# THE EFFECTS OF SUBMINIMUM DOSES OF STRYCHNINE IN NEPHRECTOMIZED RABBITS.

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(*A Study from the Rockefeller Institute for Medical Research.*[1])

By whatever method poison is introduced into the animal body, the general effect depends upon its presence within the circulating blood. The quantity present at any time is regulated by the degree of absorption of the poison into and its elimination from the blood. With a given dose of poison the effect is greater the quicker it reaches the circulation, or the more slowly it is eliminated from it. A dose which is ineffective when introduced into the alimentary canal, may produce a maximum effect when given by subcutaneous injection, or a dose which fails to produce a reaction in a single subcutaneous injection may, owing to better facilities for absorption, become distinctly effective, as was shown by Meltzer,[2] when divided into several—three or more—parts and injected into separate areas.

On the other hand, poor absorption may be compensated by restricted elimination; curare, for instance, is ineffective from the stomach. Claude Bernard and L. Hermann[3] have shown, however, that this was not the case when the ureters or the blood-vessels of the kidney are ligated. In a normal animal the degree of elimination of curare by the kidney is sufficient to keep the amount of poison within the blood below the effective minimum when its absorption is as slow as is that from the mucous membrane of the stomach. But even with this moderate absorption the amount of curare within the blood will sooner or later reach the effective dose

[1] This study was made in the Department of Pathology of the College of Physicians and Surgeons of Columbia University.

[2] Meltzer, *Jour. of Exp. Medicine*, 1901, v, p. 643.

[3] L. Hermann, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1867, p. 64.

as soon as there is no longer an eliminating power sufficient to prevent its accumulation.

These principles which were set forth by Claude Bernard, L. Hermann and others, and were recently pointed out again by E. Harnack [4] and other writers, have obviously their application also to poisoning by strychnine, the elimination of which takes place chiefly through the kidneys. M. Adam [5] discovered in a case of human poisoning the appearance of strychnine in the urine nine minutes after its injection, and Ipsen [6] states that in dogs it can be found in the urine five minutes after subcutaneous injection, and in rabbits even after two minutes.

Dragendorff [7] has shown that all the strychnine leaves the body unchanged, and Kratter [8] could, even with such a small medicinal dose as 2 mg., recover the entire quantity from the urine.

It seems, therefore, quite well established that the body of an ordinary animal, if it survives the administration, does not retain any strychnine, and nearly the entire elimination takes place through the kidney.

If the subcutaneous administration be accomplished very slowly, even more than a fatal dose may be injected without causing any characteristic effect, which is explained on the assumption that in this case enough strychnine is eliminated by the kidney to prevent the accumulation in the blood of an effective dose.[9] The reverse of this principle, i. e., that the restriction of elimination by the kidney will increase the effect of strychnine, has not yet, so far as we know, been seriously tested by experiment, but is apparently taken as self-evident. This at least is implied in an experimental argument by Carrara on the question of the ability of the animal tissues to render inert (entgiften) strychnine.

[4] E. Harnack, *Münch. med. Wochenschr.*, 1896, p. 1065.

[5] M. Adam, in T. and A. Husemann's Handbuch der Toxicologie, p. 510. Berlin, 1862-7.

[6] Ipsen, *Vierteljahrschr. f. gerichtl. Med.*, 1892, iv.

[7] Dragendorff, *Beiträge zur gerichtlichen Chemie*, iii, St. Petersburg, 1872.

[8] Kratter, *Wien. med. Wochenschr.*, 1882, pp. 214 et seq.

[9] Harnack, loc. cit.

von Czyhlarz and Donath,[10] who have made an investigation of the last mentioned question, believe that they have proven it in the affirmative by the following experiments:

The leg of a guinea-pig was tightly ligated and a fatal dose of strychnine was injected into the leg peripheral to the ligature. When the ligature was removed after a few hours, the animal survived without showing any effects of strychnine. The authors explained the result by the assumption that the tissues of the leg had fixed the poison.

