THE LOCUS OF THE ACTION OF VERATRIN.

By CHARLES L. WIBLE.

(From the Physiological Laboratory, Rutgers College, New Brunswick.)

(Received for publication, March 18, 1924.)

Maxwell (1) has shown that some nerve stimulants, such as the calcium precipitants, stimulate medullated nerve fibers alone, and he has termed these substances nerve excitants of the first class. Others, such as creatine, strychnine and picrotoxin stimulate only nerve cells and these form the second class. Tetraethyl ammonium chloride, since it stimulates both medullated nerve and nerve cells must be included in both classes. Veratrin for the reason that it directly stimulates some of the ganglia in the medulla of vertebrates must be classed as a neurophil alkaloid of the second group (2). Furthermore, in all treatises on the subject, so far as I have been able to discover, it has been assumed that veratrin also acts in a characteristic way directly on the striated muscle fibers of the vertebrates (3). It remains to be determined whether veratrin stimulates medullated nerve fibers. If this should be the case, the question is at once raised as to whether the supposed action of veratrin on muscle may not be referable to its action on nerve fibers.

In order to throw some light on the problem of the locus of action of veratrin in different forms, experiments were made with specimens of *Mnemiopsis leidyi*, *Lumbricus terrestris*, and *Musca domestica* from the invertebrate group, and frogs (*Rana pipiens*) from the vertebrate group.

All veratrin solutions were made from a sample of the amorphous powder obtained from the Mallinckrodt Chemical Company. The concentration used unless otherwise stated was 1 part by weight of veratrin to 1,345 parts of sea water or Ringer's solution as the case may be.

Mnemiopsis leidyi.

If specimens of *Mnemiopsis leidyi* (4) be immersed in a solution of veratrin made up in sea water the swimming plates immediately stop in an upright position. But, if the animal be cut into small pieces, in 2 minutes the isolated single plates and groups of plates begin beating, and continue to beat for at least half an hour in the solution of veratrin.

In the case of intact specimens there is only slight, irregular movement at the extreme tips of the plates after 15 or 20 minutes immersion in veratrin. This slight movement at the tips increases with time, although the effective stroke is at first deficient, and at the end of an hour is abnormally fast and vigorous. If during this stage, there is momentary cessation of beat, the plates do not lie prone as in the normal animal, but stop in the upright position. It can also be seen that slight mechanical stimulation at this time causes, not cessation of beat, but excitation and acceleration of beat. This abnormally vigorous type of motion of the swimming plates is maintained for a period of 6 or 7 hours, even appearing in isolated plates after disintegration of the animal.

It was also observed that the normal action of the food cilia was not influenced by immersion of the animal in veratrin. This suggests that the control mechanisms in the case of food cilia and swimming plates are not the same.

Since the first brief effects of veratrin, namely inhibition of beat, can be removed by isolation of plates, and since in the secondary stage of veratrin poisoning, mechanical stimulation causes not inhibition but excitation, it seems probable that the locus of veratrin action in *Mnemiopsis leidyi* is on the nervous mechanism.

Lumbricus terrestris.

It has been shown that the earthworm is a form favorable for the demonstration of the action of neurophil alkaloids, since the exposed ventral nerve cord is stimulated by camphor, strychnine, atropine, and picrotoxin (5).

If an intact worm be immersed in a watery solution of veratrin the result is a prolonged series of spasmodic contractions, involving both

circular and longitudinal muscles. Similar results are obtained in decapitated specimens, or in smaller pieces. Mechanical stimuli at this time do not elicit peristaltic movements, as in a normal worm, but cause spasmodic contractions of the musculature.

The action of veratrin on the ventral nerve cord was tested as follows: A decapitated animal was pinned down by the anterior and posterior ends and the intestine and ventral nerve cord removed. This left a sheet of muscle tissue, which was clamped at the posterior end and fastened by the anterior end to a counterbalanced writing lever, the whole apparatus being arranged for making a kymographic record. If this preparation of worm is now immersed, first in Ringer's solution then in a solution of veratrin, the result is that the writing lever does not leave the base line in either case. But, if the same procedure be repeated using a piece of worm containing the nerve cord, the response is of an entirely different character. Immersion in Ringer's solution results in normal peristalsis, while immersion in the solution of veratrin produces abnormally vigorous and prolonged contractions of the longitudinal muscles (Fig. 1).

