^{++}$ while tubocurarine (50 μg./ml. to 1 mg./ml.) and edrophonium (0·5 to 2 mg./ml.) depressed this. In some experiments neostigmine (25 to 150 μg./ml.) increased $^{47}Ca^{++}$-uptake, while in others this was unchanged or decreased. Decamethonium, suxamethonium, edrophonium and neostigmine all depressed $^{42}K^{+}$-uptake but tubocurarine had no apparent effect. Decamethonium, suxamethonium and neostigmine (Fig. 2)

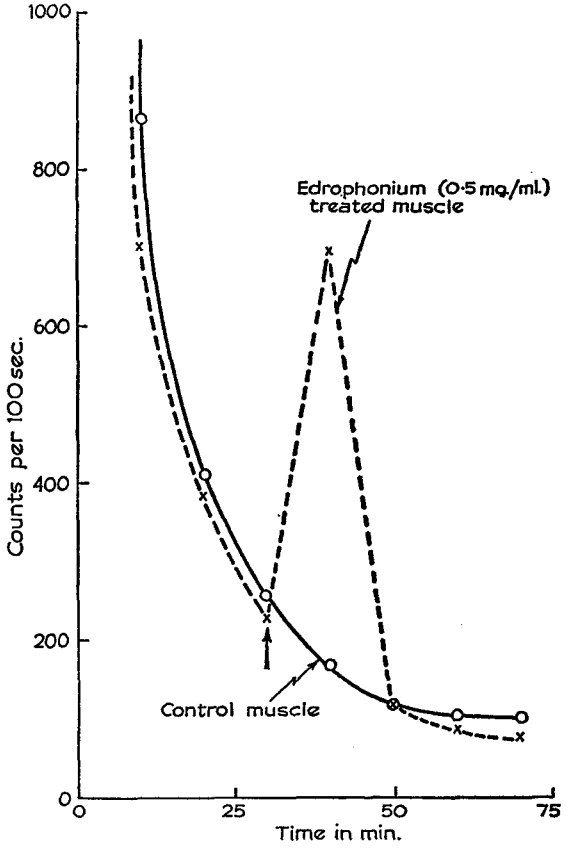

FIG. 3. Effect of edrophonium (0·5 mg./ml.) on the release of $^{47}Ca^{++}$ from resting frog sartorius muscle. Arrow indicates point of exposure of the test muscle to the drug.

increased $^{24}Na^{+}$-uptake but tubocurarine and edrophonium had no apparent effect on this. Suxamethonium, edrophonium (Fig. 3) and neostigmine also caused release of $^{47}Ca^{++}$. Large doses of tubocurarine (0·5 to 1 mg./ml.) caused release of $^{47}Ca^{++}$ but decamethonium had no effect. Decamethonium (Fig. 4), suxamethonium, and neostigmine all caused release of $^{42}K^{+}$. The latter confirms the observations of Klupp and Kraupp (1954) and Kraupp and his colleagues (1960) on decamethonium and suxamethonium. Tubocurarine and edrophonium had no effect.

It has been suggested by Frankenhaeuser and Hodgkin (1957) that depolarization acts by removing Ca^{++} from combination with a sodium carrier and our results tend to support this view and are not unexpected in view of the report by Robertson (1960) that acetylcholine increases $^{45}Ca^{++}$-uptake in depolarised smooth muscle.

It seems therefore that the application of depolarising drugs is associated with increased uptake of $^{47}Ca^{++}$ and $^{24}Na^{+}$ and increased release of $^{42}K^{+}$. The non-

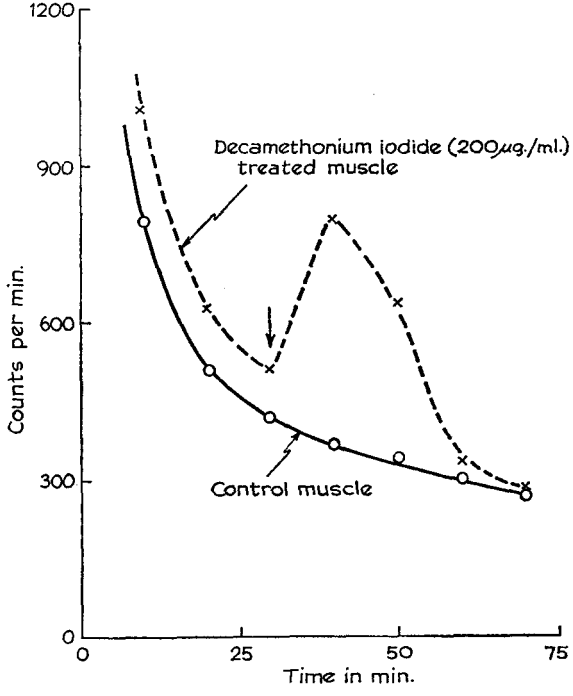

FIG. 4. Effect of decamethonium iodide (200 µg./ml.) on the release of $^{42}K^{+}$ from resting frog sartorius muscle. Arrow indicates point of exposure of the test muscle to the drug.

depolarising drug, tubocurarine, depresses uptake of $^{47}Ca^{++}$, causes no change in the uptake of $^{24}Na^{+}$ and does not release $^{42}K^{+}$. We hope to report our findings in more detail later.

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December 13, 1960.

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Tolerance to Tremorine

SIR,—The tremor-producing drug 1,4-dipyrrolidino-2-butyne, Tremorine, is becoming increasingly used in the screening of anti-Parkinsonism substances. In this connection it may be of some interest to record the observation made in this laboratory, that a surprisingly rapid tolerance to tremorine develops in the mouse. The tolerance is easily observed as soon as the third treatment of 6 to 18 mg./kg. intraperitoneally or subcutaneously is given, and is essentially complete after five to six administrations made at two-days intervals. Tolerance comprises all of the three main central effects of the drug, namely, tremor-producing action, analgesic action and anaesthesia-prolonging action. Sensitivity to tremorine returns after discontinuing of drug administration for 2 or 3 weeks.

The property of tremorine to cause tolerance should be taken into account when used in routine pharmacological screening.

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Antifertility Agents

SIR,—Petrow (1960) has recently proposed the generic term, "claudogens," for steroidal antifertility agents.

This term appears less general, less descriptive, and (to me) less euphonious than the increasing importance of this new class of pharmacologic agents would seem to require. For this reason, I wish to propose the more general, alternative term, "genotropic agent," which has been in use for some time in this laboratory and has met with acceptance by all who have had occasion to use it; in informal usage, "genotropic agent" often becomes simply "genotrope."

The adjective, "genotropic" (soft g), was coined from the Greek roots γενως = population (or γενναν = reproductive) and τρωπος = changing, affecting, altering. The resulting word expresses precisely what is intended and also conveys the sociologic connotation that is fundamental to the problem of fertility control.

The term, "genotropic," need not imply any specific site, mode, direction, or degree of action by any limited type of agent upon the reproductive process, but requires, for its proper usage, only that the agent in question (steroidal or non-steroidal) have a net effect upon the number of normal progeny produced by an individual of the species under study.

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