Physiology. — *"On the progress of the veratrin-poisoning of the striated frog-muscle"*. By ARIE QUERIDO. (Communicated by Prof. G. VAN RIJNBERK).

(Communicated at the meeting of October 28, 1922.)

1. *Concentration and dose.*

The nature of the action of veratrin on the striated muscular tissue still has not been sufficiently revealed, partly because of the lack of knowledge of the conditions, associating the poisoning. Repeatedly we read with various authors the remark, how fickle and incalculable the veratrin-phenomenon is in its appearance, seemingly independent of the quantity of poison used and the time it could act. It is true in 1904 MOSTINSKY [1]) examined the factors cooperating in the formation of a definite shape of curve and he succeeded in ascertaining the conditions incidental to this; the modifications however of these conditions in the course of an experiment, i. e. the alterations during the poisoning of the balance between muscle-metabolism and poison-action of which the curve is a result, are unknown as yet. Closely connected with this is the question, in what way the shape of the curve corresponds with the rate of poisoning of the muscle. On this subject we have some information, that is two types of contraction-shape are distinguished, viz. the type with two and with one top (fusion type), the latter of which corresponds to a stronger rate of poisoning (BOEHM [2]), DEELMAN [3])).

In order to study these questions further, I irritated muscle-nerve-preparations, after their immersion in a veratrin-Ringer-solution, by induction-shocks with so long a pause between the stimulations, that the influence of a contraction on the following need not be taken into account (three minutes).

In this way I collected a great number of curves of veratrin-poisonings for different concentrations of the poison. On contemplating the modifications in the veratrinogram, we can get an idea of the relation between curve and rate of poisoning, for if a poisoning is seen to progress in the direction of a diminishing or vanishing

[1]) Arch. f. exp. Path. u. Pharm., 51, 1904,

[2]) Idem 71, 1913.

[3]) Contrib. to Biology from the Amsterdam University 1914—15.

poison-influence, proved by the final appearance of normal, single, rapid contractions, we see, before this stage is reached, the second shortening becoming lower, of a shorter duration and appearing after a longer latent period; conversely it follows that a strong poisoning will be expressed by a high, prolonged, second shortening, having a short latent period and that the "fusion type" indeed corresponds with a stronger rate of poisoning than one with two tops, for with the former the latent period has reached its minimum, i.e. has grown equal to that of the first shortening; moreover the height is greater than that of a non-fusion second top. These magnitudes therefore, which may be expressed in the corresponding magnitudes of the first contraction, give a relative standard, holding for each separate muscle during the course of an experiment, for the poisoning at the moment of contraction, enabling us to picture to ourselves the progress of the poisoning, without our being dependent on the direct result, viz. the shape of the curve.

On studying the poisoning-process in this way, we notice in the series of curves peculiar differences, dependent on the concentrations, in which the poison has been applied.

1. In concentrations of 1 : 1000 and higher the muscle contracts as soon as it is brought into touch with the solution and maintains that shortening. On being stimulated the muscle shows either a very

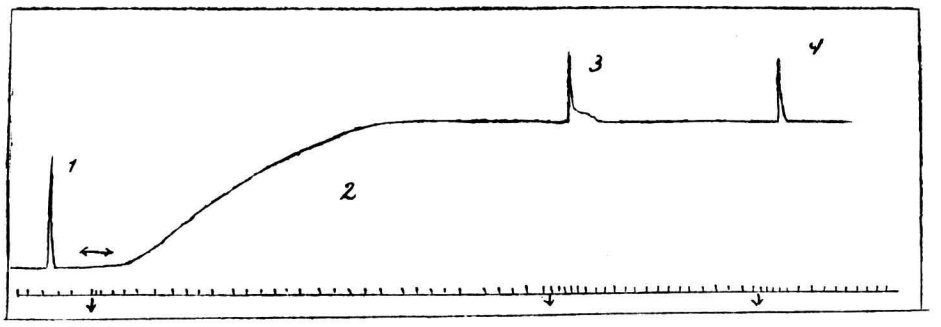

Fig. 1.

