THE EFFECT OF CHANGES IN THE CALCIUM CONTENT OF THE CEREBROSPINAL FLUID ON SPINAL REFLEX ACTIVITY IN THE DOG[1]

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Although the cerebrospinal fluid system has been the subject of extensive study, most workers have concerned themselves mainly with the mechanics of the formation and reabsorption of this fluid, relatively little attention having been paid to the relationships existing between the cerebrospinal fluid and the tissue fluid of the brain and spinal cord. The probability of free communication between the subarachnoid space and the tissue spaces of the nervous system has been demonstrated by Mott (1910), Weed (1914), and others. If such communication does exist, it is reasonable to assume that any change in the composition of the cerebrospinal fluid will be reflected by a similar change in the composition of the interstitial fluids, and thus possibly by modified activity of the neurones bathed by the latter.

Several workers have reported spontaneous neuromuscular activity following the intrathecal administration of various solutions. Collip (1920) observed tetanoid behavior in dogs following the introduction of small volumes of $NaHCO_3$, hypertonic NaCl and KCl, and Na_2HPO_4 into the lumbar subarachnoid space. $CaCl_2$ antagonized this motor activity, and it was concluded that the tetany was due to a disturbance in the concentrations of the various cations, especially of calcium. Huggins and Hastings (1933) observed that the injection of sodium citrate into the cisterna magna of the dog produced motor excitation which was antagonized by calcium chloride. Calcium citrate provoked no motor response. Mullin, Hastings, and Lees (1938) produced tetany by the cisternal injection of salt solutions which were ionically balanced except for the absence of calcium. Similar results were obtained with the injection of sodium citrate. Bathing the lower spinal cord with the calcium-free solution was ineffective, although here too citrate produced spontaneous neuromuscular activity.

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METHODS. The action of low calcium solutions on the spinal cord has been reinvestigated using a method of continuous perfusion through the spinal subarachnoid space at constant pressure and temperature (Merlis and Lawson, 1939). Dogs were anesthetized with sodium barbital (0.25 gram/kgm. intravenously), the spinal cord was sectioned at T10, and the caudal segment of the cord was prepared for perfusion. As an indicator of the effect of changes in the calcium content of the perfusion fluid on the activity of simple functional neural units, the flexion reflex of the tibialis anticus muscle was elicited by stimulating the posterior tibial nerve at 5 to 8 second intervals with single shocks applied from a thyratron stimulator. The tension developed by the muscle was recorded on smoked paper by means of a torsion wire myograph.

The control perfusion fluid was an artificial cerebrospinal fluid of the following composition (moles/liter): Na—0.141, K—0.0033, Ca—0.00125, Mg—0.0012, Cl—0.152, HPO_4—0.00048, HCO_3—0.021, glucose—0.0034, urea—0.0022. A change in the calcium concentration was always compensated by an opposite change in NaCl concentration so as to have control and test solutions isosmolar. All solutions were brought to a pH about 7.4 with CO_2, using phenol red as the indicator.

The rate of flow through the spinal subarachnoid space was varied either by changing the perfusion head of pressure, or by maintaining a constant pressure head and partially obstructing the outflow by means of a constriction. Rates varying from 2 to 25 cc./minute with perfusion pressures of 8 mm. Hg or higher were used.

RESULTS. The subarachnoid perfusion of the balanced artificial cerebrospinal fluid had, in most cases, no effect on the flexion reflex. Occasionally a slight diminution of the reflex was apparent, but augmentation was never seen. When the calcium-free solution was substituted for the balanced solution, there was marked augmentation of the reflex, an increase in the tone of the muscle, and spontaneous twitching of the muscles of the lower half of the body (fig. 1). These effects usually appeared in from 1 to 5 minutes, at least a portion of the latency being accounted for by the 3 cc. dead space between the fluid reservoirs and the spinal subarachnoid space. Replacement by the balanced salt solution was followed by subsidence of these effects, usually within less than 5 minutes.

Sodium citrate (0.1–5.0 per cent) intrathecally was followed by similar motor activity, which was more intense than that seen with calcium-free perfusions.

Solutions containing an excess of calcium, in concentrations as high as four times the control value, had no demonstrable effect on the flexion reflex. Higher concentrations than these were not studied.

The effects of a calcium-free perfusion appeared to be more intimately connected with the rate of flow through the spinal subarachnoid space than

with the perfusion head of pressure. Successful perfusions were obtained with pressures as low as 8 mm. Hg and as high as 100 mm. Hg. The pressures were always kept well below mean carotid pressure to avoid the possibility of marked reduction in blood flow through the cord with consequent anoxic effects. With perfusion pressure constant, an increase in the rate of perfusion produced increased effects (fig. 2).

