THE PHARMACOLOGICAL ACTIONS OF MAGNESIUM IONS WITH PARTICULAR REFERENCE TO THE NEUROMUSCULAR AND THE CARDIOVASCULAR SYSTEM

INDEXED

LISE ENGBAEK

The Institute of Neurophysiology, University of Copenhagen, Denmark

In reviewing an extensive field such as the pharmacology of magnesium ions it is necessary to limit the subject. The present review will deal mainly with the effects of an increase in magnesium concentration on the neuromuscular and cardiovascular system. Work on the effects of magnesium deficiency, the metabolism and therapeutic effect of magnesium ions will not be considered. Magnesium metabolism and the effects of magnesium deprivation have been reviewed by Schmidt and Greenberg (116) and Greenberg (58). The therapeutic effects were dealt with by Smith, Winkler and Hoff (123), who reviewed the pharmacological effects and the absorption and excretion of magnesium salts. Variations in serum magnesium concentration in various disease conditions were covered by Haury (67).

Central versus Peripheral Action of Magnesium in the Nervous System. The depressant action of magnesium on the neuromuscular system was first demonstrated by Jolyet and Cahours (75) who found a peripheral curare-like action in both cold- and warm-blooded animals. Meltzer and Auer (96), in an extensive series of papers on the anesthetic effect of magnesium salts, confirmed the depressant action but attributed it solely to a central anesthetic effect, a point of view they later revised by further attributing to magnesium a peripheral depressant action on neuromuscular transmission. At about the same time it was shown beyond doubt that magnesium in man has a depressant effect on the cortex, since unconsciousness is produced by injection of magnesium salts (110).

The problem of the site of action of magnesium, especially the effect on different parts of the central nervous system and the sensitivity of the central nervous system as compared with that of the neuromuscular junction, was the object of numerous discussions in the following years.

The first systematic investigations were made by Matthews and Brooks (94) using cross-circulation experiments in dogs. They found a central depressant action on respiration, though this was not as easily produced as the peripheral paralysing effect. More recent investigations of the action on the spinal reflexes show some discrepancies. The experiments of Hoff, Smith and Winkler (73) on decerebrate cats demonstrated that magnesium chiefly affects neuromuscular transmission and not the central nervous system, although the experiments do not exclude a central effect. They correlated the motor response produced by electrical stimulation of the motor nerve at different frequencies with the serum magnesium concentration. At high concentrations of serum magnesium, high frequencies of stimulation were required to produce a motor response. The different reflexes disappeared at different concentrations of serum magnesium: tendon reflexes at about 5 mM magnesium; respiration at approximately 10 mM;

whereas the corneal reflex did not disappear until a concentration of 15–18 mM of magnesium was reached. The order of disappearance of reflexes was correlated with the frequency of spontaneous reflex discharge characteristic of the motor element of the reflex, the reflexes with slow discharge frequency disappearing first. After tetanic stimulation of the afferent nerve, the reflexes could be made to reappear, suggesting to the authors that the site of effect on the reflexes was at the neuromuscular junction. On the other hand, Bryant *et al.* (22a), using a different technique, concluded that the action of magnesium is primarily central. They compared reflex activity and motor response to stimulation of the motor nerve during magnesium anesthesia, and found that spinal reflexes were abolished while neuromuscular transmission was still unimpaired. More recently a similar technique has yielded different results. The site of the depressant action of magnesium was investigated by recording the threshold for the crossed extensor reflex and the threshold for the response to electrical stimulation of the motor nerve. At the time of respiratory paralysis produced by magnesium salts, a reduced peripheral but unchanged central irritability was demonstrated. By further increasing the degree of anesthesia (during artificial respiration) a depression of the reflex centre occurred very soon (42). Hoff *et al.* (73) suggested that the discrepant findings of Bryant *et al.* (22a) might be due to the use of different frequency of stimulation. However, a high stimulation frequency can hardly be the explanation since Engbæk (42) with 100 c.p.s. still found primarily a peripheral action of magnesium.

In summarizing we may conclude that both central and peripheral depressant actions of magnesium have been demonstrated. The relative sensitivity seems to be greatest in the periphery of the motor system although this question is not finally settled.

Central Nervous System Effect of Magnesium. As mentioned above the intravenous injection of magnesium produces unconsciousness. The effect of magnesium on spontaneous electrical activity of the brain was first examined on the olfactory bulb of the isolated frog brain (85). Magnesium in concentrations of the same order of magnitude as the anesthetic concentrations in warmblooded animals (3–10 mM added to the magnesium-free Ringer's solution) produced slowing of the normal 6 per sec. rhythm. The electrocorticogram of cats under light pentobarbital anesthesia was likewise modified by intravenous infusion of magnesium, which at first produced a transient slowing (115), the effect being rather small as compared with the effect of aliphatic anesthetics. The slowing of the electrical activity reappeared later, simultaneously with electrocardiographic changes similar to those produced by calcium and potassium. But calcium and potassium did not alter the electrocorticogram until the heart was affected. As pointed out by Rubin *et al.* (115), the early transient changes in the electrocorticogram might be secondary to the fall in blood pressure simultaneously produced. However, before the heart is affected, the mean arterial blood pressure does not fall below 100 mm Hg (72), a fall in blood pressure which according to Beecher *et al.* (7) is not likely to produce changes in the electrocorticogram. A slowing in the electroencephalographic rhythm of the unanesthetized rabbit was

also found by Bertrand *et al.* (9), but these experiments were performed without artificial respiration, and the effect might be due to the anoxemia produced by respiratory failure.

Interference by the cardiovascular effects of magnesium makes it difficult to demonstrate the effect of the ion on the spontaneous electrical activity of the brain in the intact animal. If it exists at all, it is rather small as compared with the effect of the aliphatic anesthetics.

Nerve Fibers. Block of conduction in peripheral nerve occurs at concentrations which are 10–20 times those needed for blocking neuromuscular transmission (138, 46, 29, 42). At this high concentration it is doubtful if the blocking is due to a specific effect of magnesium ions. To obtain this concentration either all the sodium of the Ringer's solution must be replaced by magnesium, which suggests that the lack of sodium in itself establishes conduction block (71), or the magnesium must be applied in a hypertonic solution. Moreover, the action potential of single nerve fibers of the frog is unaffected by concentrations up to 66 mM magnesium (68) (the concentration of a magnesium solution isotonic with frog Ringer's solution being approximately 80 mM). The effect of magnesium (20 mM $MgCl_2$) upon the membrane potential of the nerve consists in a slight initial increase, followed by slight depolarization after 2 to 3 hours (87). Even after 24 hours this depolarization amounted to only a few millivolts (about 10 per cent) and the nerve fibers could still conduct impulses without detectable abnormality. Excess of calcium exerted a similar effect, but its action was more rapid and less reversible than that of magnesium. The depolarization produced by anoxia was delayed by magnesium, the magnesium treated part of the anoxic nerve staying excitable for some time (87). A similarity in effects of magnesium and calcium on peripheral nerve was found in the effect on the temperature sensitivity of the spike potential, in that both magnesium and calcium lower the optimum temperature for maximum spike height (83).

It seems well established that the depressant action of magnesium on motor nerves is much less pronounced than its effect on neuromuscular transmission and on the central nervous system.

A certain local anesthetic effect has been claimed by several authors (96, 139, 63). As for the motor nerves, however, the experiments were made with hypertonic solutions or with sodium-free isotonic solutions, and the specific blocking effect on sensory nerves is doubtful.

Neuromuscular Transmission and Muscle. The primary peripheral effect of magnesium is its blocking effect on neuromuscular transmission. When a nerve-muscle preparation is treated with magnesium (20–50 mM) it becomes inexcitable to stimulation through the nerve, while the irritability of the muscle remains unchanged (75, 77, 5, 29). After prolonged applications, changes in the irritability of the muscle occur (10, 63, 138, 5, 36, 1). During magnesium anesthesia of the intact animal, the height of contraction of directly stimulated muscle was found to be unaffected, while stimulation of the motor nerve failed to produce a response. This was the case with a serum magnesium concentration up to 15 mM (73). The magnesium effect on the muscle has been examined on curarized and

denervated muscle in order to avoid stimulation of the intramuscular nerve endings. In curarized frog muscle the height of contraction was reversibly reduced by 20 mM magnesium (138), 10 mM producing a transient small effect on contraction and a significant decrease in excitability (42). The threshold magnesium concentration for effect on the muscle is 10 mM in cold-blooded animals, while it must be higher for mammalian muscle (73).