Experimental-process, when a veratrin-Ringer solution 1 : 1000 is poured on a muscle-nerve-preparation.

1: Contraction before poisoning: Af ←→: pouring on the solution. 2: subsequent contraction of the muscle; 3 and 4: contraction after electric stimulation, three, resp. six minutes after application of the solution: at ↓ the cylinder stopped. Time $^1/_5$ sec.

slight veratin-effect, or there is no result at all of the veratrin-poisoning, and the concentration is undistinguishable from the contraction yielded by an unpoisoned muscle on single stimulation.

(Fig. 1). This reaction is soon succeeded by complete insensibility for stimulation.

2. If the muscle has been put into a veratrin-solution weaker than 1 : 1000, but stronger than 1 : 100000 a series of curves is obtained, of which either the first or a following gives the strongest picture of the typical veratrin-poisoning, after which this effect diminishes till it finally disappears, so that the muscle, just as before the poisoning, responds to the stimulation with a single, rapid contraction, if at least it has not become insensitive, before this stage has been reached.

3. If solutions of 1 : 100000 and weaker are employed, a definite effect of the veratrin-action is obtained, which can maintain itself for hours together when the preparation is regularly stimulated.

There are three hypotheses which might explain the process described sub 1 and 2.

A. When the muscle has absorbed a certain quantity of poison and gradually diminishes the effect of this by its contractions — no matter how this happens — it is no more able to stand the influence of veratrin again.

B. The quantity of poison in the solution is not sufficient to supply the quantity abolished by the muscle.

C. In the period between two contractions the muscle modifies its character in such a way, that it grows less sensitive to veratrin-influence.

Hypothesis *A* may be omitted: a muscle once poisoned by veratrin can very well be influenced by veratrin-action again, after the veratrin-effect has been abolished by repeated contracting (e.g. by frequent stimulation), as the experiment teaches.

Hypothesis *B* may also be omitted, because VON FREY'S [1]) experiments show, that minimum quantities are already sufficient to poison a muscle. Therefore the hypothesis remains, that the muscle alters its character in the period of time between two stimulations, a modification which can only be attributed to the action of veratrin, for if all circumstances are left unchanged and only the veratrin-concentration is altered, a definite rate of poisoning occurs, which appears to be constant (third process).

Evidently there exists, besides the veratrin-effect on the striated muscle, causing the well-known second shortening, another action, having an unfavourable influence on the effect first-mentioned, and causing a rapid and exhaustive effect in strong concentrations, in

[1]) Sitzungsber. der Physik.-Med. Geselsch., Wurzburg, 1912.

less strong ones a slow and gradual effect; while below a certain concentration it can no more occur.

If the poisoning-process in a calf-muscle, which is left in situ is studied here — again with a stimulation-interval of three minutes — the process mentioned sub 1 is never observed, because the veratrin-concentration in the blood never reaches a sufficient height. On employing large doses (e. g. 15 mgr. per 50 Gr. frog) the heart is arrested after a short time as BOEHM [1]) describes it and the muscle is in no other relation — not considering a more intensive contact with the veratrin-solution — than in a muscle-trough of KEITH LUCAS, filled with a solution of the concentration at which the process mentioned sub 2 occurs; the conduct of the muscle is indeed in absolute accordance with this. On using smaller doses (1—2 mgr. per 50 Gr. frog), the heart, at least during the first hours after poisoning, keeps beating, only gradually diminishing its frequency; consequently the quantity of veratrin carried to the muscle is steadily increased and it should be borne in mind, that when the veratrin-concentration exceeds a definite threshold, the second effect of veratrin mentioned above will make its influence felt, i.o.w. the poisoning will seem less intensive: conversely every contraction will abolish part of the veratrin-effect and it may be supposed that in this way interference takes place between the influence of the two factors, determining the effect of the rate of poisoning, viz. the application and the rendering inactive of veratrin, when their two causes, i.e. the heart-action and the lapse of time between two contractions, occur in a definite proportion. As a result of this interference a periodicity occurs in the poisoning-process, i.e. the effects of stronger poisoning (higher, more prolonged second top) vary with those of less strong poisoning. At length the regularity of these oscillations is interrupted, because the heart-action diminishes under influence of the effect of the poison and the relation above-mentiond exists no more.