In order, finally, to determine whether veratrin acts on the ventral nerve cord alone, experiments were made in which a worm was decapitated and the anterior portion of the ventral cord was exposed for a distance of about 3 cm. A piece of the cord about one-half this length was dissected free from the surrounding tissue, the latter being entirely removed leaving $1\frac{1}{2}$ cm. of the nerve cord free. The specimen was arranged in the usual way for making kymographic records. If now the free portion of nerve cord be immersed in Ringer's solution, normal peristalsis follows; but, if Ringer's solution be replaced by a solution of veratrin the result is a very great and immediate increase in the strength of circular and longitudinal muscular contractions (Fig. 2).

If the free end of the nerve cord of an earthworm preparation be treated with a solution of veratrin, characteristic reactions of both circular and longitudinal muscles follow. On the other hand, identical treatment of a muscle preparation from which the nerve cord has been removed has no result in stimulation of the muscles. It must therefore be concluded that veratrin does not act on the musculature of

the worm nor on nerve endings in the muscle, but upon the nerve cord alone. Hence veratrin, in the case of *Lumbricus* acts as a neurophil alkaloid of the second group.

Musca domestica.

Several house-flies kept under ventilated glass jars were given a syrupy solution of veratrin. This they drank readily. Within 5 minutes the first effects were apparent. The symptoms developed in three distinct stages.

1. Extreme elevation of the anterior portion of the body, the action so pronounced that it is suggestive of opisthotonos.

2. Loss of control of legs, which are spread apart. Repeated spasmodic attempts to fly.

3. Complete loss of equilibrium, usually ending with the fly lying ventral side upward, with legs flexed close to body. At this time if an individual be stimulated mechanically the result is shown in convulsive spasms and inability to make progressive movements. If now, these individuals be decapitated, the legs relax and extend so that the animal regains its equilibrium. In some cases there are more or less successful attempts to fly. In fact, from successful attempts of this kind specimens were lost to the experiment. If the third stage is allowed to progress too long, the animal dies and consequently decapitation is without effect.

Since the effects of poisoning by veratrin, namely rigid flexion of the legs and loss of equilibrium, can be removed by decapitation, the necessary conclusion is that in *Musca domestica* veratrin acts on the brain alone, and therefore as a neurophil alkaloid has a specific action on the neurones of the head ganglia. For this reason veratrin must be included in the second group of nerve excitants.

Nerve Fibers of Rana pipiens.

It has been shown that nerve fibers of the frog's nerve-muscle preparation can be stimulated chemically. For example, a solution of sodium citrate applied to the sciatic nerve results in irregular contractions of the gastrocnemius muscle. Similarly, if the sciatic nerve is immersed for 20 minutes in a solution of veratrin made up in Ringer's

solution, more or less rhythmical contractions of the gastrocnemius result. This can be demonstrated in the following way. The usual nerve-muscle preparation of the frog is made, and the preparation set up in such fashion that the nerve lies in a glass nerve holder containing the solution of veratrin. Precautions are taken to see that the nerve is free from tension, and that it does not become dry in the space between the nerve holder and the muscle. A vaseline ring is placed around the nerve to make sure that none of the solution of veratrin reaches the muscle by capillary action. After a latent period of 15 to 25 minutes spontaneous muscle twitchings begin in the gastrocnemius (Fig. 3).

It can be proven that veratrin does not spread *through* the nerve fibers to the muscle. For if during the muscle twitchings shown in Fig. 3, the nerve be clipped between the point of immersion in the veratrin solution and the point of attachment to the preparation, the writing lever immediately falls to the base line and remains at rest (Fig. 4). This proves that the action of veratrin in this case is on the medullated nerve fiber and not on muscle. Veratrin, therefore, is a neurophil alkaloid of the first class. Since it also belongs to the second class, veratrin is in the same category as tetraethyl ammonium chloride.

In further experiments it was found that a typical veratrin contracture results if the muscle be directly stimulated with single induction shocks ("break shocks," with coil set at 8 cm.) after the sciatic nerve is immersed in veratrin solution for a few moments, but before the spontaneous muscle contractions begin. If however, the sciatic be sectioned and electrical stimuli again applied, the contracture disappears and the period of relaxation becomes normal (Fig. 5). This proves that the characteristic veratrin curve of the frog's gastrocnemius can be reproduced without the muscle itself ever having been subjected to the action of veratrin, if only the end of the sciatic nerve be veratrinized.