In denervated frog muscle Ashkenaz (1) found a decrease in excitability, after application of 67 mM $MgCl_2$ in Ringer's solution, of the same order of magnitude as in normal muscle. The effect was, however, less reversible in the denervated than in the normal muscle.

Intravenous injection of magnesium decreased the twitch height of both the innervated and the denervated anterior tibialis muscle in the dog (90a). The depressant action was restored within a few minutes in the case of muscle with intact nerve supply, whereas the denervated muscle did not recover within half an hour. It seems advisable, however, not to transfer findings on denervated muscle directly to normal muscle. Several substances have a different mode of action on denervated muscle, possibly due to a difference in permeability. Denervated muscle is known to be more permeable than normal muscle to potassium ions (26), and the permeability of normal muscle for magnesium is very low (see p. 405).

A comparison of electrical stimulation of the single endplate in the lizard with stimulation of its nerve showed that the neuromuscular transmission was reversibly blocked for nerve stimulation by 4–8 mM magnesium, whereas the response to direct stimulation of the endplate remained unaffected at concentrations up to 80 mM (42). Lizard muscle was found to be less susceptible to magnesium than frog muscle.

The blocking effect of magnesium at the neuromuscular junction is in certain respects similar to that of curare. Curare is known to depress the sensitivity of the muscle to intra-arterially injected acetylcholine (21) and to acetylcholine applied directly to the single endplate (24, 25, 80). Also magnesium produces a decreased sensitivity to the stimulating effect of acetylcholine on the single endplate (42). However, the site of action of magnesium and curare is presumably different, as magnesium blocks the stimulating effect of potassium as well. This was examined on the sympathetic ganglion, in which magnesium blocked transmission and prevented the stimulating effect of acetylcholine and potassium, whereas curare blocked the response to acetylcholine, but not to potassium (126). The contracture produced by acetylcholine and by potassium in the frog's rectus abdominis was antagonized by magnesium, the necessary concentration being about 10 times less for potassium than for acetylcholine (71a). Whether magnesium also blocks the muscular contraction produced by potassium remains to be shown. Some further light might be thrown on the site of action of magnesium at the neuromuscular junction by electrophysiological methods. At present it is unknown whether magnesium depolarizes or stabilizes the membrane potential at the site of the endplate.

In crabs, in which the neuromuscular system is unaffected by acetylcholine and

curare, raising of magnesium concentration about 2.5 times above the normal blood level produced a complete neuromuscular block, whereas higher magnesium concentrations were required to affect the muscle substance itself. The normal blood magnesium level in crabs (about 26 mM) was about half that present in sea water and is well above the level which blocks neuromuscular transmission in vertebrates. The electric response of the nerve was increased by magnesium. Potassium in concentrations about 3 times the normal blood level produced contracture, which was prevented by magnesium (78). The neuromuscular transmission was facilitated by perfusion with fluids containing 5–20 per cent of the normal blood concentration of magnesium (11). It seems that the blocking effect of magnesium is very widespread, and obtained even when the mechanism of neuromuscular transmission is different and not influenced by curare, supporting the view of different sites of action of the two substances.

Investigations of the effect of tetanic stimulation during progressive neuromuscular block caused by curare or magnesium point in the same direction (16, 90, 107, 108). By stimulation of the motor nerve of mammalian muscle *in situ* during partial curarization there is a brief phase of facilitation; when a single stimulus is blocked, two stimuli of the same strength in rapid succession may still excite. The facilitatory effect of a single volley is maximum after about 3 msec. During tetanic stimulation of the nerve this effect is found only with the first few stimuli; it is followed by a decrease in height of contraction, which occurs even with a series of twitches elicited at 10-second intervals. This implies that the inhibitory after-effect of the single stimulus has a minimum duration of 10 seconds in partially curarized muscles (113a). The decline in tension is the more marked, the higher the frequency of stimulation (frequency of stimulation 20–120 per sec.) (16). Moreover, during partial curarization tetanic stimulation through the nerve is followed by a period of post-tetanic potentiation, during which the response to a single volley is enhanced. This post-tetanic effect lasts several minutes depending on the duration and the frequency of the conditioning tetanus (14, 113a).

These three effects are also found during a partial block produced by magnesium but there are certain differences. Magnesium produces an early facilitation with a time course identical for both substances (90). However, the decrease in tension of contraction, which is pronounced after curare, is slight and transient during magnesium and, in contrast to findings with curare, an increase in stimulation frequency reduces the period during which the contractions are decreased (16). This was confirmed by Naess (107) who applied a somewhat different method of stimulation using periods of different stimulation frequencies (1–300 c.p.s.). He found that curare produced a pronounced inhibition to high stimulation frequencies, whereas contractions produced by single stimuli and low frequencies showed less reduction in amplitude. Magnesium produced inhibition at low stimulation frequencies, with comparatively little reduction in contraction amplitude at high frequencies (107). When the neuromuscular transmission was nearly blocked, neostigmine in curarized muscle could only restore the single twitches, whereas after magnesium it restored the response to all frequencies of stimulation (107, 108).

The post-tetanic effect found with curare was present with magnesium as well (88). The degree and the duration of the post-tetanic potentiation vary in the same way with the conditioning tetanus under magnesium and curare (15). But with magnesium the post-tetanic twitches are maximal immediately after the tetanus and decline gradually; with curare the amplitude of the first post-tetanic twitches is relatively low, and become progressively higher until a maximum is reached, which suggests that the post-tetanic potentiation is gradually unmasked (16).

The early short period of facilitation during partial curarization has been explained as being due to summation of the endplate potentials which reach threshold for the establishment of transmission of impulses (39). The time course of facilitation was approximately the same for curare and magnesium, which suggests the same mechanism of facilitation. The post-tetanic potentiation outlasts the stimulation of the nerve for several minutes, and it is improbable that acetylcholine can account for it. Rosenblueth and Morrison (113a) assumed that it might result from potassium liberated during stimulation of the nerve. That potassium actually can cause a potentiation similar to that after electrical stimulation of the nerve was shown by Brown and v. Euler (22). Potassium was assumed to lower the threshold of muscle fibers previously unexcitable. It is in accordance herewith that potassium can counteract curarization and antagonize the magnesium block (20).

An explanation for the decrease in contraction height found during tetanic nerve stimulation of the partially curarized muscle, and for the differences between curare and magnesium effects is still lacking. There are several hypotheses which deal with changes in the amount of acetylcholine released or changes in threshold (113a, 113, 90), but none seems fully satisfactory. That neostigmine after curarization reestablishes only single twitches but not a response to high frequency stimulation indicates a complex mode of action of curarine (108).

Cardiovascular System. The depressant action of magnesium on the heart was known before Meltzer and Auer's studies stimulated interest in the anesthetic action and demonstrated that the cause of death after parenteral administration was respiratory failure. That magnesium causes a fall in blood pressure was demonstrated by Meltzer and Auer (96) and later confirmed by several authors (94, 133, 93). The main interest of recent investigators was an attempt to find the site of the complicated action on the cardiovascular system.

Electrocardiographic examinations demonstrated a depressant action on rhythm and conduction. Magnesium prolonged the P-Q conduction time and extended the QRS complex, while the cardiac rate was lowered (100, 114, 101, 122).

The action upon the conduction system of the heart was shown to be independent of vagus impulses (34). More recently it was demonstrated that slowing of the heart rate is caused partly by blocking of the cardioaccelerator ganglion (see p. 403), but in addition there is a direct inhibitory action on the spontaneous rhythm of the heart (126). At a time when the conduction system was affected, the heart muscle itself was relatively unimpaired, the systole being vigorous until

the heart stopped (122). Mechanical stimulation was still possible after cardiac arrest (114). This is in agreement with recent investigations on the effect of magnesium on the isolated papillary muscle. Calcium and magnesium produced a slight rise in threshold, the effect of magnesium was least pronounced and very small compared with the effect of potassium. Magnesium had no demonstrable effect on either myogram or electrogram (61, 56). Direct investigations on the heart-lung preparations demonstrated that increase of the magnesium concentration in serum diminished the competence of the heart and decreased the systemic output. The total output remained nearly constant and, because of the decrease in rate, the stroke volume was increased (127). Competence of the heart could be restored by calcium, which is in contrast to the findings of Smith, Winkler and Hoff (122), who found that the serum magnesium concentration necessary to stop the heart action was not increased by addition of calcium.