A constant poisoning in a muscle in situ can only then be obtained when the poison is applied without interference of the heart, e.g. by subcutaneous muscular injection (BUCHANAN) [2]).

2. *Combination of veratrin and curare.*

DE BOER [3]) communicates the possibility of leaving only the second shortening by simultaneous application of veratrin and curare. He

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[1]) Arch. f. exp. Path. u. Pharm., 71, 1913.

[2]) Journ. of Physiol. 1899.

[3]) Contributions Amsterdam 1914—15 and Zeitschr. f. Biol. 65.

gives few particulars however, so that I did not think it superfluous to repeat this experiment. It appears that quite different processes may arise, dependent on the lapse of time between the application of the two drugs.

A. If veratrin is first injected and the application of curare is put off till a distinct veratrinogram appears, the curare-injection remains without perceptible effect, the veratrin-poisoning proceeds as usual.

B. If curare is injected either simultaneously with veratrin-or so short a time after, that the veratrin-effect has not yet become manifest in the shape of a curve, in the further course of the experiment a typical veratrinogram appears, which shows that the two parts are equally effected by curare, so that both of them diminish till complete indirect insensibility; on direct stimulation the muscle even then gives a typical veratrinogram.

C. If veratrin is applied, if there is already an outspoken curare-poisoning, no veratrin-effect is shown, the poisoning behaves as a common curare-action till complete indirect insensibility.

D. If veratrin is injected while there are slight effects of the curare-action — it is of course impossible to mention objective data on this subject — in the further progress a veratrinogram appears with a usually very striking second top, wich is afterwards modified into a normal-looking veratrinogram, which further behaves as such.

E. Finally veratrin may be injected between the stages *C* and *D*; then there arises neither a rapid contraction nor a veratrinogram, but a muscle-contraction, which should be identified the second shortening of the veratrin-curve. On direct stimulation there is also formed a typical veratrinogram in that case. (Fig. 2). The further process may lead to complete indirect insensibility, or to the fact that before this slow contraction there occurs a rapid one, causing another typical veratrinogram. In shape the shortening thus obtained is identical to the second contraction of a veratrinogram, when this succeeds the first in isolated condition, as it is sometimes seen during a poisoning-process.

Examined on a quick-turning cylinder its latent period appears to be twice or four times as long again as that of a normal single contraction; no top is formed, the highest part of the contraction is a horizontal line; the crescent is much less steep than the decrescent; the duration amounts to one to four seconds.

3. *Temperature.*

As to the influence of temperature, I agree in general with BRUNTON

and CASH [1]), according to whom both high and low temperatures have an unfavourable influence on the veratrin-phenomenon.

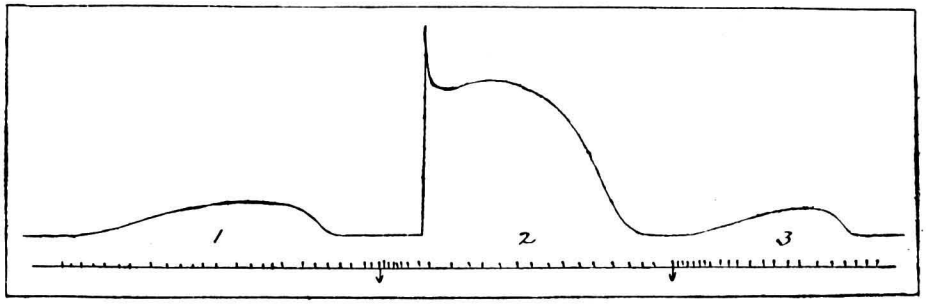


Fig. 2.