In a series of experiments, Meltzer and Langmann[11] have shown that the results are positive only when minimum doses are used, and that in their opinion the most probable explanation of the fact is that this prolonged application of a tight ligature affects the absorbing capacity of the tissue of the leg to such a degree as to permit, after the removal of the constriction, of only very little absorption of strychnine at a time. This process is similar to that belonging to very slow injections, the continual elimination through the kidneys preventing the accumulation of strychnine in the blood to an effective dose. Against this interpretation, Carrara[12] brought forward an apparently very striking experiment. He repeated successfully the experiment of Czyhlarz and Donath on guinea-pigs, the kidneys of which were removed, and he argues that, if the absence of the effects of strychnine in the ligatured animals be due to slow absorption, the effect ought to appear in the nephrectomized animals in whom the strychnine cannot be eliminated, and consequently must accumulate in the blood to an effective dose. Here it is presupposed as a matter that is self-evident, that in a nephrectomized animal subminimum doses will soon aggregate within the blood to an effective dose, simply because an eliminating organ is removed.

It seemed to us, however, that this supposition, though very plausible and generally acknowledged, should not be accepted with-

[10] v. Czyhlarz and Donath, *Centralb. f. inn. Med.*, 1900, p. 321.

[11] Meltzer and Langmann, Ibid., 1900, p. 992, and *Medical News*, 1900, lxxvii, p. 685.

[12] Carrara, *Centralb. f. innere Med.*, 1901, No. 20.

out a direct test. Could it not be the case that after the removal of the kidneys other organs assume the task of elimination? This hypothesis is not new. Urea, for instance, is under normal conditions eliminated through the kidneys. We know, nevertheless, that in advanced destructive renal diseases urea is excreted through all kinds of organs, even through the lungs and the skin. Thus we have been led to make a series of experiments with the view of testing the validity of the principle in question, at least so far as strychnine is concerned. In other words, we have tried to ascertain whether repeated injections of subminimum doses, separated by shorter or longer intervals, become effective as soon as the sum total reaches an effective minimum.

Experiments were made on rabbits, which are very sensitive to strychnine. The subcutaneous method was employed exclusively. With regard to the effective doses, distinction is to be made between a fatal dose (dosis lethalis) and a dose which is only poisonous (dosis toxica)—tetanic attack with recovery. In nearly all of the experiments nitrate of strychnine was employed in a solution of 1 to 1000. We have made a few control experiments on normal rabbits. From these experiments, as well as from the very many data in the literature on this subject, it can be stated that 0.45 to 0.5 mg. are toxic doses, while 0.55 to 0.6 mg. are fatal doses of strychnine nitrate per kilo of rabbit in a single injection. Once in a while an exception is met with, when even such a single dose as 0.3 mg. per kilo produces a tetanus. But it hardly ever happens that 0.6 mg. per kilo fails to cause a fatal tetanus. The time between the injection and the outbreak of convulsions varies inversely as the dose; i. e., the larger the dose the shorter the interval; fifteen minutes is the average time for minimum doses, but it may vary from 4 to 40 minutes. In normal rabbits we have rarely seen a tetanus appear later than 30 minutes, and never after 40 minutes. In our experiments we relied on convulsions as the characteristic reaction; hyperæsthesia alone, especially in rabbits, is not characteristic enough to serve as a basis for comparison.

Knowing that in rabbits strychnine appears in the urine even a

few minutes after hypodermic injections (2 minutes, Ipsen), and knowing further that it takes from 10 to 15 minutes before a reaction sets in after a minimum dose, we may safely state that the quantity of the minimum dose of strychnine required to be present within the blood to call forth a reaction is equal to the amount absorbed minus the amount eliminated in the interval between injection and reaction. When we now remove the kidneys—the main path of elimination—we might expect that the minimum dose for the blood will be attained by the absorption of an amount which is perceptibly less than the normal amount of absorption; in other words, in nephrectomized animals we might expect to bring out a reaction with a smaller dose than is required in normal rabbits.

Our experiments did not confirm these expectations. The following is a table of some of the experiments with single injections in nephrectomized rabbits:

| No. of exp. | Hours after nephrectomy. | Dose per kilo injected. | Results. |
|---|---|---|---|
| 1 | 26 | 0.45 mg. | Slight short convulsion after 64 minutes; recovered. |
| 2 | 1 | 0.5 mg. | Tetanus after 7 minutes; recovered. |
| 3 | 3 | 0.5 mg. | No reaction. |
| 4 | 23 | 0.5 mg. | Tetanus after 15 minutes; recovered. |
| 5 | 24 | 0.55 mg. | Fatal tetanus after 15 minutes. |

Comparing these results with the data above given for normal animals, we see that the required minimum doses in nephrectomized animals are not smaller than those for normal ones; on the contrary, there is a suggestion rather that the nephrectomized animals require a somewhat higher dose. However, the difference is too small and not constant enough to merit a further discussion. But it remains a noteworthy fact, that the removal of the kidney does not decrease the required amount of the minimum dose.

