"On the Physiological Action of the Poison of the Hydrophidæ. Part II.—Action on the Circulatory, Respiratory, and Nervous Systems." By LEONARD ROGERS, M.D., B.S. (Lond.), M.R.C.P., F.R.C.S., I.M.S., late acting Professor of Pathology, Medical College, Calcutta. Communicated by Dr. A. D. WALLER, F.R.S. Received June 6,—Read June 18, 1903.

(From the Physiological Laboratory of the University of London.)

In my previous paper I dealt with the action of the poison of the Sea snakes as far as it was possible to examine it under the conditions of work in Calcutta, and reserved the consideration of certain questions until I was able to test them with the aid of a well-equipped laboratory. This I have now been able to do in the Physiological Laboratory of the London University, by the courtesy of Dr. A. D. Waller, with results which appear to be worthy of being placed on record in a further paper.

Blood Pressure and Respiratory Curves.

The effects of the poison on the blood pressure and on the rate and amplitude of the respirations have been studied by taking tracings of the former by a Gad's manometer and of the latter with Sandström's recorder, large but varying doses being administered intravenously in chloroformed cats and rabbits. The results uniformly showed a primary failure of respiration followed by a marked rise of blood pressure with the increasing venosity of the blood, respiratory convulsions (except when the respiratory failure was extremely rapid), and a final sudden fall of blood pressure some minutes after complete cessation of respiration. The general results obtained may be conveniently summarised in the following table :—

No.	Animal.	Dose per kilo.	Respiration failing.	Blood pressure rising.	Respiration ceased.	Convulsions ceased.	Blood pressure fell rapidly.
		mgrm.	min.	min.	min.	min.	min.
1....	Cat.....	1	6	8	12	8	12½
2....	Rabbit..	1	3½	6	9	10½	13½
3....	Rabbit..	2	2½	4	6	6	10
4....	Cat.....	2	3	4½	(?)	(10)	(22)*
5....	Rabbit..	4	1	1½	2½	Nil	3½

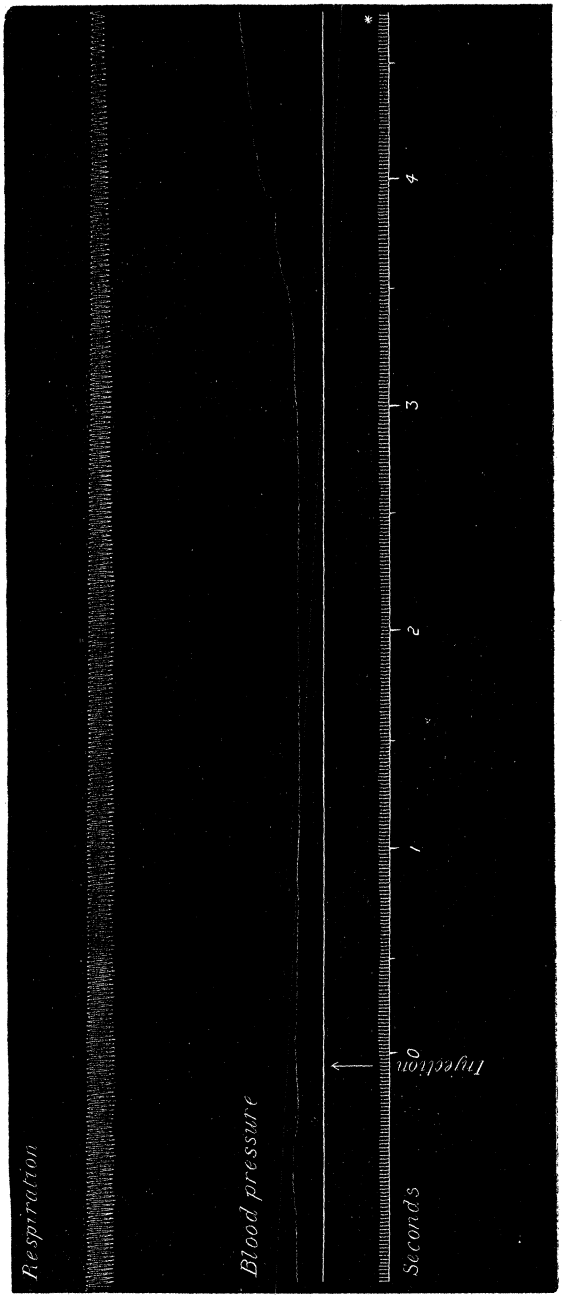
* Artificial respiration.

In the fourth experiment the onset of convulsions and the final failure of the circulation, as evidenced by the sudden fall in blood pressure, were both delayed by the use of artificial respiration. With this exception, it will be seen from the table that both the respiration and the circulation fail more and more rapidly as the dose of the poison is increased, until with a dose of 4 milligrammes per kilo. weight (1/250,000 of the body weight) the respiration was affected in 1 minute and had entirely ceased in $2\frac{1}{2}$ minutes, while the circulation failed in $3\frac{1}{2}$ minutes. The exact sequence of events can be best illustrated by the data and tracings of the following two typical experiments, being Nos. 3 and 5 in the above table.

Experiment 3.

Rabbit, weight $1\frac{1}{2}$ kilos., under chloroform. Cannula in the carotid artery, connected with a Gad's manometer. Respirations recorded with Sandström's instrument. 3 milligrammes (2 milligrammes per kilo. weight) of dried Enhydrina poison injected into the external jugular vein dissolved in 0·75 c.c. of 0·9 per cent. NaCl.