Combined action of veratrin and curare; 1 and 3: contraction on indirect stimulation; 2: contraction on direct stimulation; period between contractions: three minutes; at ↓ the cylinder stopped. Time $^1/_5$ sec.

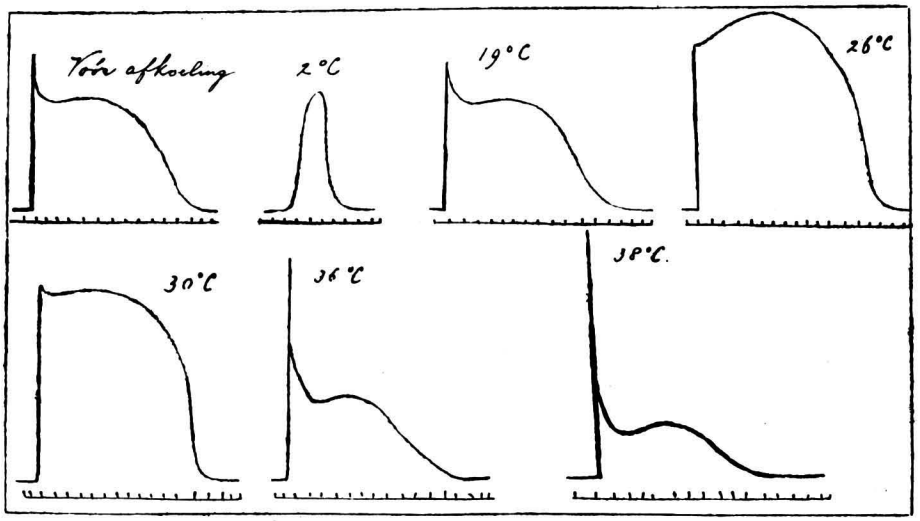
Here too a number of details are to be observed with respect to the modifications, the veratrinogram undergoes at various temperatures.

If a frog is cooled to 4° C. or lower and a veratrin-injection is given after that, no poisoning-effect is observed; the muscle behaves as an unpoisoned, cooled muscle, giving a relatively long and low contraction on induction-irritation. If the frog is subsequently heated, the second shortening gradually appears, first rapidly and of a short duration; above 14° C. the normal veratrinogram appears; conversely if a frog already poisoned is cooled, the second shortening disappears in quite the same way as it appears in the reverse experiment. Here too the cooled muscle behaves like an unpoisoned one. On heating above room-temperature the second shortening is seen to increase (in height as well as in duration). The first also increases its height as the contraction of an unpoisoned muscle would do, the second however increases more rapidly and consequently soon ecxeeds the first in size, so that a "fusion" type of curve arises.

At about 30 degrees the second shortening still increases in size, now however the first grows more rapidly and at ± 36° the second shortening begins to decrease also absolutely, the first behaves exactly as thecontraction of an unpoisoned muscle would do; till the muscle has become insensitive in consequence of heat-stiffness, there is still some veratrin-effect left. (Fig. 3). All this occurs quite independently of the poisoning-process; from every temperature with its corresponding

[1]) Journ. of Physiol. 1883.

curve-shape, we can return to room-temperature and see a typical veratrinogram arise.



Voor afkoeling = Before cooling.

Shapes of veratrinogram, yielded by one muscle at various temperatures. Time $^1/_5$ sec.

4. *Strength of stimulus.*

I have not succeeded in exercising an influence on one of the two parts of the veratrinogram separately by means of the strength of the stimulus. If the strength of the stimulus is gradually diminished, we may observe as MOSTINSKY [1]) describes, the critical progress of the excitability of the veratrin-muscle, i.e. below a definite limit, which is very exact, no reaction occurs on irritation, above this limit a reaction, differing but little from the maximal; moreover this always is a complete veratrinogram.

„A more detailed research concerning the problem of veratrin will appear in the „Archives de Physiologie Nêerl.""

[1]) loc. cit.