It has been observed by previous investigators, that with gastrocnemius muscles of frogs veratrinized by injection into the dorsal lymph sac the descending limb of the curve of muscle contraction often showed distinct and somewhat rhythmical waves (6). Such rhythmical waves can also be observed in nerve-muscle preparations, when

electrically stimulated, if the sciatic nerve alone be immersed in a solution of veratrin (Fig. 6). From this it is clear that the characteristic waves in the relaxation curve of the muscle are caused by the action of veratrin on the nerve fibers alone. Presumably the waves in the relaxation curve of muscle, observed by former investigators, were caused by the same mechanism, namely the action of veratrin on the nerve fibers.

These results make it seem possible that the characteristic effects of veratrin heretofore ascribed to action of the drug on muscle cells may be due instead to the excitatory action of veratrin on the nerve fibers within the muscle.(7)[1] An answer can be given to the question by means of experiments on the sartorius muscle, since the upper part of this muscle contains nerve fibers while the lower part does not. With this purpose in view an entire sartorius muscle was dissected out and arranged in the usual way for making kymographic records.

If such a muscle preparation be bathed with a solution of veratrin the muscle always responds with a prolonged contraction as a result of veratrin stimulation (Fig. 7). Electrical stimulation applied after the contraction has ceased results in the characteristic veratrin muscle curve (Fig. 8). If a fresh sartorius be sectioned transversely through the middle, and the lower half which presumably consists of muscle cells without nerve fibers, be bathed in a solution of veratrin, there is no contraction as with the entire muscle, and electrical stimulation as a rule gives a normal contraction and relaxation (Fig. 9). The occasional exceptions to this rule may perhaps be accounted for on the ground that the region of the sartorius muscle which is supplied with nerves varies in different preparations, and that it is impossible in every case to section the muscle exactly on a line between the part containing nerve fibers and that which does not.

On the other hand if the upper half of the muscle which is supplied with nerve fibers is bathed in a solution of veratrin the result is always a prolonged contraction (Fig. 10), and subsequent electrical stimulation elicits a contraction with a delayed relaxation period (Fig. 11).

[1] Wyman (7) has found that the action of veratrin in causing an expansion of chromatophores is probably caused by excitation of the nerves supplying the chromatophores.

The facts show then that while the entire sartorius and the upper half of the muscle respond in characteristic fashion to veratrin poisoning, the lower half of the muscle which contains no nerve fibers does not contract spontaneously when bathed in veratrin solution, nor does the lower half when stimulated electrically show a delayed relaxation in the muscle curve. For these reasons it must be concluded that veratrin produces its characteristic effects upon an entire muscle through its excitatory action on medullated nerve fibers of the muscle and that it is very questionable whether veratrin is capable of affecting functional striated muscle fibers.

SUMMARY.

1. In *Mnemiopsis* veratrin shows two stages of veratrin poisoning. First, inhibition of the beats of the plates which disappears on cutting them away either singly or in small groups. Second, after half an hour mechanical stimulation excites the beat of the plates in the intact veratrinized animal. It is concluded that veratrin acts on nervous tissue and not on the substance of the swimming plates.

2. In *Lumbricus*, veratrin acts on the ventral nerve cord alone, and not on the muscles and peripheral nerves.

3. In *Musca*, veratrin first causes opisthotonos, then spasms and extreme flexion of the legs. Decapitation causes these effects to disappear hence veratrin acts on the cerebral ganglia of the fly.

4. Veratrin applied to the sciatic nerve of the frog causes, after a latent period of 20 minutes, irregular contractions of the gastrocnemius which persist for an hour or more. Veratrin is thus a neurophil alkaloid of the first class as well as second and in this way resembles tetraethyl ammonium chloride.

5. If the end of a sciatic nerve is dipped into veratrin solution, then direct stimulation of the gastrocnemius muscle results in contraction with delayed relaxation, although the muscle itself is not subject to the action of veratrin.

6. By means of preparations of the sartorius muscle of the frog it is shown that veratrin acts not on the muscle cells directly but on the nerve fibers. Hence veratrin produces the characteristic muscle curve showing delayed relaxation by its action on the nervous elements.

BIBLIOGRAPHY.

1. Maxwell, S. S., *Am. J. Physiol.*, 1918–19, xlvii, 283. See also Moore, A. R., *Proc. Nat. Acad. Sc.*, 1917, iii, 598.
2. Gottlieb, R., and Meyer, H., Pharmacology, clinical and experimental, Philadelphia and London, 1914, 425.
3. Querido, A., *Arch. néerl. physiol. homme et animaux*, 1923, viii, 307.
4. Wible, C., *Proc. Soc. Exp. Biol. and Med.*, 1923, xxi, 53.
5. Moore, A. R., *J. Gen. Physiol.*, 1921–22, iv, 29.
6. Brunton, T. L., and Cash, J. T., *J. Physiol.*, 1883–84, iv, 1.
7. Wyman, L. C., *J. Exp. Zool.*, 1924, xxxix, 113.