Time.	Blood pressure in mm. Hg.	Respirations per minute.	Amplitude of respirations.	Remarks.
			mm.	
Before injection ..	92	51	4	
After 1 min.	89	50	4	
„ 2 „	91	49	$3\frac{1}{2}$	
„ 3 „	94	46	3	Respiration failing.
„ 4 „	140	43	$2\frac{1}{2}$	Blood pressure rising.
„ 5 „	166	39	2	
„ 6 „	180	34	1	Convulsions beginning.
„ 7 „	200	21	$\frac{1}{2}$	Respiration ceased.
„ 8 „	195	0	—	Convulsions violent.
„ 9 „	166	—	—	
„ 10 „	90	—	—	Blood pressure falling.
„ 11 „	70	—	—	



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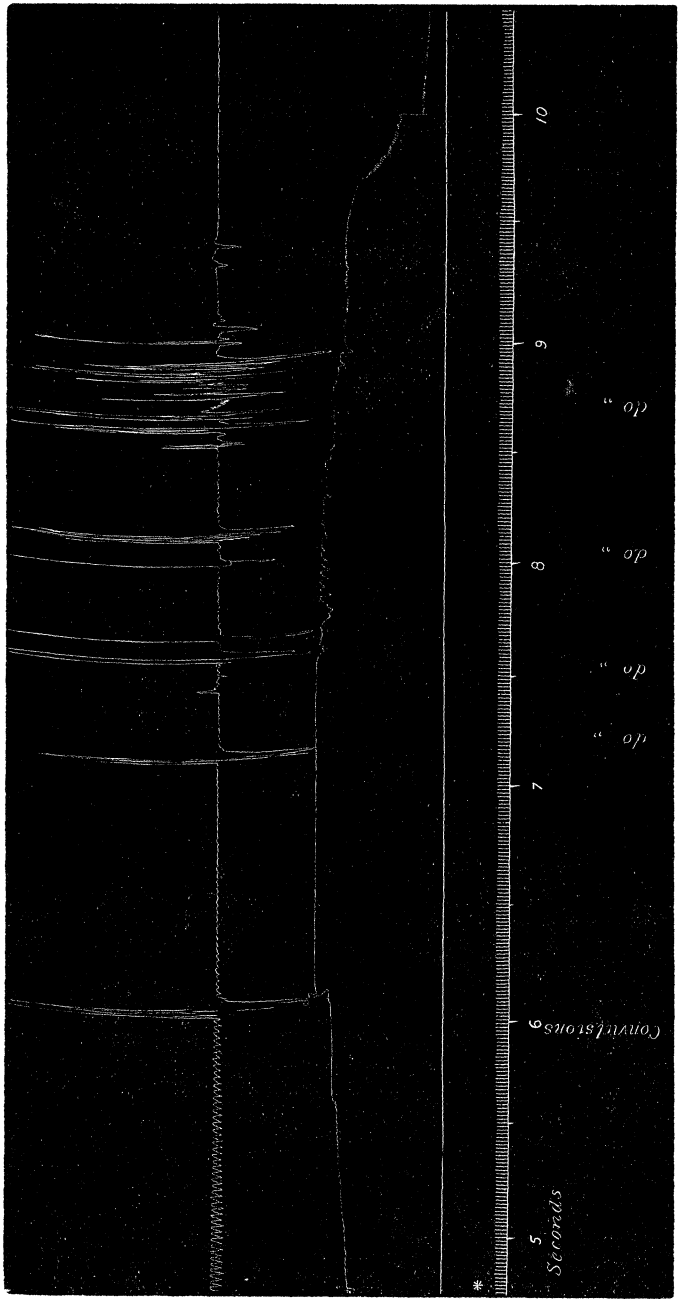


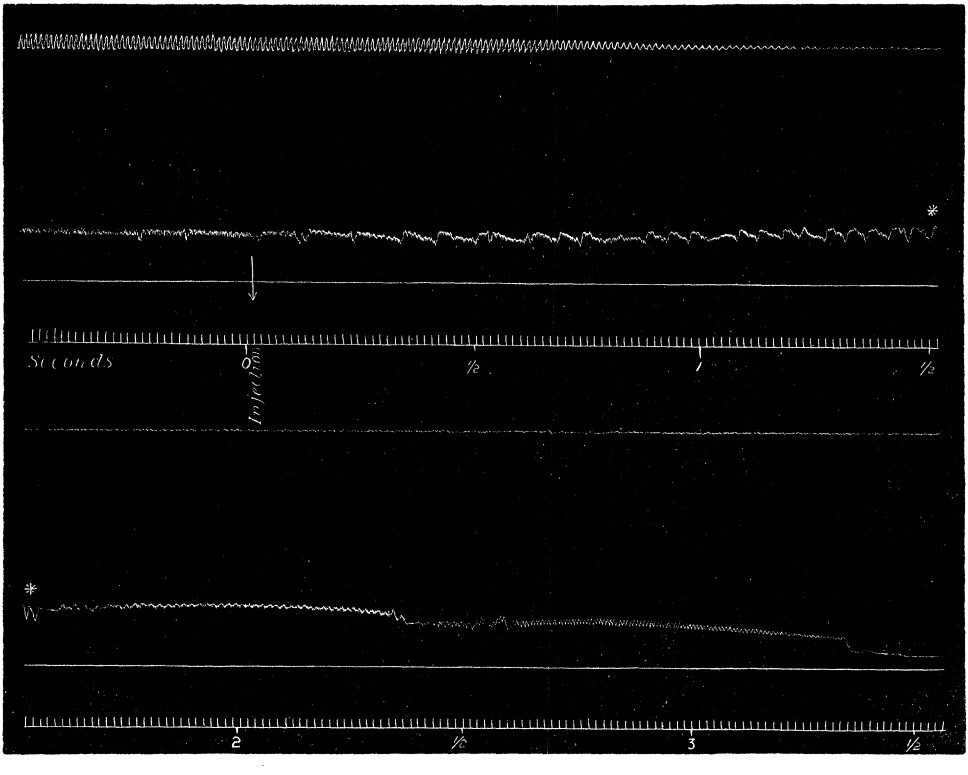
TRACING I (2ND HALF).

Experiment 5.