The serum concentrations associated with depression of the cardiac conduction system were examined by Smith, Winkler and Hoff (122). After a transient accelerating effect (probably produced by the fall in blood pressure caused by magnesium), the effect on conduction from auricles to ventricles occurred at approximately 3–5 mM magnesium, and cardiac arrest usually occurred within a serum magnesium range of 15–22 mM, the effective concentration depending on the rate of infusion. Cardiac failure occurred at considerably higher concentrations than those necessary for respiratory failure. In heart-lung preparations the rate of discharge of the sino-atrial node was progressively depressed by increasing concentrations of magnesium in the serum, a 10 per cent fall in heart rate being found with a serum concentration of about 4 mM of magnesium (127). This is in good agreement with findings in intact animals and indicates that the slowing effect on heart rate begins at about the same concentration as the depressant action on the reflexes.

Magnesium has especially been claimed to counteract extranodal cardiac rhythms arising spontaneously or during therapy with digitalis glycosides. In digitalized dogs, magnesium increased the degree of block by producing a further delay in auriculo-ventricular conduction time and produced impulses of ectopic origin (102). In dog heart-lung preparations, cardiac irregularity and fatal doses of digoxin were not altered by increasing serum magnesium concentrations (up to approximately 10 mM). But ventricular irregularities caused by digoxin could be temporarily eliminated by injection of large amounts of magnesium solution in the heart-lung preparation, in normal dogs and in dogs with denervated hearts. The serum magnesium concentration was determined in one case to be approximately 4 mM magnesium when the rhythm returned to normal (127). The explanation of these discrepancies is not clear. The authors suppose that massive doses temporarily produce a concentration of magnesium in the coronary circulation which may be sufficient to stop foci of origin of heterotopic rhythms, but which would be lethal if maintained over a long period.

The initial depressing action on the *blood pressure* occurs at very low serum magnesium concentrations (1–3 mM magnesium) (72), and is accompanied by a pronounced vasodilatation. As the serum concentration of magnesium is grad-

ually increased, the fall in blood pressure continues until the heart stops. During more recent years, interest was concentrated on the possible mechanisms responsible for the blood pressure lowering effect of magnesium. The main factor seems to be a *blocking action on the sympathetic ganglia.* If magnesium is given during stimulation of the cervical sympathetic nerve, a brief relaxation of the nictitating membrane is observed. The principal site of this activity is located in the ganglion, since concentrations which block the ganglionic potential and ganglionic transmission have no influence on the conduction of impulses in pre- and post-ganglionic fibers. A blockage of impulse transmission by magnesium was found for the superior cervical, stellate and inferior mesenteric ganglia. This general blocking effect on sympathetic ganglia is assumed to be an important factor in the blood pressure lowering effect of magnesium (126). As stressed by Stanbury (126) other factors might be involved as well. (a) An effect on the vasomotor center; (b) a direct effect on peripheral vessels; and (c) change in cardiac output. A depressant effect on the *vasomotor center* was considered unlikely by this author, since the intracarotid injection of magnesium produced a *rise* in blood pressure even after exclusion of vasomotor reflexes by evulsion of the cranial nerves IX through XII (126). Perfusion of the cerebral ventricles with a solution containing an excessive magnesium concentration (approximately 10 mM magnesium), caused a fall in arterial pressure and a depression of the vasomotor reflexes. This effect of magnesium was similar to that of calcium but less pronounced (84), and its central origin can hardly be doubted. The concentrations which evoke this depression are rather high as compared with those required for neuromuscular block. A depression of carotid vasomotor reflexes could be produced by magnesium injection and was antagonized by calcium (68a). By the technique applied, the site of action could not be decided. It is not known whether calcium antagonizes the blocking effect of magnesium on sympathetic ganglia.

A *direct effect on peripheral vessels* can hardly be excluded. Magnesium produced a fall in blood pressure in spinal dogs, and a vasodilatation was observed in frogs with the central nervous system destroyed (66). This was confirmed on spinal cats and in a cat in which sympathetic tone was eliminated by stabilizing the blood pressure at a high level, injection of magnesium still causing a fall in blood pressure (126). Stanbury (126) emphasizes that it is not possible on the basis of these observations to differentiate between an effect on the cardiac output and a direct effect on peripheral vessels.

As to a decrease in *cardiac output* as the cause of the fall in blood pressure produced by magnesium, experiments on isolated heart-lung preparations demonstrated a decrease in cardiac competence, but the total output and the mean arterial pressure remained very nearly constant during successive additions of magnesium chloride (concentrations of the order of magnitude of 7 mM magnesium) (see p. 402). It seems improbable, therefore, that depression of the heart plays an important role in the lowering of blood pressure after magnesium in anesthetic concentrations.

It may be concluded that the depressant action of magnesium on the blood pressure is complicated; hardly any of the possible mechanisms can be excluded;

most important is its blocking effect on sympathetic tonus, but still more experimental work is needed to clear up the relative influence of the individual mechanisms of action.

Smooth Muscle. A direct effect of magnesium on smooth muscle of peripheral vessels remains to be shown. It seems rather likely that it exists since there is a direct depressant effect on other smooth muscles. A direct inhibitory action on the nictitating membrane was found in addition to the inhibition produced by blocking the superior cervical ganglion (126). A bronchodilator action of magnesium was found by perfusion of excised guinea pig lungs (65). In isolated intestine, magnesium chloride reduced the contraction height obtained with acetylcholine and decreased the sensitivity of the muscle (140). In uterine muscle, an increase in magnesium concentration above that of the usual Ringer's solution increased sensitivity to oxytocic hormone, whereas it interrupted or prevented the spontaneous rhythm. This effect has been employed in the biological assay of oxytocin (53, 74).

Antagonists to the Depressant Action of Magnesium. The depressant action of magnesium is to a large extent antagonized by an excess of calcium. At moderate magnesium doses intravenous administration of calcium acts immediately whereas the protective action of calcium fails at high magnesium doses (97). Calcium counteracts both the central and the peripheral depression (22a). Eserine can counteract the effect of magnesium as well, but it acts more slowly (128) and the depressant action is not completely abolished. With eserine the effect on respiration is said to be the most prominent (76). The depressant action of magnesium on the neuromuscular junction is antagonized by neostigmine but not so completely as by calcium (91). A combination of pentamethylenetetrazol and neostigmine antagonizes magnesium anesthesia nearly completely, whereas pentamethylenetetrazol alone has a slight effect and only on respiration. Neostigmine alone has a somewhat more pronounced effect than pentamethylenetetrazol, but still significantly less than the combined action of the two drugs. The magnesium depression of indirect excitability of the muscle was unaffected by pentamethylenetetrazol, but counteracted by neostigmine. The interpretation of these findings is in agreement with the assumption of both a central and a peripheral site of action of magnesium, the former being antagonized by the analeptic, neostigmine affecting the peripheral point of attack, and calcium intervening in both (13).

Permeability. The effect of magnesium on the neuromuscular system is closely related to its concentration in the serum (45, 73, 104, 42), but the concentration in the central nervous system and the muscles does not increase during magnesium anesthesia. This was first demonstrated in brains of dogs dying under magnesium anesthesia (92), and was later confirmed on brain and muscle (45, 131). In more recent experiments, a slight increase of magnesium content was found in frog and mammalian muscle; this, however, was small as compared with that of serum and it might be explained by an increase of the magnesium in the extracellular space only (29, 130).

The lack of proportionality between the magnesium concentration in blood and

in tissue is presumably due to the relative impermeability of the cellular membrane to magnesium. When magnesium is administered intravenously in the intact animal, it will be distributed over 20 to 25 per cent of the body weight during the first 4 hours. This volume of distribution corresponds approximately to the volume of the extracellular space. Later, within 24 hours, but at a time when the anesthetic effect has ceased, a variable amount, which cannot be accounted for as having been excreted, will leave the extracellular space and be deposited in an unknown part of the body (124). Perfusion of the hindlegs of frogs indicates that magnesium in this preparation can penetrate cells (muscle or bone), the volume of distribution being greater than that of the extracellular space (52). Whether this discrepancy is due to differences between intact and perfused muscles, or whether there is some penetration of magnesium even in the former can not be decided at present. Experiments on excised tissues have only been performed on frog muscle and show a slow penetration of magnesium. Suspension of frog muscle in solutions containing 80 mM magnesium showed a rapid increase in magnesium concentration during the first half hour, followed by a slow constant increase. Determinations of the extracellular space by means of magnesium and inulin during the first half hour both gave 9 to 10 ml per 100 gram of muscle; hence, only the slow increase of magnesium concentration was due to actual penetration into the muscle cell (18). If isolated frog muscle is exposed for a long time (5 hours) to magnesium concentrations of the same order of magnitude as those found during anesthesia (above 4 mM) magnesium enters the muscle cell. The calculated volume of distribution was 60 per cent or more, indicating that magnesium slowly penetrates the muscle cell (52).

