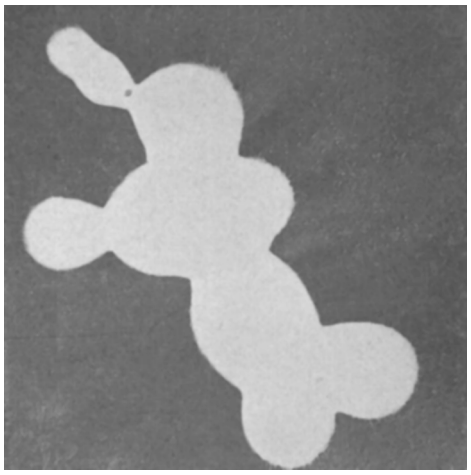

FIG. 3. The same preparation 15 min after incitation of precipitation.

rupts the formation of aggregates or at least slows down the process sufficiently to make a registration of the different stages possible.

The surface relief of the aggregates does not show but their contours suggest a granulated structure. although it is hardly possible to draw any conclusions regarding the size or shape of the individual molecules in the aggregates. However, a scrutiny of the micrographs reveals the presence of other structures besides the aggregates, *viz.*, small practically spherical bodies measuring ca. 120-180 Å. In the 0-minute specimen they are very numerous and not easily distinguishable from a mere coarseness of the carrier film. With the growth of the aggregates they decreased considerably in number and appear, in the 15-minute specimen, scarce and evenly distributed over the membrane. It would seem, therefore, that these structures represent particles present in the specimen and not only a rough surface of the collodion film. The obvious inverse correlation between the size of the aggregates and the number of small particles indicates that the larger structures are formed by aggregation of the latter. The size of the small particles is not incompatible with the assumption that they represent molecules of the macroglobulin.

Summary. A case of anemia combined with hyperglobulinemia is described. In the serum the presence of a macroglobulin with an estimated molecular weight of about one million was demonstrated. Electron micrographs of the partially purified macroglobulin under certain experimental conditions show the presence of spherical particles measuring approximately 120-180 Å.

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The Neuromuscular Mechanism of Alkalotic and Hypocalcemic Tetany.* (19367)

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The motor phenomena which characterize tetany consist of muscle fasciculations and tonic and clonic muscle spasms. Tetany is usually caused by a reduction in the concentration of ionized blood calcium or by an elevation in blood pH. Either of these alterations in the milieu interne apparently diminishes the stability of the nerve cell membrane, which diminution is reflected in an increased electrical excitability, altered afterpotentials and a reduced capacity of accommodation(1,2). At a critical level, iterative spontaneous discharges occur which are propagated along the nerve fiber(1,3). While

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most of these observations have been made on peripheral nerves, other neural structures have been demonstrated to discharge spontaneously in hypocalcemic and alkalotic states. Bronk and associates(4) have shown this to be true for sympathetic ganglion cells. Kuffler(5) found that the peripheral nerve terminations in the motor end plate display spontaneous activity in hypocalcemic states. Very little is known of the influence of hypocalcemic and alkalotic states on neurons within the central nervous system. The question of what part or parts of the neuromuscular system are the primary site or sites of irritation in tetany is still incompletely answered. The present investigation is concerned with the analysis of this problem.

Material and methods. Eleven cats and one dog anesthetized with sodium pentobarbital (35 mg/kg) were employed in this study. Artificial respiration was provided by a Harvard type respirator pump through a tracheal cannula. Alkalosis was produced by continuous intravenous infusion of an 8% solution of sodium bicarbonate or a 5% solution of sodium carbonate. Venous blood was collected under mineral oil in a hypodermic syringe from time to time and the pH determined with the Beckman glass electrode pH meter. Hypocalcemia was produced by venoclysis of a phosphate buffer with a pH of approximately 7.4 (3 g of monobasic sodium phosphate, 30 g of dibasic sodium phosphate in 600 cc of solution). The rate of administration of these solutions was adjusted so that manifest tetany was present in from 15 minutes to 30 minutes after venoclysis was begun. The influx of solution was then slowed so as to maintain a relatively steady state of tetany until the termination of the experiment. In the analysis of the neuromuscular mechanism of tetany in these experiments it was found necessary only to section selected peripheral nerves and to employ a myoneural blocking agent, although other procedures such as spinal cord transection and dorsal root section had been contemplated. The hypoglossal nerve was dissected out in five cats at the level of the hyoid bone in the floor of the mouth and loosely ligated. The brachial plexus was exposed through a dorsal approach and this, too, was loosely ligated in 5 cats and one dog. After tetany was present, these nerves were cut and the effects on the tongue and forelimb musculature observed. In these and in other experiments the direct effect of alkalosis and hypocalcemia on muscles was observed by intravenous administration of d-tubocurarine (Squibb) in doses greater than sufficient to block transmission of nerve impulses at the myoneural junction.