Rabbit, weight 1·7 kilos., under chloroform. Conditions the same as in the above experiment, except that 6·8 milligrammes (4 milligrammes per kilo. weight) was given intravenously.

Time.	Blood pressure in mm. Hg.	Respirations per ½ minute.	Amplitude of respirations.	Remarks.
			mm.	
Before injection ..	115	43	2	
After ½ min.....	115	44	2	
„ 1 „	115	38	1	Respiration failing.
„ 1½ „	120	32	½	Blood pressure rising.
„ 2 „	135	22	½	
„ 3 „	110	—	—	Respiration ceased.
„ 3½ „	82	—	—	Blood pressure falling.

TRACING II.



Both the curves of the two experiments above detailed show the same sequence of results, viz., primary failure of respiration accompanied by a rise of blood pressure, followed by a fall of the same some little time after the respirations have entirely ceased. There is one remarkable and important difference between them, namely, an entire absence of respiratory convulsions in the case of the last very rapid poisoning. This absence of convulsions may be due to the respiratory centre being so rapidly overwhelmed by the relatively enormous dose of poison injected direct into the circulation (for the amount used amounted to some 200 times the minimal lethal dose for a rabbit), that the centre was paralysed completely before the failure of the breathing had had time to render the blood sufficiently venous to produce respiratory convulsions. The comparatively slight rise of blood pressure occurring with the failure in respiration in this case as compared with comparatively large rise obtaining in the other four experiments agrees with the explanation just suggested.

Another possible explanation must, however, be considered; a paralysis of the end plates of the motor nerves, which, as we shall see presently is a marked feature of the action of the poison under consideration, might cut off the peripheral muscles from the action of the respiratory centre, in spite of its over-stimulation by venus blood. In order to test this possibility, the right leg, exclusive of the sciatic nerve, was ligatured before the poison was injected in Experiment 5, and the response of the nerves and muscles of both limbs to the interrupted induced current was tested immediately after the death of the animal, with the following result:—

	Distance of secondary coil.	Contraction of muscle.
Protracted limb, nerve	45 mm.	Good.
,, muscle.....	45 ,,	Good.
Poisoned limb, nerve	0 ,,	Nil.
,, muscle	45 ,,	Good.

Here we have a typical curara effect, the end plates of the poisoned limb only being completely paralysed. In this experiment it is therefore impossible to say how far the absence of convulsions is due to this cause and how far to failure of the respiratory centre. I shall return to this point further on, after the experiments on the action of the poison on the nerves have been related.

Direct Action on the Heart.

The next question to be dealt with is whether the poison of the Enhydrina has any direct action on the heart, which is so marked a feature in the case of Pseudechis poison,* and has also been noted in

* 'Roy. Soc. of New South Wales Proc.,' 1896.

a less marked degree by Brunton and Fayrer,* when large doses of Cobra venom are introduced directly into the circulation. (In small doses, subcutaneously administered, Cobra venom has very little action on the heart, which can be kept going for many hours after spontaneous respiration has ceased by means of artificial respiration, as shown by the Indian Snake Poison Commission.)†

I have examined this point by testing if the poison has any paralysing action on the heart of a pithed frog, tracings being taken of the contraction of the organ before and after the direct action of solutions of the poison of various strengths in normal saline solution. As a few drops of a 1-in-1000 solution of Enhydrina poison given *per venam*, and therefore further greatly diluted in the circulation, is very rapidly fatal, it is evident that the poison should produce a very marked action on the heart when directly applied to it if the lethal effect is in any degree due to cardiac paralysis. My experiments have shown that such is not the case, for a 1-in-1000 solution when directly applied to a vigorous frog's heart produced no appreciable effect in any of several trials; a 1-in-100 solution, similarly applied on two occasions, did not retard, still less arrest the action of the heart.

Effect of Artificial Respiration on the Blood Pressure and the Heart.

The absence of any direct paralytic effect of Endydrina poison on the heart was also shown by an experiment of another kind. As already mentioned, the heart can be kept going by artificial respiration for a very long time in Cobra poisoning, but this is not the case with poisoning with the venom of the Pseudechis; C. J. Martin‡ has shown that the heart fails within a very few minutes after cessation of spontaneous respiration, in spite of artificial respiration, in the case of the last-named snake poison, which also has a marked direct paralytic action on the heart. In the following experiment artificial respiration was started directly marked failure of respiration appeared and the blood pressure had begun to rise, and the effect of repeatedly stopping and recommencing it on the blood pressure was noted.

Experiment 4.