Experiments with radioactive isotopes of magnesium have not been performed as half-life of Mg^{23} and Mg^{27} unfortunately is very short (11.6 sec. and 9.6 min., respectively).

The magnesium content of the central nervous system and of muscle is high compared with the concentration in plasma. The concentration of magnesium in the central nervous system is about 4.5 to 6 mM/kg (45, 29, 41), in muscle about 8 to 12 mM/kg (30, 51, 29), in plasma about 1.2 to 1.6 mM/kg. The figures show some variation in different species and different individuals, and with different methods of assay, but the order of magnitude is the same. Although by far the greater portion of intracellular magnesium in muscle is unionized, the actual concentration of the ionized and the unionized fractions is unknown. A frog muscle suspended in magnesium-free Ringer's solution retains 80 to 90 per cent of the total magnesium up to the fifth day (31). The main loss of magnesium seems to occur within the first 5 hours, during which the loss amounts to about 14 per cent of the total magnesium content (52). It is uncertain to which anions magnesium is bound within the cell. The finding that the magnesium content of muscle cells is relatively constant in magnesium deficiency is consistent with the assumption that magnesium is mainly bound to protein and organic phosphate anions (135, 32).

Histochemical assay of magnesium in striated muscle fiber has given rather contradictory results. It is well established that micro-incineration of striated

muscle gives ash diagrams with a distribution which resembles the cross striation. It is, however, difficult to decide whether the band with high ash content corresponds to the anisotropic or to the isotropic segment of the fiber. Comparing a micro-incinerated and an adjacent stained section in mammalian muscle, Scott (117, 118) concluded that the ash lay in the anisotropic segments. In insect muscle Engström (44) compared the site of the main ultraviolet absorption and of the ash content in the same fiber in relation to well-defined reference points. In these experiments, the ash was found to be localized in the ultraviolet absorbing isotropic segments, while the anisotropic segments were practically devoid of ash. By application of emission electron microscopy it was possible to differentiate magnesium and calcium from potassium and sodium. Magnesium and calcium were present almost exclusively within the muscle, but it was uncertain where in relation to the anisotropic and isotropic segments (119).

By use of the electron beam of the electron microscope for micro-incineration, not only organic but also more volatile inorganic material is removed and the cross-striation disappears. The narrow striations of the fibril with a period of 400 A are, however, present. This periodicity is interpreted as being due to the more stable salts of calcium and magnesium (37). This would suggest that the magnesium and calcium are distributed independently of the anisotropic and isotropic segments. It seems, however, that the interpretations of the findings need further experimental support.

Mechanism of Action. Anesthesia with magnesium is the only example of anesthesia produced by a metallic electrolyte. When attempts are made to explain its mode of action, it seems natural to consider its role in the enzymatic processes considered essential for the function of the neuromuscular system. The magnesium ion has been shown to be an activator for tissue phosphatases and phosphate transferring enzymes. Its presence is of importance for the activity of several enzymes with special functions in the neuromuscular system. The best known of these are the enzymes which cause the enzymatic breakdown of adenosinetriphosphate, and the enzymes which synthesize and split acetylcholine in tissue. However, the main difficulty for all interpretations is that the magnesium ion penetrates the cell slowly, while its depressant action occurs soon after an intravenous injection and is abolished almost instantaneously by calcium. Consequently it has often been suggested that the site of the anesthetic action of magnesium ion must be primarily at the cell membrane, especially at that of the neuromuscular junction. This is in accordance with the small increase in magneseium concentration in brain and muscle found during magnesium anesthesia.

According to current concepts, the following sequence of events occurs in neuromuscular transmission: The arrival of the nerve impulse with subsequent liberation of acetylcholine, which depolarizes the endplate; the depolarization initiates a propagated impulse in the muscle fiber. Accordingly several mechanisms could be involved in the blocking action of magnesium: the acetylcholine production might be reduced; the endplate membrane might be prevented from responding normally; or the excitability of the muscle fiber membrane or the contractility of the fiber substance might be decreased. The magnesium effect seems

primarily to be localized to the endplate membrane, as the sensitivity to acetylcholine was found to be decreased while the excitability of the muscle was still unimpaired. Sooner or later the muscle fiber is also affected. At the moment, it is solely a matter for speculation whether magnesium, like curarine, decreases the endplate potential, depolarizes it or acts by some other unknown mechanism. As mentioned before, the experimental evidence seems to indicate a mechanism of action which is different from that of curarine.

The explanation of the antagonistic effect of calcium is likewise obscure, though a little more is known about the effect of calcium on the endplate potential. Calcium produces a more general decurarizing effect, as curarization likewise was found to be counteracted by calcium (50). Excess of calcium (4 to 5 times normal content) increased the size of the endplate potential in curarized frog muscle without appreciably altering its time course. Calcium raised the threshold potential required for the initiation of muscle spikes with a given concentration of curarine (40, 79, 28). In isolated fibers from frog muscle, a moderate excess of calcium first caused a delay in onset of the spike, which indicates a decrease in excitability of the muscle fiber. The amplitude of the endplate potential was not increased by calcium. A further increase in the calcium concentration caused neuromuscular block (81). The decurarizing effect of calcium has been explained as being due to increased liberation of acetylcholine (33). In curarized muscle, in contrast to normal, excess calcium increased the amplitude of the endplate potential. This increase was not accompanied by an increase in sensitivity to the depolarizing action of acetylcholine in the endplate region. This suggests that the increase in amplitude may be due to an increase in the amount of acetylcholine released at the endplate (28).

It is difficult to explain the magnesium-calcium antagonism on the basis of these findings. We do not know the influence of calcium excess on the endplate potential in a magnesium-treated muscle; and if calcium produces an increased acetylcholine release this might be accompanied by a decrease in excitability of the muscle fiber.

At higher concentrations of magnesium, a decrease in excitability and contractility of the muscle develops slowly, probably produced by the slowly penetrating magnesium. As mentioned above, magnesium ion influences the enzymatic breakdown of adenosinetriphosphate (ATP), at present believed to be the final link between the energy-yielding chemical reactions and mechanical activity.

The finding that myosin acts as an ATP-ase (43) links the process of energy transfer to the contractile structure. The enzymatic activity of myosin and actomyosin (3, 132) was found to be strongly influenced by the electrolyte medium; among other ions it was activated by calcium (2), whereas magnesium inhibited ATP-ase activity (86). The ATP-ase activity of myosin and actomyosin was differently influenced by magnesium. Pure myosin was inhibited at even the lowest concentrations of magnesium, whereas phosphatase activity of actomyosin was enhanced by magnesium with an optimum between 1 and 5 mM magnesium. At high ionic strength (100 mM KCl), the activity of actomyosin was inhibited by all magnesium concentrations, which was explained as being due to a dissocia-

tion of the protein complex into actin and myosin. Calcium strongly enhanced the enzymatic activity at concentrations between 1 and 100 mM; this enhancing effect was inhibited by all concentrations of magnesium (4). The phosphate uptake of actomyosin threads with low ATP-ase activity was enhanced by magnesium (100 mM) (23).