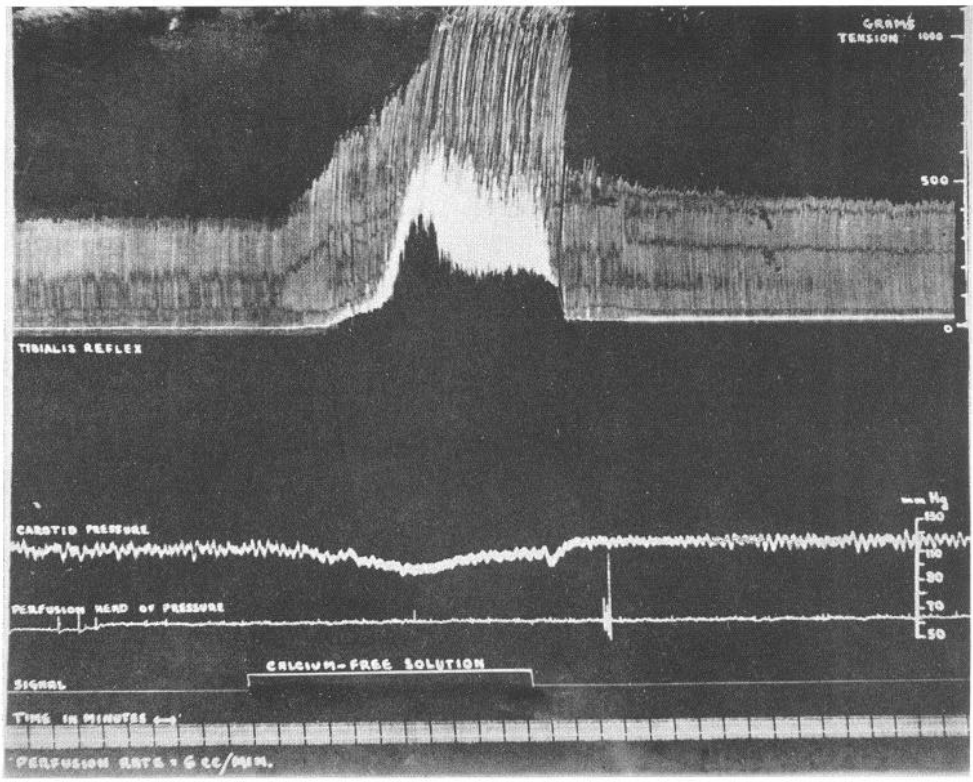

Fig. 1. The effect of a calcium-free perfusion. Irregular fibrillary contractions are recorded in the intervals between reflex contractions during the response.

Discussion. The failure of Mullin and his collaborators (1938) to produce tetany by bathing the spinal cord with low calcium solutions may be attributable to the method employed in their studies. If the action of these solutions is due to a lowering of the calcium content of the fluids bathing the cells of the central nervous system, it is essential that a diffusion gradient of sufficient magnitude be set up between the subarachnoid fluid and the interstitial fluids. Simple bathing of the exposed spinal cord with small volumes of salt solution might very well fail to achieve this end. The

effectiveness of the method of continuous subarachnoid perfusion may be ascribed to maintenance of an effective gradient.

It has been shown by Lehmann (1937) and by Brink and Bronk (1938) that a decrease in the calcium content of the fluids bathing peripheral nerve produces increased excitability of the nerve fibers and may provoke