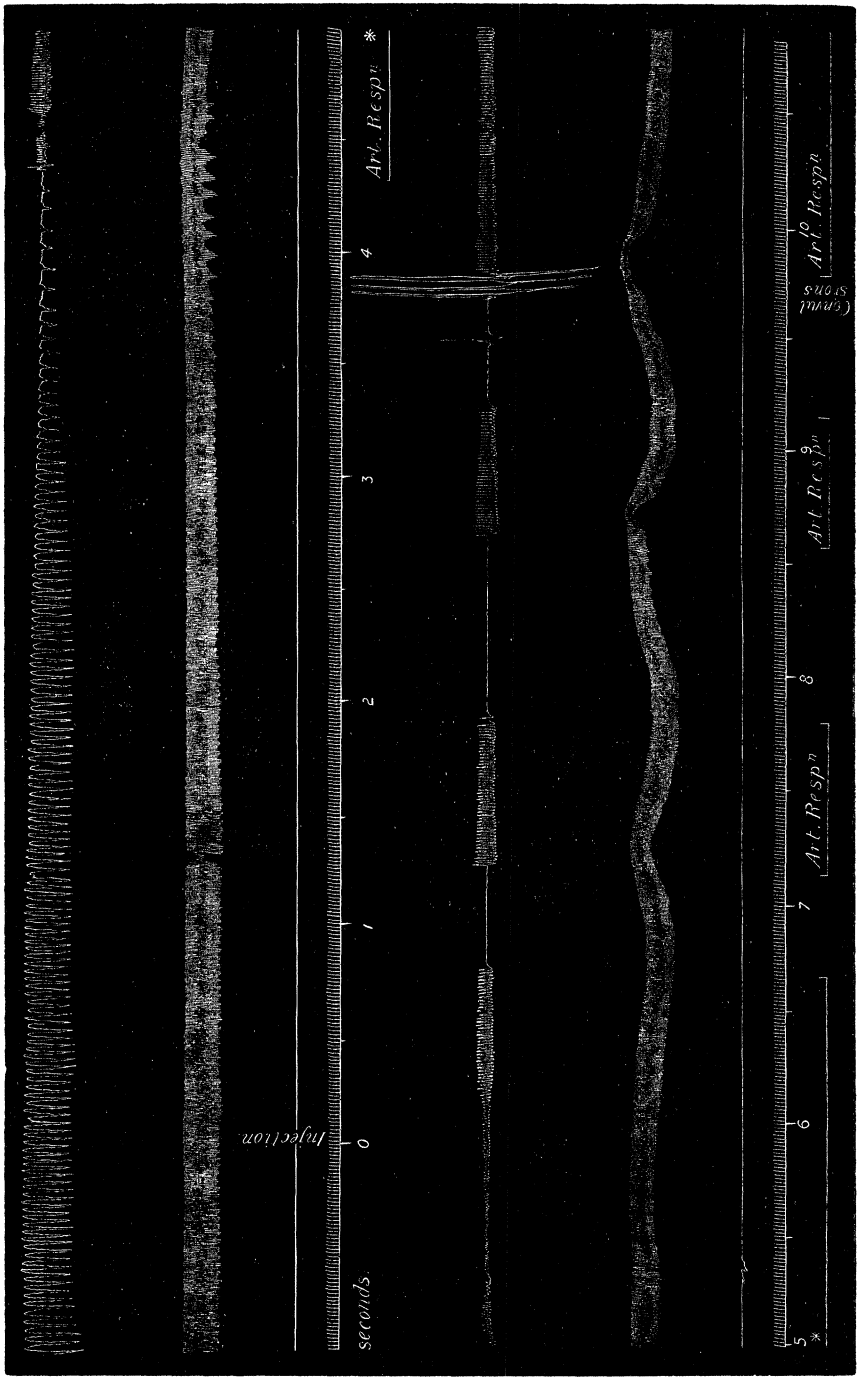
Cat, 3½ kilos., under chloroform. Cannula in the carotid artery, connected with a Gad's manometer. Respirations recorded with a Sandström's instrument. 7 milligrammes (2 milligrammes per kilo. weight) in 1·75 c.c. 0·9 per cent. NaCl injected into the jugular vein.

* 'Roy. Soc. Proc.,' vols. 21, 22, and 23.

† 'Indian Medical Gazette,' 1873, p. 119.

‡ 'Roy. Soc. of New South Wales Proc.,' 1896.

Time.	Blood pressure.	Respirations.		Remarks.
		No.	Amplitude.	
	Mm.		Mm.	
Before injection	160	28	7	
After 1 min...	160	29	$6\frac{1}{2}$	
„ 2 „ ..	160	27	$5\frac{1}{2}$	
„ 3 „ ..	160	24	4	Respirations beginning to fail.
„ 4 „ ..	170	16	2	Blood pressure beginning to rise. Artificial respiration commenced.
„ $4\frac{1}{2}$ „ ..	—	—	—	
„ $6\frac{3}{4}$ „ ..	150	—	—	Blood pressure fallen again. Artificial respiration stopped.
„ $7\frac{1}{2}$ „ ..	170	—	—	Blood pressure risen again. Artificial respiration resumed.
„ $7\frac{3}{4}$ „ ..	148	—	—	Blood pressure fallen again. Artificial respiration stopped again.
„ $8\frac{3}{4}$ „ ..	180	—	—	Blood pressure risen again. Artificial respiration resumed again.
„ $9\frac{1}{4}$ „ ..	150	—	—	Blood pressure fallen again. Artificial respiration stopped again.
„ $9\frac{3}{4}$ „ ..	185	—	—	Blood pressure risen higher than previously; convulsions commencing. Artificial respiration resumed.
„ $11\frac{1}{4}$ „ ..	140	—	—	Convulsions stopped; blood pressure fallen again. Artificial respiration stopped again.
„ $12\frac{1}{2}$ „ ..	195	—	—	Blood pressure risen, convulsions recommenced. Artificial respiration resumed.
„ $13\frac{3}{4}$ „ ..	160	—	—	Convulsions stopped again; blood pressure fallen. Artificial respiration stopped.
„ $15\frac{1}{4}$ „ ..	198	—	—	Blood pressure very high; feeble convulsions. Artificial respiration resumed.
„ $18\frac{3}{4}$ „ ..	140	—	—	Blood pressure fallen. Artificial respiration finally stopped.
„ 21 „ ..	190	—	—	Blood pressure high again, but no convulsions.
„ $22\frac{1}{2}$ „ ..	105	—	—	Final fall of blood pressure beginning.
„ 24 „ ..	70	—	—	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TRACING III.

After the final failure of the circulation the sciatic nerves were tested with an interrupted induced current, and stimulation of both the nerves and the muscles directly caused contractions, showing that the smaller dose of poison used in this experiment had not caused paralysis of the end plates, although after the final cessation of artificial respiration no convulsions followed the rise in blood pressure. This points to complete exhaustion of the respiratory centre, occurring before paralysis of the end plates, having been the cause of the absence of terminal convulsions.