The antagonistic action of magnesium-calcium in muscle was confirmed by Greville and Lehmann (62) who tried to correlate it to the magnesium-calcium antagonism of magnesium anesthesia. The rôle of magnesium ions in enzymatic processes in living muscle is, however, complicated by its dependence on several factors which are not sufficiently elucidated: the mutual relation between actomyosin and myosin, intramuscular pH, ionic strength and the concentration of other activators and inhibitors. As mentioned before, the degree of ionization and the localization of magnesium in the fiber are uncertain and several contradictory statements about the effect of magnesium ions are met with. On the basis of a study of the magnesium-calcium antagonism under a variety of conditions it was concluded that myosin ATP-ase under the conditions met with in muscle is probably subjected to the inhibitory action of magnesium (103). On the other hand, ATP-ase activity of isolated myofibrosils (which retained considerable organisation of structure) was found to be activated by magnesium (maximum of activation at 5 mM) even in the presence of 100 mM KCl. Destruction of structural organisation by 1.0 M KCl or by storing transformed the magnesium activation to inhibition. Evidence was presented that the ATP-splitting enzyme was actomyosin, and it was presumed that the state of organisation of actomyosin in the fibrils was responsible for this difference in behaviour (111). In view of these discrepancies and of our incomplete knowledge of the conditions for enzymatic activity in living muscle fiber, it seems hardly possible at present to give a consistent explanation for the depressant action of magnesium on muscle.

The question of the effect of magnesium on ATP-ase activity in living tissues was treated from another point of view by examining the amount of ATP in muscle and in brain during magnesium anesthesia. The ATP gain was higher in muscle and in brain samples taken during magnesium anesthesia than in those from the unanesthetised animal. There was a difference between animals in ether anesthesia and in magnesium anesthesia as well, which was claimed to indicate that the decrease in ATP-content was not solely caused by the increased muscular activity in unanesthetised animals. The increased content of ATP found in muscles after application of magnesium was interpreted as being due to magnesium inhibition of the enzymatic breakdown at ATP into ADP and inorganic phosphate (38). These findings in muscle were confirmed by Stoner (130) who, on the basis of experiments with P^{32}, interpreted the differences in another way. The specific activity (counts per min. per mg P) of (ATP)P and phosphocreatine P in magnesium-treated animals was significantly higher than in the controls, whereas the specific activity of total organic P and the sum of (ATP)P acid and stable ester P were unaltered. It was suggested that magnesium reduces the utilization of ATP and of phosphocreatine in glycolysis and leads to an accumulation of high energy phosphate bonds. That magnesium anesthesia increases the ATP

content of the muscle is well established, but more experimental work is needed to determine the mechanisms involved. Experiments on curarized animals could possibly be of assistance in clarifying how far the increased amount of ATP in muscle after magnesium is due to the absence of muscular movements.

A group of enzymes which are of great importance for the special functions of the neuromuscular system and which are affected by the magnesium ion are acetylcholinesterase and acetylcholinacetylase.

The acetylcholine-splitting enzymes are known to be activated by magnesium ions. Most investigations are concerned with the non-specific cholinesterase (98, 54, 55), but even the specific acetylcholinesterase from the electric organ of torpedo was activated by magnesium in physiological concentrations, up to 2 mM. Calcium likewise acted as an activator (105). It would be interesting to know whether the concentrations obtained during magnesium anesthesia also produce activation of cholinesterase, as an increase in the enzymatic breakdown of acetylcholine would inhibit neuromuscular transmission as well. The antagonistic effect of calcium cannot, however, be explained by the effect on cholinesterase.

Acetylcholine can be synthesised in cell-free solution. The enzyme responsible for the synthesis was termed choline acetylase (106). This synthesis was diminished by calcium ions (49) and activated by magnesium ions in the presence of citrate, the optimal concentration of magnesium being 4 mM (47, 48). As the concentration of magnesium in the central nervous system is about 5 mM, it seems plausible that an increase in the concentration of magnesium ions in the extracellular fluid might inhibit the synthesis of acetylcholine. Experiments on the cholineacetylase activity of brain tissue during magnesium anesthesia might contribute to the solution of this problem.

Assay of Magnesium. The earliest method for the determination of magnesium in biological fluids is based upon its precipitation as magnesium ammonium phosphate. Magnesium is determined as phosphate by reduction of phosphomolybdic acid to a blue substance, the amount of which is measured colorimetrically (19, 35, 99, 124). Moreover, phosphate can be determined as the yellow molybdivanadophosphoric acid, which is claimed to be stable over a longer time than the molybdenum blue (120, 121).

Another principle for the determination of magnesium is precipitation with 8-hydroxyquinoline. The hydroxyquinoline bound to magnesium can be determined in several ways:

Bromination of the magnesium hydroxyquinoline was described by Greenberg and Mackey (60), the chief source of error being a loss of bromine through evaporation (59). A cerimetric titration of the hydroxyquinoline has the advantage, that the amount of ceric sulphate consumed by the same amount of hydroxyquinoline is about 8 times greater than that of bromate in bromatometric titration (125). Colorimetric determination may be used after addition of diazotised sulphanilic acid (112). Another possibility is to measure the hydroxyquinoline micromanometrically by direct combustion of the hydroxyquinoline complex, the carbon being determined as CO_2. This method was claimed to be more sensitive than the colorimetric methods since the precipitate contains 18 carbon atoms per atom of Mg (70). The formation of the insoluble Mg-hydroxyquinolate is also the basis for the polarographic determination of magnesium. Direct polarographic determination is impossible, but the excess of oxyquinoline left after precipitation with magnesium is determined by this technique (129, 136).

Before the determination of magnesium with ammonium phosphate or hydroxyquinoline

it is necessary to precipitate the calcium. One of the main difficulties in the removal of calcium as oxalate is the separation of magnesium and calcium, as some magnesium oxalate is always occluded. The difficulty arises chiefly when the quantity of magnesium exceeds that of calcium (8, 12). In normal serum the separation of calcium and magnesium apparently has not presented difficulties for the estimation of magnesium. In magnesium anaesthesia, when the serum contains about 10 times the value of the normal serum, the separation of calcium results in a loss of 4 to 8 per cent of the total magnesium (42). This error could be overcome by using slow precipitation at high temperature (100°C.) (8) at low p*H* (3 to 4) (89, 137).

Several methods exist which can be used in the presence of calcium. The colorimetric method for the determination of magnesium by means of Titan yellow is based on the development of a red magnesium hydroxide-titan yellow complex (6, 64, 69, 82). With this method calcium need not be removed, since it does not interfere with the colour produced by magnesium in concentrations ordinarily found in blood (57). The sensitivity of the method was increased 10-fold by Orange and Rhein (109). This method might prove suitable with larger amounts of magnesium in plasma as well.

In addition to precipitation, magnesium in body fluids can be determined by direct spectroscopy (17, 27).

A method for the determination of magnesium based upon isolation by electro-deposition in the presence of calcium has been developed by Terkildsen (134).

REFERENCES

(1) Ashkenaz, E. W. Magnesium narcosis in muscle. J. Cell. & Comp. Physiol., **11**: 163–174, 1938.

(2) Bailey, K. Myosin and adenosinetriphosphatase. Biochem. J., **36**: 121–139, 1942.

(3) Banga, I. and Szent-Györgyi, A. Preparation and properties of myosin A and B. Stud. Inst. Med. Chem. Univ. Szeged, **1**: 5–15, 1942.

(4) Banga, I. and Szent-Györgyi, A. The influence of salts on the phosphatase action of myosin. Stud. Inst. Med. Chem. Univ. Szeged, **3**: 72–75, 1943.

(5) Baumecker, W. Experimentelle Beiträge zum Antagonismus der Magnesium- und Calciumionen. Biochem. Ztschr., **142**: 142–158. 1923.

(6) Bécka, J. Über die praktische Anwendung der kolorimetrischen Magnesiumbestimmung in der Biochemie. Biochem. Ztschr., **233**: 118–128, 1931.

(7) Beecher, H. K., McDonough, F. K. and Forbes, A. Effects of blood pressure changes on cortical potentials during anesthesia. J. Neurophysiol., **1**: 324–331, 1938.

(8) Benedetti-Pichler, A. Microversuche über quantitative Trennungen. 1. Mitteilung. Versuche unter Benutzung des Filterstäbschens. Ztschr. f. analyt. Chemie, **64**: 409–436, 1924.

(9) Bertrand, J., Gayet-Hallion, Th. and Quivy, D. Influence des sels de magnésium sur l'electro-encéphalogramme du Lapin. Compt. rend. Soc. biol., **141**: 145–147, 1947.

(10) Binet, P. Recherches comparatives sur l'action physiologique des metaux alcalins et alcalino-terreux. Rev. méd. Suisse Rom., **12**: 523-550, 587-611, 1892.

(11) Boardman, D. L. and Collier, H. O. J. The effect of magnesium deficiency on neuro-muscular transmission in the shore crab, carcinus maenas. J. Physiol., **104**: 377–383, 1946.