Results. The objective manifestations of tetany in cats and the one dog were identical whether produced by alkalosis or by hypocalcemia. Muscle fasciculations appeared first and were most easily seen early in the tongue. These soon spread to involve other muscles. In the alkalosis series, the blood pH at the onset of fasciculations ranged from 7.65 to 7.80. Tonic muscle spasms were observed usually soon after the onset of fasciculations. The muscles of mastication and the forelimb muscles were the first to be so affected. Tonic spasm of the former muscles produced a firm elevation of the mandible. Tonic spasm of the latter muscles produced a sustained flexion at the wrist joint and in more extreme cases also an extension at the other joints, although the posture of the tonically contracted limb differed somewhat in different animals. The hindlimb and trunk musculature participated in the tonic spasms only in extreme tetany, which was induced by administering the phosphate or alkaline solution at a rapid rate after tetany was already present. This was a hazardous procedure, however, for cardiac arrest frequently supervened. At no time were clonic spasms observed.

Hypoglossal nerve section. The hypoglossal nerve was cut unilaterally proximal to its point of entry into the tongue in 5 cats after tetany was produced. Fasciculations were present in the tongue at the time of section. In three animals, there occurred a complete or partial cessation of muscle fasciculations in the ipsilateral half of the tongue. After a few seconds, fasciculations reappeared and shortly thereafter there was no perceptible difference in motor activity between the 2 halves of the tongue. In the remaining two experiments,

section of the hypoglossal nerve produced no alterations in the muscle fasciculations at any time.

Brachial plexus section. In 6 experiments the brachial plexus was cut on one side after muscle fasciculations and tonic spasms were present in the forelimbs. In 2 animals there occurred a moderate relaxation of the tonic spasm which lasted for several seconds and then was followed by a return to the previous state, the motor activity being identical in the experimental and control forelimb. In a third animal, a temporary exaggeration of the tonic spasm occurred upon cutting the brachial plexus. In another 2, section of the brachial plexus did not alter the muscle spasms of the ipsilateral forelimb. Fasciculations behaved as the tonic spasms in all these experiments.

d-Tubocurarine injection. In four experiments, d-tubocurarine was injected into the femoral vein when tetany was marked. Within a few seconds, complete cessation of all motor phenomena occurred, and persisted even after the rate of administration of phosphate or alkaline solution was increased.

Discussion. The reports in the literature dealing with this problem are frequently conflicting. Thus, some investigators(6-10) found that transection of the spinal cord at the thoracic level abolished tetany in the hindlimbs, whereas others(11,12) claimed that this procedure had no effect on tetany. Paton *et al.*(6) reported that section of the peripheral nerve abolished all manifestations of tetany in the denervated limb. West(12), however, found that this procedure, and deafferentation by dorsal nerve root section as well, blocked the tonic and clonic muscle spasms of tetany but that muscle fasciculations still occurred. The latter investigator concluded that the tonic and clonic muscle spasms in tetany depend upon the integrity of the spinal reflex arc and that the muscle fasciculations result from a direct action of the blood on the muscle fibers. The above-mentioned discrepancies in results may be attributed to two factors: the method of producing tetany, and the use of the hindlimb musculature. These investigators, with one exception(10) produced tetany by extirpation of the parathyroid glands. Parathyroidectomized animals have periods of manifest tetany alternating with latent tetany. Furthermore, they may rapidly become resistant to the hypoparathyroid state(13). This form of tetany is evidently difficult to control or regulate. The second factor, perhaps even more significant than the first, is the muscle groups singled out for the study of neuromuscular mechanism of tetany. The hindlimb musculature of mammals is relatively resistant to tetany as compared with the forelimb muscles. In spite of this, however, previous studies have employed the hindlimb muscles in analyzing the role of the nervous system. Negative results in such studies, *i.e.*, failure to observe manifestations of tetany, are not necessarily conclusive. Positive results when recorded are significant.