The repeated lowering of the blood pressure and disappearance of the commencing convulsions following immediately upon the performance of artificial respirations go to show that the rise of blood pressure and the convulsions are secondary in nature to the paralysis of the respiratory centre, and due to the increasing venosity of the blood. Further, it is evident that the poison has no powerful direct paralysing effect on the heart itself, as is the case with Pseudechis venom. The result of the above experiment is also of interest in connection with one by Vincent Richards,* in which a dog was bitten by an Enhydrina, and artificial respiration was kept up for 24 hours and 35 minutes after the failure of respiration, sensibility being restored, and at the same time convulsions recurred, pointing to partial recovery of the respiratory centre from the condition of complete paralysis. Death finally occurred by accidental arrest of artificial respiration. In his experiment the dose given was a small one, as respiration did not cease until after two hours, but it is evident that the poison exerted no injurious action on the heart.

Action on Nerves and End-Plates.

Brunton and Fayrer† first showed that Cobra venom exerts a paralysing action on the muscles' end-plates like curara; this was confirmed by Ragotzi,‡ and the last-mentioned observer attributed the failure of respiration to paralysis of the end-plates of the diaphragm. We have already seen (Experiment 5) that a similar motor nerve paralysis may result from Enhydrina poisoning, so that it is necessary to inquire whether this is due to an effect on the nerve trunk or on the end-plates. If the nerve trunk is itself poisoned, so as to lose its power of conductivity, then the negative variation of the current of injury should be greatly reduced or entirely abolished by very dilute solutions of the venom. This has been tested by Dr. Waller's method by placing the sciatic nerves of frogs in dilute solutions of the venom, and measuring the negative variation of the current of injury with a galvanometer both before and after exposure to the poison. The nerves were first

* 'Indian Medical Gazette,' 1873, p. 119.

† 'Roy. Soc. Proc.,' vols. 21, 22, and 23.

‡ 'Virchow's Archiv,' vol. 122, p. 232.

placed in 0·83 per cent. NaCl for about two hours, this strength having been found recently by Dr. N. H. Alcock (to whom I am indebted for much help throughout this investigation) to be the optimum one for nerves.

The poison was used in strengths of from 10^{-6} to 10^{-3}, and the nerves were exposed to their influence for from 1—5 minutes in the case of the stronger solutions, and up to 1 hour in the weaker one, but with entirely negative results. In one experiment a 1 per cent. solution was used up to 5 minutes without any poisonous effect on the nerve being produced, although this is a stronger solution than I have ever used for injection. It is evident, then, that the poison of the Enhydrina does not produce paralysis by any direct action on the nerve fibres.

That it does act by paralysing the muscle end-plates, as in the case of Cobra venom, is shown by the following experiments on etherised frogs.

Frog. Weight 20 grammes. Etherised. Right thigh ligatured, excluding the sciatic nerve. 0·2 milligramme Enhydrina poison in 0·2 c.c. 0·9 per cent. NaCl injected into dorsal lymph sac. (10 milligrammes per kilo. = 20 minimal lethal doses.)

Respirations per minute.

Before injection	56	5th min.	19
1st min.	68	6th ,, ...	5
2nd ,, ...	64	7th ,, ...	9
3rd ,, ...	50	8th ,, ...	0
4th ,, ...	32	9th ,, ...	0

Respirations finally ceased. Heart still beating 4 hours later. After cessation of respiration the frog was pithed, and when the spinal cord was destroyed, the ligatured (protected) limb only showed contraction of the muscles. Both the sciatic nerves and leg muscles were then tested with the interrupted faradic current, with the following results:—

	Distance of secondary coil.	Contraction of muscle.
Protected limb, nerve	40 mm.	Good.
,, muscle	30 ,,	Good.
Poisoned limb, nerve	0 ,,	Nil.
,, muscle	30 ,,	Good.

This is a typical curara effect, and on proceeding to test the negative variation of the current of injury of the sciatic nerves of each limb, after placing them in 0·83 per cent. NaCl for 2 hours, both nerves were found to give it well, that of the poisoned limb being slightly the stronger of the two, probably owing to partial drying of that of the protected limb over the ligature.

The above experiment was repeated with a dose of 5 milligrammes

per kilo. weight, with a precisely similar result, including the presence of the negative variation of the current of injury in each sciatic nerve. In two more experiments doses of 5 and 1 milligrammes per kilo. respectively were injected without previous ligaturing of a limb, and in both cases stimulation of the nerves of each limb caused no muscle response, although they contracted when directly stimulated. In each case both nerves showed well-marked negative variation of the current of injury, proving that their conducting powers were intact, so that it is clear that the end-plates must have been paralysed.

Action on the End-Plates of the Phrenic Nerves.

The marked action on the motor end-plates of the poison of the Enhydrina once more brings it into line with that of Cobra venom, but on the other hand constitutes a marked difference from Pseudechis venom, which C. J. Martin showed had no such action. He also found that the stimulation of the phrenic nerves still produced normal contraction of the diaphragm after total cessation of respiration due to the latter poison.

In order to ascertain how far the paralysis of respiration produced by Enhydrina venom is due to paralysis of the respiratory centre, and how far, if at all, to poisoning of the motor end-plates of the phrenic nerves, the following experiments were performed.

Cat, weight $3\frac{1}{2}$ kilos., under chloroform. Tracheal cannula connected with a recorder inserted. Left phrenic nerve exposed in the neck, $3\frac{1}{2}$ milligrammes of Enhydrina poison injected into external jugular vein (1 milligramme per kilo.). Phrenic nerve stimulated by an interrupted induced current at intervals of one minute.

Occasional feeble inspirations produced by movement of the chest walls only, continued up to the 24th minute, when they finally ceased. At the 27th minute the final rapid fall of blood pressure to 50 mm. took place. The sciatic nerves were tested at this point, and the right when stimulated with the secondary coil at 30 mm. produced a good muscular response, as did the left with the secondary coil at $27\frac{1}{2}$ mm. It appears from this that the phrenic nerve was paralysed completely before any very marked loss of function of the sciatic nerves had taken place. The respirations, however, were very greatly reduced in both frequency and amplitude several minutes before any weakening of the phrenics had occurred, so that the first and most important action of the poison appears to be its effect on the respiratory centre, although the paralysis of the phrenics speedily ensues and is a very important feature of the action of the venom. If a very large dose is given, as in Experiment 5, then the end-plates of the muscles in general are also paralysed at the same time or very soon after the failure of the respiratory centre and the phrenics.