(12) Bobtelsky, M. and Malkowa-Janowski. Über die Löslichkeit von Magnesiumoxalat und ihre Bedeutung für die Calcium-Magnesium Trennung. Ztschr. f. angew. Chemie, **40**: 1434–1436, 1927.

(13) Borglin, N. E. and Lindstén, T. On the combined effect of neostigmine and pentamethylenetetrazol or β-phenylisopropylamine on magnesium anaesthesia. Acta pharmacol. et toxicol., **5**: 309–320, 1949.

(14) Boyd, T. E. Recovery of the tongue from curare paralysis, following prolonged stimulation of the hypoglossal nerve. Am. J. Physiol., **100**: 569–575, 1932.

(15) Boyd, T. E. and Brosnan, J. J. Decurarizing effect of repetitive stimulation of a motor nerve. Proc. Soc. Exper. Biol. & Med., **35**: 404–405, 1936.

(16) Boyd, T. E., Brosnan, J. J. and Maaske, C. A. The summation of facilitating and inhibitory effects at the mammalian neuromuscular junction. J. Neurophysiol., **1**: 497–507, 1938.

(17) Boyle, A. J., Whitehead, T., Bird, E. J., Batchelor, Th. M., Iseri, L. T., Jacobson, S. D. and Myers, G. B. The use of the emission spectrograph for the quantitative determination of Na, K, Ca, Mg, and Fe in plasma and urine. J. Lab. & Clin. Med., **34**: 625–630, 1949.

(18) Boyle, P. J., Conway, E. J., Kane, F. and O'Reilly, H. L. Volume of interfibre spaces in frog muscle and the calculation of concentrations in the fibre water. J. Physiol., **99**: 401–414, 1941.

(19) Briggs, A. P. A colorimetric method for the determination of small amounts of magnesium. J. Biol. Chem., **52**: 349–355, 1922.

(20) Brosnan, J. J. and Boyd, T. E. Agents which antagonize the curare-like action of magnesium. Am. J. Physiol. **119**: 281–282, 1937.

(21) Brown, G. L., Dale, H. H. and Feldberg, W. Reactions of the normal mammalian muscle to acetylcholine and to eserine. J. Physiol., 87: 394–424, 1936.
(22) Brown, G. L. and von Euler, U. S. The after effect of a tetanus on mammalian muscle. J. Physiol., 93: 39–60, 1938.
(22a) Bryant, G. W., Lehmann, G. and Knoefel, P. K. The action of magnesium on the central nervous system and its antagonism by calcium. J. Pharmacol. & Exper. Therap., 65: 318–321, 1939.
(23) Buchthal, F., Deutsch, A., Knappeis, G. G., and Munch-Petersen, A. Phosphorylation and adenine nucleotide uptake of actomyosin and actin-free myosin. Acta physiol. Scandinav., 16: 326–344, 1949.
(24) Buchthal, F. and Lindhard, J. Direct application of acetylcholine to motor end plates of voluntary muscle fibres. J. Physiol., 90: 82P–83P, 1937.
(25) Buchthal, F. and Lindhard, J. Transmission of impulses from nerve to muscle fibre. Acta physiol. Scandinav., 4: 136–148, 1942.
(26) Cannon, W. B. and Rosenblueth, A. The supersensitivity of denervated structures. A law of denervation. The Macmillan Co., New York, 1949, p. 245.
(27) Cassen, B. A method for rapid determination of magnesium in body fluids and some preliminary results on clinical material. J. Lab. & Clin. Med., 25: 411–413, 1940.
(28) Castillo, J. del and Stark, L. The effect of calcium ions on the motor end-plate potentials. J. Physiol., 116: 507–515, 1952.
(29) Cloetta, M., Fischer, H. and van der Loeff, M. R. Die Verteilung und die Wirkung des Magnesiums im Organismus und deren Beeinflussung durch Calcium. Arch. exper. Path. u. Pharmacol., 200: 6–42, 1942–1943.
(30) Conway, E. J. The physiological significance of inorganic levels in the internal medium of animals. Biol. Rev. 20: 56–72, 1945.
(31) Conway, E. J. and Crueas-Callaghan, G., Magnesium and chloride "permeations" in muscle. Biochem. J., 31: 828–836, 1937.
(32) Cotlove, E., Holliday, M. A., Schwartz, R., and Wallace, W. M. Effect of electrolyte depletion and acid-base disturbance on muscle cations. Am. J. Physiol., 167: 665–675, 1951.
(33) Cowan, S. L. The actions of eserine-like compounds upon frog's nerve-muscle preparations, and conditions in which a single shock can evoke an augmented muscular response. Proc. Roy. Soc., London, s. B., 129: 356–391. 1940.
(34) Dellen, T. R. Van and Miller, J. R. Electrocardiographic changes following the intravenous administration of magnesium sulfate. II. An experimental study on dogs. J. Lab. & Clin. Med., 24: 840–843, 1939.
(35) Denis, W. The determination of magnesium in blood, plasma and serum. J. Biol. Chem., 52: 411–415, 1922.
(36) Desprès, M. Action curarisante du chlorure de magnésium. Compt. rend. Soc. biol., 107: 602–604, 1931.
(37) Draper, M. H. and Hodge, A. J. Sub-microscopic localization of minerals in skeletal muscle by internal "micro-incineration" within the electron microscope. Nature, London, 163: 576–577, 1949.
(38) Dubois, K. P., Albaum, H. G. and Potter, V. R. Adenosine triphosphate in magnesium anesthesia. J. Biol. Chem., 147: 699–704, 1943.
(39) Eccles, J. C., Katz, B. and Kuffler, S. W. Nature of the "endplate potential" in curarized muscle. J. Neurophysiol., 4: 362–387, 1941.
(40) Eccles, J. C., Katz, B., and Kuffler, S. W. Effect of eserine on neuromuscular transmission. J. Neurophysiol., 5: 211–230, 1942.
(41) Eichelberger, L., Kollros, J. J. and Walker, A. E. Water, nitrogen and electrolyte content of brain following cerebral concussion. Am. J. Physiol., 156: 129–136, 1949.
(42) Engbaek, L. Investigations on the course and localization of magnesium anesthesia. A comparison with ether anesthesia. Acta pharmacol. et toxicol., 4: Suppl. 1, Pp. 189, 1948.
(43) Engelhardt, W. A. and Ljubimowa, M. N. Myosine and adenosine-triphosphatase. Nature, London, 144: 668–669, 1939.
(44) Engström, A. The localization of mineral salts in striated muscle fibers. Acta physiol. Scandinav., 8: 137–151, 1944.
(45) Eskelund, V. Undersøgelser over magniumnarkosen og forholdet mellem magnium og calcium ved denne og nogle andre narkoser. Diss. Copenhagen 1931. Pp. 130.
(46) Essen, K. W. Untersuchungen über die Narkosevertiefung nach Grosshirnexstirpation. Arch. exper. Path. u. Pharmakol., 159: 387–400, 1931.
(47) Feldberg, W. and Hebb, C. Effect of magnesium ions on the enzymic formation of acetylcholine. J. Physiol., 104: 42P, 1945–1946.
(48) Feldberg, W. and Hebb, C. The effects of magnesium ions and of creatine phosphate on the synthesis of acetylcholine. J. Physiol., 106: 8–17, 1947.
(49) Feldberg, W. and Mann, T. Formation of acetylcholine in cell-free extracts from brain. J. Physiol., 104: 8–20, 1945.
(50) Feng, T. P. Studies on the neuromuscular junction. I. The inhibition at the neuromuscular junction. Chinese J. Physiol., 10: 417–434, 1936.
(51) Fenn, W. O. Electrolytes in muscle. Physiol. Rev., 16: 450–487, 1936.
(52) Fenn, W. O. and Haege, L. F. The penetration of magnesium into frog muscle. J. Cell. & Comp. Physiol. 19: 37–46. 1942.
(53) Fraser, A. M. The effect of magnesium on the response of the uterus to posterior pituitary hormones. J. Pharmacol. & Exper. Therap., 66: 85–94, 1939.
(54) Frommel, E., Herschberg, A. D. and Piquet, J. Effets des ions inorganiques sur l'activité de la cholinestérase sérique. I. Etudes, in vitro, sur le sérum de cheval. Helvet. physiol. et pharmacol. acta, 2: 169–191, 1944.