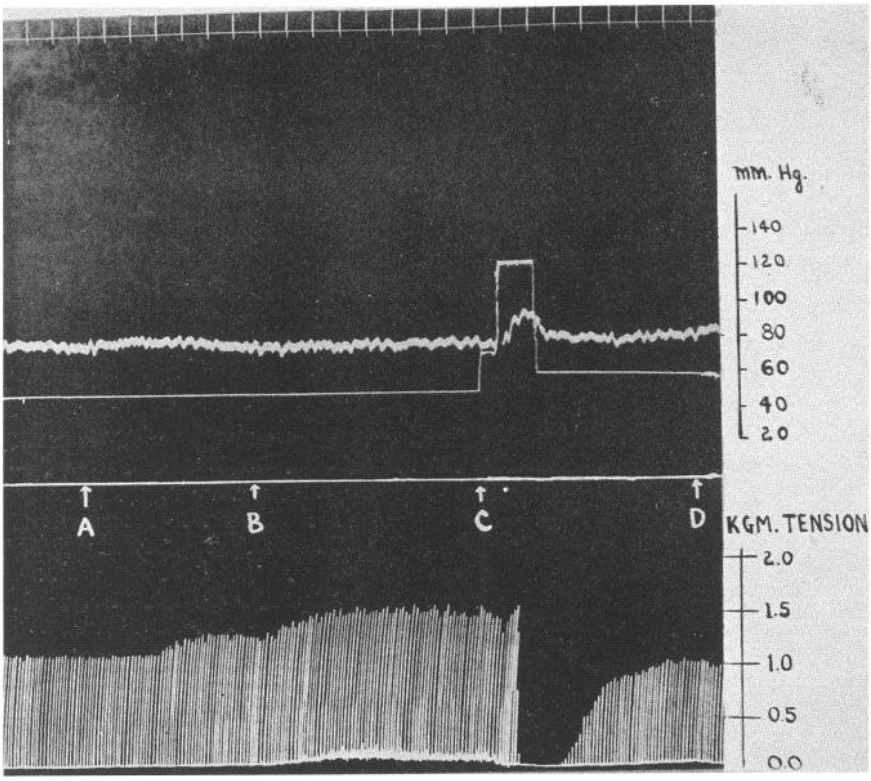

Fig. 2. Tracings from above downward: 1, time in minutes; 2, carotid pressure; 3, perfusion head of pressure; 4, signal; 5, tibialis anticus reflex. At *A*, calcium-free perfusion begun, perfusion rate: 3.4 cc./min. At *B*, perfusion rate increased to 16 cc./min. At *C*, perfusion pressure raised above carotid pressure. At *D*, calcium-free solution replaced by balanced salt solution. Fibrillary contractions are just visible in the intervals between reflex contractions during the Calcium-free perfusion, and disappear with the reflex during cord ischemia at *C*.

spontaneous discharge in many of them. The tetany produced by the spinal subarachnoid perfusion of calcium-free solutions might be due to an action of these fluids not in the cord itself but rather on the ventral rootlets in their intrathecal course. If this were true, synaptic transmision through the cord would not be essential, at least insofar as the spontaneous

twitching is concerned. This possibility has been tested by blocking synaptic transmission through the medium of the ischemia which is produced when perfusion pressure is raised above blood pressure (Luckhardt and Montgomery, 1929; Merlis and Lawson, 1939). When synaptic transmission was abolished by such a procedure, not only was the flexion reflex abolished, but so too were the spontaneous twitchings (fig. 2). It is therefore apparent that the muscular twitchings are not caused by spontaneous firing of the motoneurones of the cord, or of the nerve fibers of the anterior roots.

Two possibilities still remain: 1, the action is on the afferent limb of the reflex arc, i.e., spontaneous discharge of the dorsal roots or internuncial neurones, or 2, there is no spontaneous firing, but rather an increase in the excitability of the cord neurones, so that they respond more effectively to the normally incident flow of afferent impulses arising in the periphery. When the dorsal roots of the perfused cord segments were sectioned extradurally, spontaneous twitching was completely abolished, although synaptic transmission was still possible as was shown by eliciting reflex responses by mechanical stimulation of the central ends of the cut dorsal roots. This can mean only that the spontaneous tetanic manifestations depend upon the receipt of afferent impulses from the periphery and are not due to spontaneous firing of neurones of the cord, or of dorsal or ventral root fibers.

In the case of citrate perfusions, the situation is somewhat different. Ischemic blocking of synaptic transmission through the cord diminished, but did not abolish, the twitching which results from a citrate perfusion. Citrate, therefore, does cause spontaneous discharge of the motoneurones of the cord, or of the intrathecal ventral root fibers, or of both. Corroborative evidence was obtained from the deafferented preparation, where again there was diminution, but not abolition of twitching.

It is of interest to note the parallelism between the tetanic manifestations produced by calcium-free cerebrospinal fluid and those reported by West (1935) in his studies on parathyroid tetany. The results of his experiments led West to conclude that there were three neuromuscular manifestations of parathyroid tetany: fibrillary twitching, tonic, and clonic contractions. The clonic and tonic contractions were abolished by deafferentation of the cord, although the fibrillary movements were still in evidence after this operation. This finding is quite similar to that reported in this study, in which the tetany, although definitely central in origin, does depend upon the integrity of the somatic reflex arcs before it may be manifested.

SUMMARY AND CONCLUSIONS

1. The effect of changes in the calcium content of balanced salt solutions perfused through the lower spinal subarachnoid space at constant pressure

and temperature was studied in barbitalized dogs with spinal cord sectioned at T10.

2. Calcium-free solutions produced an augmentation of the spinal flexion reflex, an increase in muscle tone, and spontaneous twitching of the muscles of the lower half of the body.

3. The tetany produced by calcium-free perfusions is not due to spontaneous firing of the motoneurones nor of the dorsal or ventral rootlets. The twitching requires the integrity of the spinal reflex arcs, for it disappears when these arcs are broken. It appears to be due to an increased responsiveness of the cord neurones to the normally incident afferent impulses from the periphery.

4. Similar motor activity was seen with sodium citrate perfusions, differing in that the twitching persisted, with diminution, when synaptic transmission was abolished. Citrate appeared to cause spontaneous firing of the motoneurones, or of the dorsal and ventral rootlets, or both.

5. High calcium solutions, up to four times normal concentrations, were without effect.

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