Time.	Blood pressure.	Respirations per minute.	Phrenic nerve.		Remarks.
			Coil at	Contraction of diaphragm.	
Before injection...	mm. 150	45	25 mm. only	Good.	
After 3 min.	165	41	,,	,,	
,, 6 ,,	169	33	,,	,,	
,, 9 ,,	170	17	,,	,,	
,, 12 ,,	170	10	,,	Slight.	
,, 14 ,,	150	8	,,	Nil.	
			20 ,,	Slight.	
,, 16 ,,	150	3	20 ,,	Nil.	Respirations nearly ceased, phrenics weakened.
			15 ,,	Slight.	
,, 17 ,,	100	Convulsions	15 ,,	Nil.	
			10 ,,	Good.	
,, 19 ,,	100	Do.	0 ,,	Nil.	Phrenics completely paralysed. Blood pressure falling.

The above experiment was repeated in a rabbit, with a precisely similar result to that just detailed, the respiratory centre failing first, quickly followed by paralysis of the phrenics, although the diaphragm still responded to direct excitation. The muscles of both limbs (one of which was ligatured before the injection of the poison) contracted well immediately after death to both direct stimulation and that through the sciatic nerves. In this experiment the respirations failed very rapidly, ceasing at the end of two minutes, and no convulsions ensued, in spite of the motor end-plates not being paralysed, so that in this instance the absence of convulsions could not be due to muscular paralysis, but only to complete paralysis of the respiratory centre.

Action of the Spinal Cord Reflexes.

In the case of Cobra poisoning Brunton and Fayrer showed that the spinal cord is paralysed from below upwards, the hind legs being first affected. C. J. Martin also found that a direct poisonous action on the spinal cord was produced by Pseudechis venom.

In order to test this point a frog was etherised, and after a ligature had been tied round the right thigh, excluding the sciatic nerve, a dose of 5 milligrammes per kilo. of Enhydrina poison was injected into the dorsal lymph sac, and the reflexes induced by stimulating the skin of different parts of the body with an interrupted induced current with

the secondary coil at 5 mm. were observed. Respiration finally ceased at the end of 40 minutes. The sequence of events as regards reflexes was as follows: During the first 25 minutes, stimulation of the left foot produced contractions in both the legs and arms, as did also stimulation of either arm, showing that the reflexes were intact. After $27\frac{1}{2}$ minutes, stimulation of the left foot still produced good movement in the arms, as well as in the legs, but stimulation of an arm now produced only a feeble movement of the legs. After 35 minutes, stimulation of the left leg produced only feeble movement in it, although the right (protected) limb still responded well, the motor end plates in the poisoned limb being now partially paralysed. Stimulation of one arm now produced no movement of the poisoned leg, but both arms contracted well. After 40 minutes, in addition to the conditions just noted, it was found that when the current was applied to the eye directly, movement occurred in all four limbs, showing that a powerful stimulus still produced a cord reflex. When, however, the current was applied over the lower end of the vertebral column, the legs only contracted, and when applied over the dorsal region the arms only moved, showing some impairment of the functions of the spinal cord so far as conduction in its long axis was concerned. On stimulating one arm, however, both upper limbs contracted, showing conduction transversely in the upper part of the cord still persisted. After 45 minutes the transverse conduction had also disappeared, for stimulation of one upper extremity only caused contraction of the irritated limb, and not of the opposite one, although when the electrodes were placed over the upper cord itself both limbs responded. On applying the electrodes to the eye directly at this stage, the protected limb contracted well, and the three poisoned ones feebly only, while $7\frac{1}{2}$ minutes later this powerful stimulus produced a reflex action of a very feeble nature in the protected leg only. The heart was still beating, but respiration had ceased for some minutes, the animal being quite flaccid, and apparently dead; the nerve trunks of the limbs were now exposed, and stimulated directly, to ascertain how far the end-plates were paralysed, with the following results: The muscles of all four limbs still responded to direct stimulation. The sciatic nerve of the left (poisoned limb) gave no response at all with the secondary coil at 0. That of the right (protected limb) responded with the coil at 15 mm. On testing the arm nerves, contractions were produced with the coil at $7\frac{1}{2}$ mm., but not at 10 mm., showing only partial paralysis of the end plates of the arm muscles at a time when those of the poisoned lower limb were completely paralysed; an important point, which must be taken into account in considering how far the changes in the reflexes detailed above can be taken as evidence of loss of function of the spinal cord, as apart from the affection of the motor end-plates. The loss of the transverse reflex in the upper cord when the motor end

plates of the muscles of the upper extremities were not paralysed, points to a diminution of the reflex functions of the spinal cord. On the other hand, the marked reflex contraction of all four limbs on applying a strong current to the eye just after respiration had ceased, shows that the reflex functions of the cord were not abolished at this period, although they rapidly declined within a few minutes of complete respiratory paralysis, as would be expected. The less rapid affection of the motor end-plates of the upper extremity, as compared with those of the lower limbs, accounts for the ascending paralysis apart from any interference with the functions of the spinal cord itself.

We must conclude, then, that the respiratory paralysis is complete before the reflex functions of the spinal cord are abolished, although they may be diminished at an earlier stage, so that the action of Enhydrina poison on the spinal cord itself is of quite secondary importance as compared with the paralysis of the respiration and of the motor end-plates of the muscles.

Conclusions.

1. In lethal doses, Enhydrina poison has no direct depressing action on the heart. The marked rise in blood pressure observed is secondary to failure of respiration, producing venosity of the blood.