(55) FROMMEL, E., HERSCHBERG, A. D. AND PIQUET, J. Effets des ions inorganiques sur l'activité de la cholinestérase sérique. II. Etudes, in vivo, sur le cobaye. Helvet. physiol. et pharmacol. acta, 2: 193-201, 1944.
(56) GARB, S. The effects of potassium, ammonium, calcium, strontium and magnesium on the electrogram and myogram of mammalian heart muscle. J. Pharmacol. & Exper. Therap., 101: 317-326, 1951.
(57) GARNER, R. J. Colorimetric determination of magnesium in plasma or serum by means of Titan yellow. Biochem. J., 40: 828-831, 1946.
(58) GREENBERG, D. M. Mineral metabolism. Calcium, magnesium, and phosphorus. Ann. Rev. Biochem., 8: 269-300, 1939.
(59) GREENBERG, D. M., ANDERSON, C. AND TUFTS, E. V. A note on a closed titration flash for use in the bromometric determination of magnesium with 8-hydroxyquinoline. Application to the estimation of magnesium in the tissues and urine. J. Biol. Chem., 111: 561-565, 1935.
(60) GREENBERG, D. M. AND MACKEY, M. A. The determination of magnesium in blood with 8-hydroxyquinoline. J. Biol. Chem., 96: 419-429, 1932.
(61) GREINER, TH. H. AND GARB, S. The influence of drugs on the irritability and automacity of heart muscle. J. Pharmacol. & Exper. Therap., 98: 215-223, 1950.
(62) GREVILLE, G. D. AND LEHMANN, H. Magnesium-calcium antagonism in muscle. Nature, London, 152: 81-82, 1943.
(63) GUTHRIE, C. C. AND RYAN, A. H. On the alleged specific anaesthetic properties of magnesium salt. Am. J. Physiol., 26: 329-346, 1910.
(64) HAURY, V. G. Modification of the Titan yellow method for the determination of small amounts of magnesium in biological fluids. J. Lab. & Clin. Med., 23: 1079-1084, 1938.
(65) HAURY, V. G. The broncho-dilator action of magnesium and its antagonistic action (dilator action) against pilocarpine, histamine and barium chloride. J. Pharmacol. & Exper. Therap., 64: 58-64, 1938.
(66) HAURY, V. G. The effect of intravenous injections of magnesium sulfate on the vascular system. J. Pharmacol. & Exper. Therap., 65: 453-460, 1939.
(67) HAURY, V. G. Variations in serum magnesium in health and disease: a review. J. Lab. & Clin. Med., 27: 1361-1375, 1942.
(68) HERTZ, H. Action potential and diameter of isolated fibres under various conditions. Acta physiol. Scandinav., 13: suppl. 43, pp. 91, 1947.
(68a) HEYMANS, C. AND CAPET, L. Influences du magnésium et du calcium sur la regulation proprioceptive de la pression artérielle. Arch. internat. de pharmacodyn. et de thérap. 71: 164-172, 1945.
(69) HIRSCHFELDER, A. D. AND SERLES, E. R. A simple adaptation of Kolthoff's colorimetric method for the determination of magnesium in biological fluids. J. Biol. Chem., 104: 635-645, 1934.
(70) HOAGLAND, C. Micromanometric determination of magnesium. J. Biol. Chem., 136: 553-558, 1940.
(71) HODGKIN, A. L. AND KATZ, B. The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol., 108: 37-77, 1949.
(71a) HOF, C. VON AND SCHNEIDER, H.-H. Über die Wirkungsweise von Magnesium im Gegensatz zu Curarin und Nikotin. Arch. exper. Path. u. Pharmakol., 214: 176-184, 1952.
(72) HOFF, H. E., SMITH, P. K. AND WINKLER, A. W. The relation of blood pressure and concentration in serum of potassium, calcium and magnesium. Am. J. Physiol., 127: 722-730, 1939.
(73) HOFF, H. E., SMITH, P. K. AND WINKLER, A. W. Effects of magnesium on the nervous system in relation to its concentration in serum. Am. J. Physiol., 130: 292-297, 1940.
(74) HSU, YU-CHUN. The influence of the magnesium concentration in the Ringer's solution used for the assay of oxytocin in pituitary (posterior lobe) extracts. J. Pharm. & Pharmacol., 21: 146-151, 1948.
(75) JOLYET, F. AND CAHOURS. Sur l'action physiologique des sulfates de potasse, de soude et de magnésie en injection dans le sang. Arch. physiol. norm. et path., 2: 113-120, 1869.
(76) JOSEPH, R. AND MELTZER, S. J. The life-saving action of physostigmin in poisoning by magnesium salts. J. Pharmacol. & Exper. Therap., 1: 369-387, 1909-1910.
(77) JOSEPH, D. R. AND MELTZER, S. J. Der hemmende Einfluss des Magnesiums auf die indirekte und direkte Erregbarkeit von Froschmuskeln und die antagonistischen Effekte von Natrium und Calcium auf diesen Einfluss. Zentralbl. Physiol., 24: 7-8. 1910.
(78) KATZ, B. Neuro-muscular transmission in crabs. J. Physiol., 87: 199-221, 1936.
(79) KATZ, B. Impedance changes in frog's muscle associated with electronic and "endplate" potentials. J. Neurophysiol., 5: 169-184, 1942.
(80) KUFFLER, S. W. Specific excitability of the endplate region in normal and denervated muscle. J. Neurophysiol., 6: 99-110, 1943.
(81) KUFFLER, S. W. The effect of calcium on the neuro-muscular junction. J. Neurophysiol., 7: 17-26, 1944.
(82) KUNKEL, H. O., PEARSON, P. B. AND SCHWEIGERT, B. A. The photoelectric determination of magnesium in body fluids. J. Lab. & Clin. Med., 32: 1027-1033, 1947.
(83) LAGET, P. AND LUNDBERG, A. Further observations on environmental factors influencing the temperature sensitivity of mammalian nerve fibre. Effects of Ca^{++} and other agents. Acta physiol. Scandinav., 18: 121-127, 1949.
(84) LEUSEN, I. The influence of calcium, potassium and magnesium ions in cerebrospinal fluid on vasomotor system. J. Physiol., 110: 319-329, 1949.
(85) LIBET, B. AND GERARD, R. W. Control of the potential rhythm of the isolated frog brain. J. Neurophysiol., 2: 153-169, 1939.
(86) LJUBIMOVA, M. N. AND PEVSNER, D. Cit. from (2). Biokhimya, 6: 178, 1941.