The most striking result, however, we obtained in experiments in which we employed subminimum doses repeated at shorter or longer intervals. Assuming that any dose of strychnine which cannot be eliminated by the kidneys remains in an effective state within the blood, which is the prevailing view, we might expect, no matter how small the doses and how long the intervals between the injections,

that, as soon as the sum total should reach the minimum of 0.45 or 0.5 mg. per kilo, there ought to occur immediately a tetanic outbreak, and that as there is no elimination, the tetanus ought to repeat itself indefinitely. Furthermore, it might appear that when the sum total reached the dose of 0.6 mg. per kilo, the first tetanic outbreak ought to terminate fatally. The results of our experiments, however, were entirely at variance with these expectations. On account of the importance of the results, we shall illustrate them by a number of protocols.

*Exp. 6.* Weight of rabbit, 1550 grm., double nephrectomy, Aug. 14, 1901, 12 M.

Aug. 14, 4.45 P. M., 0.3 mg. strychnine per kilo.
" 15, 12.45 P. M., 0.3 mg. " "
" 15, 4 P. M., 0.3 mg. " "
" 16, 1.30 P. M., 0.2 mg. " "
" 16, 5 P. M., 0.2 mg. " "

Rabbit died Aug. 17, at 10 A. M., 17 hours after the last injection, without having had convulsions. This rabbit then had received within 48 hours 1.3 mg. per kilo—that is, more than twice the fatal dose—without exhibiting any effects of strychnine poisoning.

*Exp. 7.* Rabbit, 1520 grm., double nephrectomy, Aug. 14, 12.30 P. M.

Aug. 14, 4.45 P. M., 0.3 mg. strychnine per kilo.
" 15, 12.45 P. M., 0.3 mg. " "
" 15, 4 P. M., 0.3 mg. " "
" 16, 1.45 P. M., 0.2 mg. " "
" 16, 4 P. M., 0.2 mg. " "
" 17, 9.30 A. M., 0.2 mg. " "

The animal was alive the same afternoon; was found dead the next morning. This rabbit received 1.5 mg. per kilo without any reaction.

*Exp. 8.* Weight of rabbit, 1900 grm.

Aug. 12, 1 P. M., double nephrectomy.
" 12, 4.20 P. M., 0.3 mg. per kilo.
" 13, 1 P. M., 0.3 mg. "
" 13, 5 P. M., 0.3 mg. "
" 13, 8 P. M., 0.2 mg. "
" 14, 12.30 A. M., 0.2 mg. "
" 14, 8 A. M., 0.2 mg. "

The rabbit died Aug. 15, at 10 A. M., or 26 hours after receiving the last dose of strychnine. This animal, too, had 1.5 mg. strychnine per kilo in the blood without showing any reaction.

*Exp. 9.* Weight of rabbit 1250 grm.

July 31, 3 P. M., double nephrectomy.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aug. | 1, | from | 1 | to | 2 P. M., | injected slowly, 0.43 mg. | per kilo. |
| " | 1, | " | 3 | to | 4 P. M., | 0.2 mg. | " |
| " | 1, | " | 5 | to | 6 P. M., | 0.2 mg. | " |
| " | 1, | " | 7 | to | 8 P. M., | 0.2 mg. | " |
| " | 1, | " | 9 | to | 10 P. M., | 0.25 mg. | " |
| " | 2, | " | 7 | to | 8 A. M., | 0.32 mg. | " |

The rabbit died Aug. 3, at 8.30 A. M., or 24 hours after the last dose of strychnine had been injected, without any reaction. He received altogether 1.6 mg. per kilo of strychnine.

*Exp. 10.* Aug. 20, double nephrectomy, 4 P. M.

At 5.45 P. M. started giving hourly injections of 0.2 mg. per kilo until 11.50 P. M., altogether seven injections, 1.4 mg. per kilo, without any reaction.

Aug. 21 at 9.40 P. M. again hourly injections of 0.2 mg. per kilo, 20 minutes after the 3rd (10th) injection, convulsions occurred: the animal recovered soon, but died on the next day without having had convulsions again. This animal without kidneys received 1.4 mg. per kilo within seven hours without any reaction, and with more than 2 mg. within its body recovered easily and lived 24 hours longer without any strychnine symptoms.