2. The primary action of the poison is the production of a respiratory paralysis by a direct action on the respiratory centre, this being very quickly followed by paralysis of the end-plates of the phrenic nerves. The latter may occur at a time when the sciatic nerves show no end plate paralysis.

3. The poison has a very marked action in paralysing the end-plates of motor nerves, but does not perceptibly affect the conducting powers of the nerve trunks themselves. In this respect it resembles Cobra venom and curara.

4. Its action on the reflex functions of the spinal cord is slight, and altogether secondary in importance to its influence on respiration.

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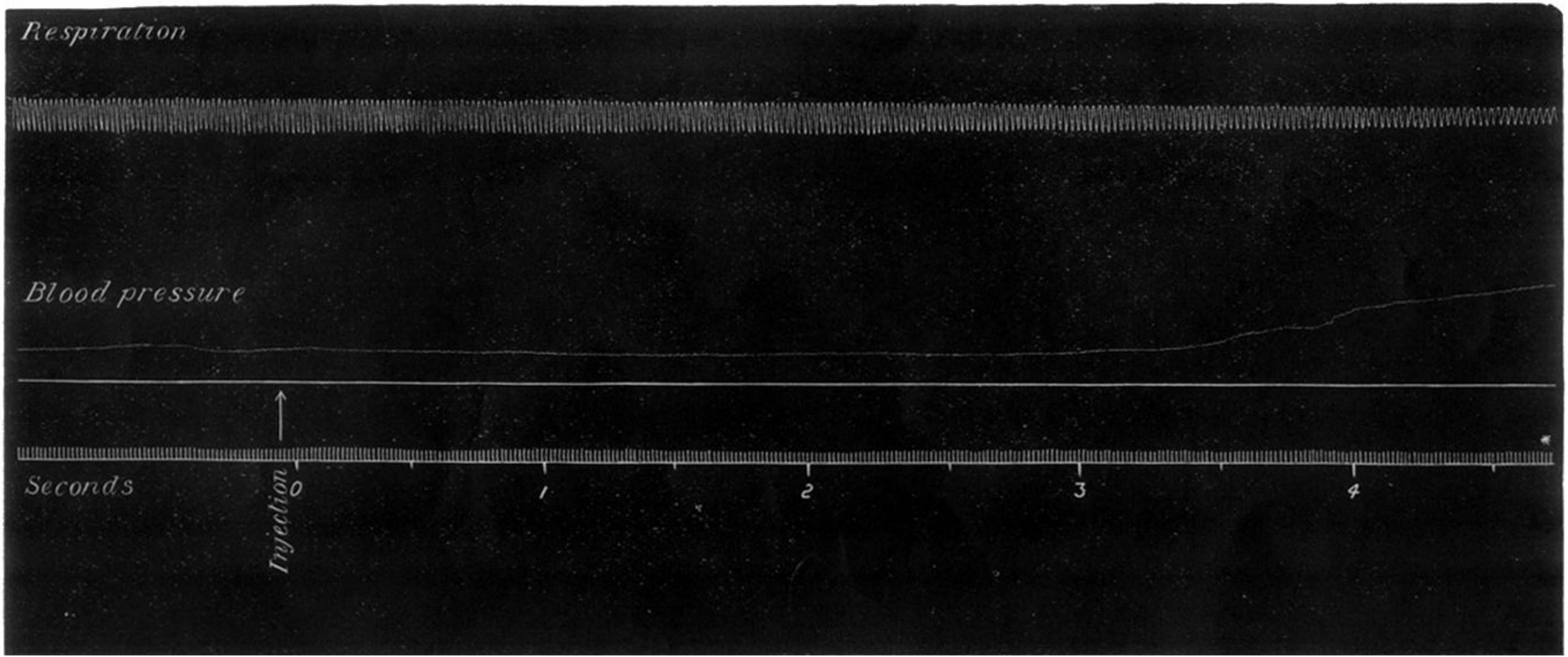