(87) LORENTE DE NÓ, R. A study of nerve physiology. Part I, no. 131 Pp. 496. Rockefeller Inst. Med. Res., New York, 1947.
(88) LUBINSKA, L. Les troubles d'origine périphérique au cours de la narcose magnésienne. Arch. internat. de physiol., 41: 456–473, 1935.
(89) LUX, H. Praktikum der quantitativen anorganischen Analyse. Berlin, 1941, p. 161.
(90) MAASKE, C. A., BOYD, T. E. AND BROSNAN, J. Inhibition and impulse summation at the mammalian neuromuscular junction. J. Neurophysiol., 1: 332–341, 1938.
(90a) MAASKE, C. A. AND GIBSON, B. The effects of magnesium upon denervated mammalian muscle. Am. J. Physiol., 127: 486–491, 1939.
(91) MALORNY, G. AND OHNESORGE, F. K. Magnesium-Kalzium Antagonismus bei Prüfung der neuromuskulären Erregbarkeit. Die Naturwissenschaften, 38: 481–482, 1951.
(92) MANSFELD, G. AND BOSÁNYI, S. Untersuchungen über das Wesen der Magnesiumnarkose. Arch. f. d. ges. Physiol., 152: 75–80, 1913.
(93) MARKWALDER, J. Experimentelle Untersuchung über Therapie des Wundstarrkrampfes mit intravenöser Magnesiuminfusion. Ztschr. f. d. ges. exper. Med., 5: 150–172, 1917.
(94) MATTHEWS, S. A. AND BROOKS, C. On the action of magnesium sulphate. J. Pharmacol. & Exper. Therap., 2: 87–99, 1910.
(95) MELTZER, S. J. Magnesiumsulfat bei Tetanus. Berl. klin. Wchnschr., 52: 261–266, 1915.
(96) MELTZER, S. J. AND AUER, J. Physiological and pharmacological studies of magnesium salts. III. The narcotizing effect of magnesium salts upon nerve fibres. Am. J. Physiol., 16: 233–251, 1906.
(97) MELTZER, S. J. AND AUER, J. The antagonistic action of calcium upon the inhibitory effect of magnesium. Am. J. Physiol., 21: 400–419, 1908.
(98) MENDEL, B., MUNDELL, D. AND STRELITZ, F. Cholinesterase and electrolytes. Nature, London, 144: 479–480, 1939.
(99) MICHAELS, G. D., ANDERSON, C. T., MARGEN, S. AND KINSELL, L. W. A method for the colorimetric determination of calcium and magnesium in small amounts of urine, stool, and food. J. Biol. Chem., 180: 175–180, 1949.
(100) MIKI, Y. Experimentelle und klinische Untersuchungen über die Dauer des K-Ekg (Kammer-Elektrokardiogramms). Ztschr. f. d. ges. exper. Med., 27: 323–388, 1922.
(101) MILLER, J. R. AND VAN DELLEN, T. R. Electrocardiographic changes following the intravenous administration of magnesium sulfate. An experimental study on dogs. J. Lab. & Clin. Med., 23: 914–918, 1937–1938.
(102) MILLER, J. R. AND VAN DELLEN, T. R. Electrocardiographic changes following the intravenous administration of magnesium sulfate. III. Combined effect with digitalis. J. Lab. & Clin. Med., 26: 1116–1120, 1940–1941.
(103) MOMMAERTS, W. F. H. M. AND SERAIDARIAN, K. A study of the adenosine triphosphatase activity of myosin and actomyosin. J. Gen. Physiol., 30: 401–422, 1947.
(104) MOORE, R. M. AND WINGO, W. J. Blood level of magnesium ion in relation to the lethal, anesthetic, analgesic and antitetanic effects. Am. J. Physiol., 135: 492–495, 1942.
(105) NACHMANSOHN, D. Action of ions on choline esterase. Nature, London, 145: 513–514, 1940.
(106) NACHMANSOHN, D. AND MACHADO, A. L. The formation of acetylcholine. A new enzyme: "choline acetylase". J. Neurophysiol., 6: 397–403, 1943.
(107) NAESS, K. The peripheral effects of magnesium and curare. Acta pharmacol. et toxicol., 8: 137–148, 1952.
(108) NAESS, K. The mechanism of action of curare. Acta pharmacol. et toxicol., 8: 149–163, 1952.
(109) ORANGE, M. AND RHEIN, H. C. Microestimation of magnesium in body fluids. J. Biol. Chem., 189: 379–386, 1951.
(110) PECK, C. H. AND MELTZER, S. J. Anesthesia in human beings by intravenous injection of magnesium sulfate. J. Am. M. A., 67: 1131–1133, 1916.
(111) PERRY, S. V. The adenosinetriphosphatase activity of myofibrils isolated from skeletal muscle. Biochem. J., 48: 257–265, 1951.
(112) PIGNARD, P. Microdosage colorimétrique du magnésium sanguin par la 8 hydroxyquinoléine. Bull. Soc. chim. biol., 32: 401–408, 1950.
(113) ROSENBLUETH, A. The transmission of nerve impulses at neuroeffector junctions and peripheral synapses. John Wiley & Sons, Inc., New York, 1950, p. 325.
(113a) ROSENBLUETH, A. AND MORISON, R. S. Curarization, fatigue and Wedensky inhibition. Am. J. Physiol., 119: 236–256, 1937.
(114) ROTHBERGER, C. J. AND ZWILLINGER, L. Über die Wirkung von Magnesium auf die Strophantin- und die Barium-Tachykardie. Arch. exper. Path. u. Pharmakol., 181: 301–316, 1936.
(115) RUBIN, M. A., HOFF, H. E., WINKLER, A. W. AND SMITH, P. K. Intravenous potassium, calcium and magnesium and the cortical electrogram of the cat. J. Neurophysiol., 6: 23–28, 1943.
(116) SCHMIDT, C. L. A. AND GREENBERG, D. M. Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. Physiol. Rev., 15: 297–434, 1935.
(117) SCOTT, G. H. Distribution of mineral ash in striated muscle cells. Proc. Soc. Exper. Biol. & Med., 29: 349–351, 1932.
(118) SCOTT, G. H. The localization of mineral salts in cells of some mammalian tissues by micro-incineration. Am. J. Anat., 53: 243–287, 1933.
(119) SCOTT, G. H. AND PACKER, D. M. An electron microscope study of magnesium and calcium in striated muscle. Anat. Rec., 74: 31–45, 1939.
(120) SIMONSEN, D. G., WERTMAN, M., WESTOVER, L. M. AND MEHL, J. W. The determination of serum phosphate by the molybdivanadate method. J. Biol. Chem., 166: 747–755, 1946.

(121) SIMONSEN, D. G., WESTOVER, L. M. AND WERTMAN, M. The determination of serum magnesium by the molybdivanadate method for phosphate. J. Biol. Chem., 169: 39–47, 1947.
(122) SMITH, P. K., WINKLER, A. W. AND HOFF, H. E. Electrocardiographic changes and concentration of magnesium in serum following intravenous injection of magnesium salts. Am. J. Physiol., 126: 720–730, 1939.
(123) SMITH, P. K., WINKLER, A. W. AND HOFF, H. E. The pharmacological actions of parenterally administered magnesium salts. A review. Anesthesiology, 3: 323–330, 1942.
(124) SMITH, P. K., WINKLER, A. W. AND SCHWARTZ, B. M. The distribution of magnesium following the parenteral administration of magnesium sulfate. J. Biol. Chem., 129: 51–56, 1939.
(125) SPIER, H. W. Kombinierte gesamt-Calcium-und-Magnesium Bestimmung in Serum (Oxydimetrische Magnesiumanalyse über Mg-oxinat). Klin. Wchnschr., 29: 85–87, 1951.
(126) STANBURY, J. B. The blocking action of magnesium ion on sympathetic ganglia. J. Pharmacol. & Exper. Therap., 93: 52–62, 1948.
(127) STANBURY, J. B. AND FARAH, A. Effects of the magnesium ion on the heart and on its response to digoxin. J. Pharmacol. & Exper. Therap., 100: 445–453, 1950.
(128) STARKENSTEIN, E. Untersuchungen über die Magnesiumnarkose. I. Zentralbl. Physiol., 28: 63–70, 1914.
(129) STONE, K. G. AND FURMAN, N. H. Polarographic use of organic reagents. Magnesium with 8-hydroxyquinoline. Indust. & Engin. Chem., 16: 596–598, 1944.
(130) STONER, H. B. Effect of magnesium on P^{31} and P^{32} partition in muscle. Am. J. Physiol., 161: 387–398, 1950.
(131) STANSKY, E. Untersuchungen über die Magnesiumnarkose. Arch. exper. Path. u. Pharmakol., 78: 122–153, 1915.
(132) STRAUB, F. B. Actin. Stud. Inst. Med. Chem. Univ. Szeged, 2: 3–15, 1942.
(133) STRAUB, W. Experimentelle Untersuchung über Wesen und Aussicht der Tetanustherapie mit Magnesiumsulfat. München. med. Wchnschr., 62: 25–29, 1915.
(134) TERKILDSEN, T. CHR. Electrochemical microdetermination of magnesium. Scandinav. J. Clin. & Lab. Investigation, 4: 27–35, 1952.
(135) TUFTS, E. V. AND GREENBERG, D. M. The biochemistry of magnesium deficiency. I. Chemical changes resulting from magnesium deprivation. J. Biol. Chem., 122: 693–714, 1937–1938.
(136) WALAAS, O. Polarographic determination of magnesium in serum by aid of 8-hydroxyquinoline. Scandinav. J. Clin. & Lab. Investigation, 1: 187–190, 1949.
(137) WASHBURN, M. L. AND SHEAR, M. J. Composition of bone. VIII. Direct gravimetric determination of Ca, Mg, and PO_4. J. Biol. Chem., 99: 21–41, 1932–1933.
(138) WIECHMANN, E. Zur Theorie der Magnesiumnarkose. Arch. f. d. ges. Physiol., 182: 74–103, 1920.
(139) WIKI, B. Sur l'action anesthésiante locale du sulfate de magnésium. Arch. internat. de pharmacodyn. et de thérap., 21: 415–423, 1911.
(140) ZADINA, R. AND KRIZ, V. L'action du magnésium sur la contraction de l'intestin isolé. Compt. rend. Soc. biol., 142: 1037–1039, 1948.