EXPLANATION OF FIGURES.

FIG. 1. Curve of contraction of a preparation of earthworm containing nerve cord, showing effect of immersion in veratrin solution in greatly increased contraction of the longitudinal musculature. X indicates point at which veratrin was applied. Tracing made from right to left.

FIG. 2. Earthworm preparation with nerve cord freed at one end. When this part of the cord is immersed in veratrin solution (shown at X) the strength of peristalsis increases and tone is heightened.

FIG. 3. Tracing made by frog's gastrocnemius muscle after its sciatic nerve had been immersed in a veratrin solution for 20 minutes.

FIG. 4. Shows effect of sectioning (at X) the sciatic nerve of the preparation described in Fig. 3. The muscle at once relaxes.

FIG. 5. *a*, normal contraction curve of frog's gastrocnemius resulting from direct electrical stimulation of the muscle. *b*, same with the sciatic nerve immersed in veratrin showing delayed relaxation characteristic of veratrin poisoning. *c*, contractions of same after section of the sciatic.

FIG. 6. Showing periodic waves in the relaxation phase of muscle with sciatic nerve immersed in veratrin.

FIG. 7. Contraction curve of the frog's sartorius muscle obtained as a result of immersion in a solution of veratrin.

FIG. 8. *a*, normal contraction of the sartorius muscle, as a result of direct electrical stimulation. *b*, same after muscle has been immersed in veratrin.

FIG. 9. Contractions resulting from electrical stimulation of the veratrinized lower half of the sartorius which is not supplied with nerve fibers.

FIG. 10. Contraction obtained by immersion of the upper half of the sartorius in a solution of veratrin. This part of the sartorius contains nerve fibers.

FIG. 11. Electrical stimulation of the upper half of a sartorius which has been immersed in veratrin.

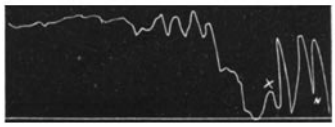


FIG. 1.

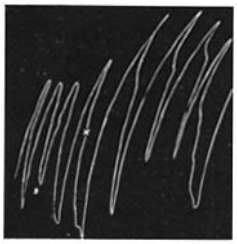


FIG. 2.

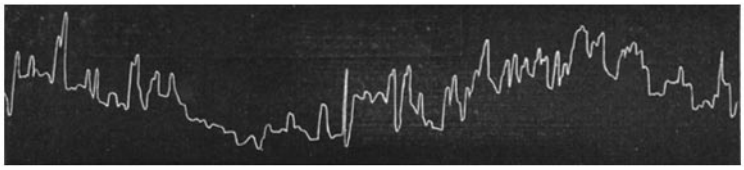


FIG. 3.

FIG. 4.

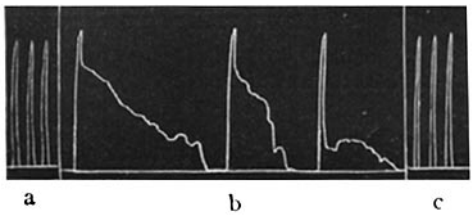


FIG. 5.

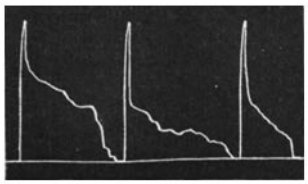
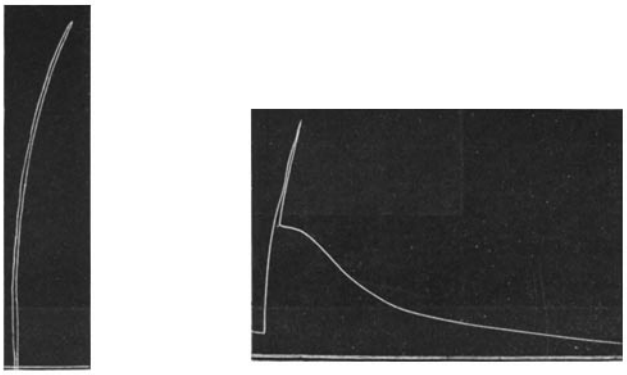


FIG. 6.

FIG. 7.



a b

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.