*Exp. 11.* Rabbit, 1150 grm. Double nephrectomy finished at 11.30 P. M., Sept. 15, 1901. From 3 P. M. on hourly injections of 0.2 mg. per kilo, given five times; the last one at 9 P. M. The animal was observed for 2 hours longer; no reaction.

Sept. 16, 3 P. M., injected 0.3 mg. per kilo; no effect. 8.55 P. M., injected 0.4 mg. per kilo; 50 minutes later the animal had a few convulsions, recovered entirely after 20 minutes.

Sept. 18 at 7 A. M. injected 0.3 mg. per kilo without effect. At 2 P. M. again 0.3 mg. per kilo; no effect. Rabbit died on the morning of Sept. 19, without having had convulsions again.

*Exp. 12.* Rabbit, weight 2175 grm. Double nephrectomy, Sept. 14, 4 P. M. From 7 P. M. an injection hourly, 0.2 mg. per kilo; 10 minutes after the fourth injection a slight convulsion occurred; the animal recovered entirely in less than 2 minutes. Again injected at 1, 3, 5 and 8 P. M., 0.2 mg. per kilo without any response. Sept. 16 at 3 P. M.

injected 0.3 mg. per kilo with no effect. At 9 P. M. injected again 0.4 mg. per kilo; 35 minutes later tetanic manifestations occurred and the animal succumbed very soon.

We shall not, of course, report in detail all our numerous experiments. The few protocols which we have quoted are sufficient to indicate the correctness of the main fact which we wish to bring out here, namely, that rabbits without their main eliminating organs, the kidneys, can nevertheless tolerate the sum total of twice and thrice the fatal dose of strychnine without showing any reaction, if only care is taken to employ proper subminimum doses at not too short intervals. From a review of all our experiments we gather the following details: If the doses do not exceed 0.3 mg. per kilo at intervals of not less than four hours, injections can be made apparently indefinitely without causing any reaction. The same applies apparently also to doses of 0.2 mg. per kilo at intervals of about three hours; with larger doses or shorter intervals, the injections sooner or later induce a tetanus. How large a dose the sum total can attain before it becomes effective, varies with different animals. For instance, with a subminimum dose of 0.2 mg. per kilo in hourly injections, in some animals eight consecutive injections—with a sum total of 1.6 mg. per kilo—could be administered before a tetanus occurred or even marked hyperæsthesia was noticed. In other animals an effect appeared some time after the fourth injection, with a sum total of 0.8 mg. per kilo. However, in all cases the total exceeded the fatal minimum dose for the normal animal, and what is more, very rarely did any of these sum-total doses cause a fatal tetanus. If the injections were discontinued the animal recovered and survived the last injection for twenty-four hours or more without having another convulsion.

Leaving the statement and discussion of other details which we have observed in our present line of experimentation for some future occasion, our experiments brought out the following points:

1. For a rabbit without its chief eliminating organs, the kidneys, the minimum toxic and fatal doses of strychnine are, nevertheless, not smaller than those for the normal animals.

2. Even in a rabbit without its kidneys a single cumulative toxic dose of strychnine induces only one attack, or a few, and the animal soon recovers and shows no further effects of strychnine. Here a toxic dose remains apparently within the body without causing a continual effect.

3. If proper subminimum doses at proper intervals are employed nephrectomized rabbits can gradually receive thrice the fatal dose of strychnine without showing any reaction. Large fatal doses of strychnine are apparently accumulated within the body without causing any effect.

How are these remarkable facts to be interpreted? What becomes of the single toxic dose and of the cumulative fatal doses of strychnine within the body? Several explanations are possible. Thus it may be said that:

1. After the removal of the kidneys the act of elimination is carried on by other organs, for instance, by the gastro-intestinal canal. We know that secreting organs can substitute one another. We may then assume substitution also among excreting organs. An instance of this was mentioned above in the elimination of urea. It may be that even normally many organs are endowed with excretory mechanisms which have no opportunity to come into play so long as the great outlet through the kidneys, the excretory organs par excellence, is unimpaired. Collateral circulation after obstruction of the main artery transforms into large vessels fine capillaries which were previously hardly noticeable.