In this study, both of these factors were taken into consideration and the experiments designed to avoid these pitfalls. The experimental production of acute hypocalcemic and alkalotic tetany by continuous intravenous infusion of phosphate and sodium carbonate and bicarbonate solutions is a reliable method which permits minute to minute control of the severity of tetany merely by regulating the influx of solution. The muscles of the tongue and forelimbs were selected for the analysis of neural mechanism of tetany since it was these muscle groups that were found to be most responsive in tetany. The results of the present investigation show clearly that not only the fasciculations but also the tonic spasms produced in alkalotic and hypocalcemic tetany can still occur in muscles shortly after section of their nerves. West's(12) failure to observe tonic spasms in acutely denervated muscles and in muscles deafferented by dorsal nerve root section can be attributed to his employment of the hindlimb muscles of parathyroidectomized dogs. The complete and enduring abolition of all motor activity in tetany which follows the administration of d-tubocurarine, a myoneural blocking agent, eliminates the possibility of a direct activation of muscle fibers in tetany. This is consistent with a report of Hartridge and West(14) that subparalytic doses of curare abolish parathyroid tetany in dogs. In spite of this finding, however, West(12) in a subsequent re-

port inferred that muscle fasciculations result from a direct action of the blood on the muscle fibers.

Under the conditions of these experiments, it is evident that the fasciculations and tonic spasms occasioned by alkalosis and hypocalcemia can occur as the result of spontaneous iterative discharges arising in peripheral motor nerve fibers or their terminations in the motor end plates. These results cannot, of course, preclude the possibility of the participation of neurons within the central nervous system, or of sensory neurons in spinal and perhaps even supraspinal reflex arcs. In fact, there is considerable evidence that sensory fibers also are irritated and discharge repetitively in human tetany(2). However, our results strongly suggest that activation of peripheral motor nerve fibers or terminations account for the motor manifestations of tetany whether caused by an elevation in blood pH or a reduction in ionized blood calcium. This view is supported by Kugelberg's(15) finding in one human subject with inveterate hypocalcemic tetany that impulses occurring in the fourth interosseous muscle of the hand and recorded electromyographically continued unaltered after apparently effective procaine block of the ulnar nerve at the elbow.

Summary. 1. Tetany was produced acutely in eleven cats and one dog with solutions of sodium bicarbonate, sodium carbonate and a phosphate buffer administered by venoclysis. Fasciculations and tonic spasms involving especially the head and forelimb musculature characterized this form of tetany. Clonic spasms were were not seen in anesthetized animals. 2. Section of the hypoglossal nerve did not significantly influence the fasciculations of the tongue musculature. Section of the brachial plexus did not significantly influence the fasciculations or tonic spasms of the forelimb musculature. 3. Paralytic doses of d-tubocurarine blocked all motor manifestations of tetany. 4. It was concluded that all of the motor phenomena characterizing alkalotic and hypocalcemic tetany could result from the spontaneous iterative discharges occurring in peripheral motor nerve fibers or their terminations.

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Some Factors Influencing Production of Ulcers in the Shay Rat. (19368)

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In our experience, the incidence of ulcers in the Shay rat varied from 100% to 25%. There are no apparent reasons for this variation, and some of the complicating, but significant, observations are recorded in this paper. Recently, Madden *et al.*(1) in a critical study of the Shay procedure concluded that the method is valuable for the study of gastric secretion and of less value for the study of the ulceration mechanism. We have used