2. It is possible that strychnine is destroyed within the circulation by the blood, liver, etc. Before it was firmly established that strychnine is eliminated by the kidney, there were many theories as to the decomposition of strychnine within the blood. Lately the assertion was made anew by von Czyhlarz and Donath [13] and others, that blood cells, liver cells, and other tissues are capable of neutralizing strychnine. For the normal human body it has been established beyond doubt that none of the strychnine becomes decomposed by the tissues. However, it may be different in a nephrectomized animal. Falk [14]

[13] von Czyhlarz and Donath, *Ztschr. f. Heilkunde*, 1901.

[14] Falk, *Centralbl. f. med. Wiss.*, 1899, p. 481.

has shown that in chickens, which are very resistant to strychnine, 90 per cent of the poison is decomposed by the tissues, and he suggests that this may be connected with the fact that there is very little liquid urinary secretion in these animals. This suggestion would hold good also for a nephrectomized animal.

3. It is further possible that within the blood of nephrectomized animals substances develop which do not decompose strychnine, but neutralize its effect upon the nervous system. Uræmic coma might be mentioned in this connection, indicating that there are indeed in the blood of animals with impaired kidneys substances which affect the functions of some nerve cells.

4. It is also possible that in nephrectomized animals absorption from the subcutaneous tissues is impaired on account of the increased blood pressure. This, however, would not explain why a toxic dose, already within the blood, does not continue to be effective. Besides, we found that a single minimum dose for a normal animal is also effective for the nephrectomized animal, which shows that absorption cannot be impaired, so that non-absorption cannot be the chief reason for the failure of the large doses of strychnine in cumulative administrations to cause some reaction.

All these hypotheses are open to experimental study which will be carried out at some future time.

We started out to test the generally acknowledged assumption, implied in the argument of Carrara, that when the kidneys, the chief eliminating organs for strychnine, are removed, subminimum doses will be effective as soon as the sum total reaches the effective toxic or fatal dose. We have shown that this assumption is incorrect, and that even thrice the fatal dose can be gradually introduced without producing any effect.

The experiments teach us also a general lesson in experimental science. Because we know that a certain organ has a certain function, it is commonly assumed that we abolish the function by removing the organ. We have seen that this is not true. Elimination is the preventive of cumulation. After removal of the eliminating organ, it might have been expected that the cumulative effect would

remain unrestricted. We have found that cumulation was nevertheless manifested to only a slight extent. Then again, if an organ is removed and a function persists, it has been assumed that normally the function does not belong to this organ. In nephrectomized animals, cumulation is very little manifested; nevertheless, we know it is the eliminating kidney which controls cumulation in normal animals. This last remark applies to a statement made by Leube in connection with our subject. Leube[15] in attempting to establish (34 years ago) the path by which strychnine is eliminated, thought of the kidney, and tied the ureters or the blood vessels, in the expectation that a small dose, which causes a mild effect in a normal animal, would have violent results in the operated one. Leube was disappointed in his results. Not only was the effect not stronger, but in one animal a certain dose, which two days previous to the operation caused a mild effect, after the operation had no effect at all. Leube explained this case as an instance of adaptation, and gave up the kidneys as the eliminating organs. We know that there is no adaptation of the body to strychnine (Hare[16] and others). We also know now that the kidney is the eliminating organ for strychnine in normal animals, and Leube's error in his conclusion is just the one to which we referred above. What Leube found is the same that we have seen in our experiments, namely, that the minimum dose for nephrectomized animals is the same as for normal ones, if not even a trifle higher.

Our results have also direct practical bearings. It has been argued by physiologists and pharmacologists (L. Hermann for instance) and has been repeatedly maintained by clinicians, that in chronic diseases of the kidneys, when the eliminating power of this organ is considerably reduced, great care should be exercised in the administration of poisonous medicines, lest they may accumulate in the blood with fatal effects. According to our experiments with strychnine on animals entirely without kidneys, fatal doses may be gradually introduced without effect, and there is a great difference between even a

[15] Leube, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1867, p. 629.
[16] Hare, *Amer. Jour. of Physiology*, 1901, v, p. 333.

maximum medicinal dose and a minimum toxic dose. The animal body apparently possesses a mechanism capable of regulating the cumulative capacities of the blood even in the absence of the kidneys. The influence of removal of the kidneys on the cumulative effect of other poisonous substances has not yet been studied. Thus the fear of cumulative effect in renal disease rests at present apparently on theoretical grounds alone.