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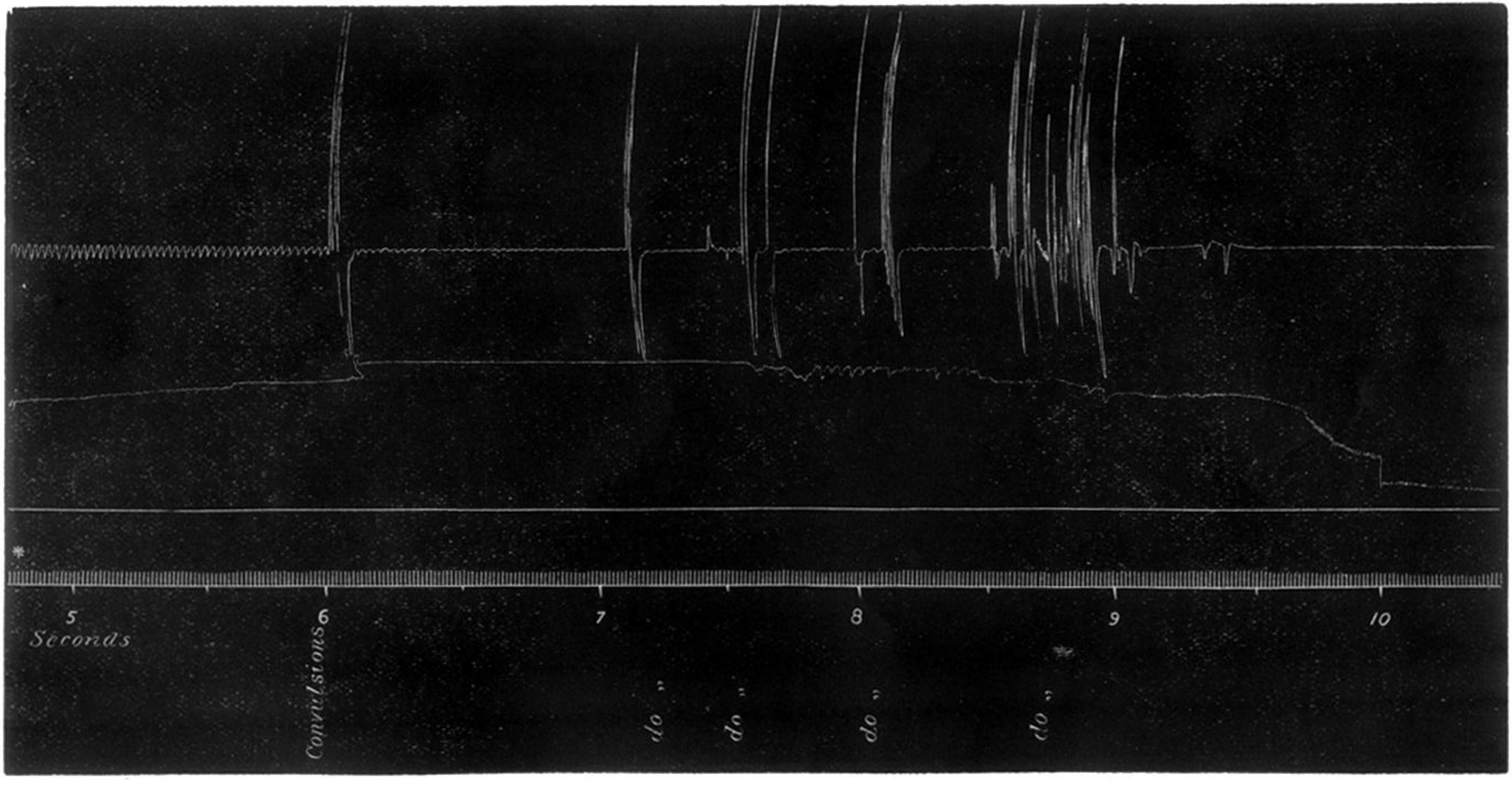

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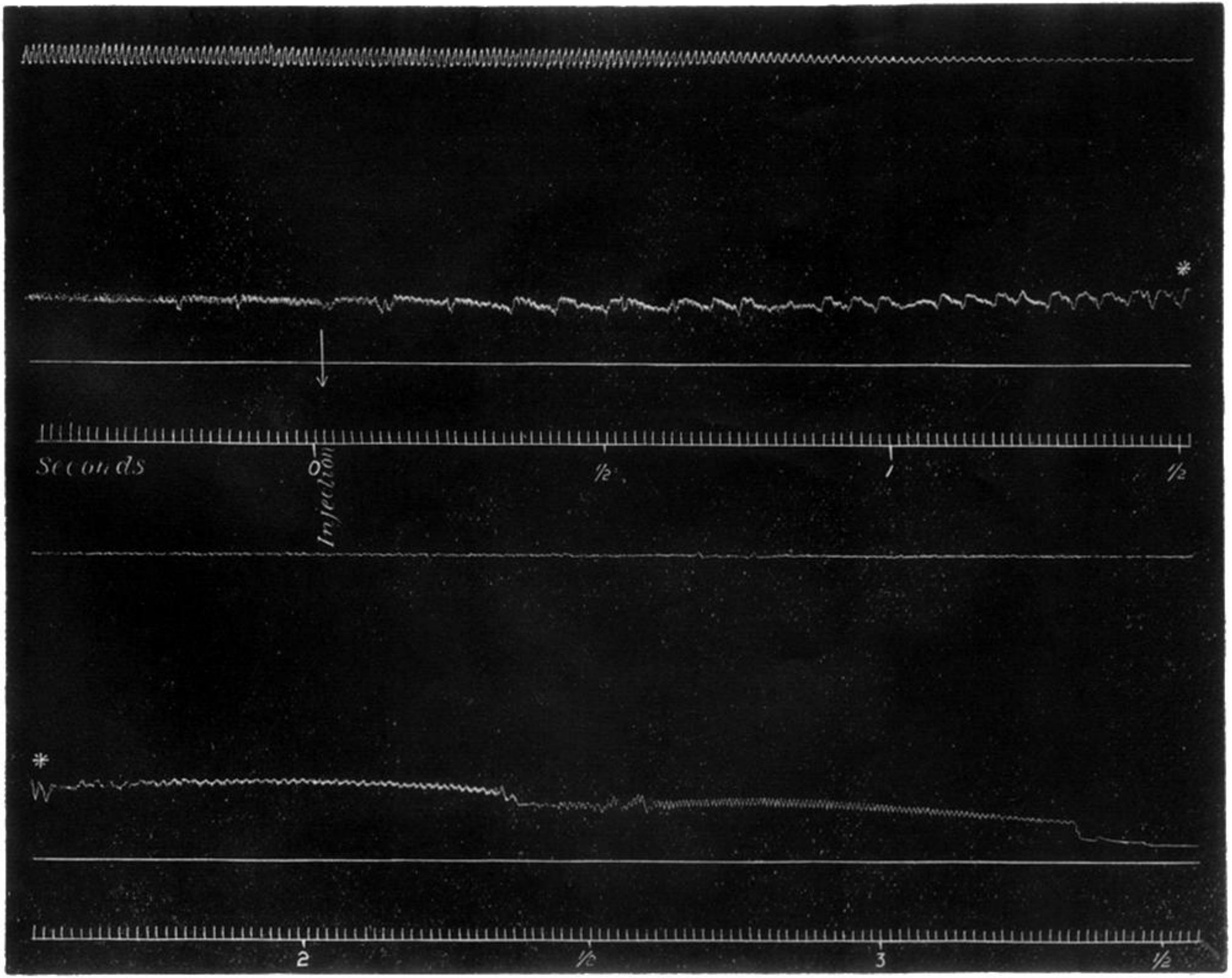

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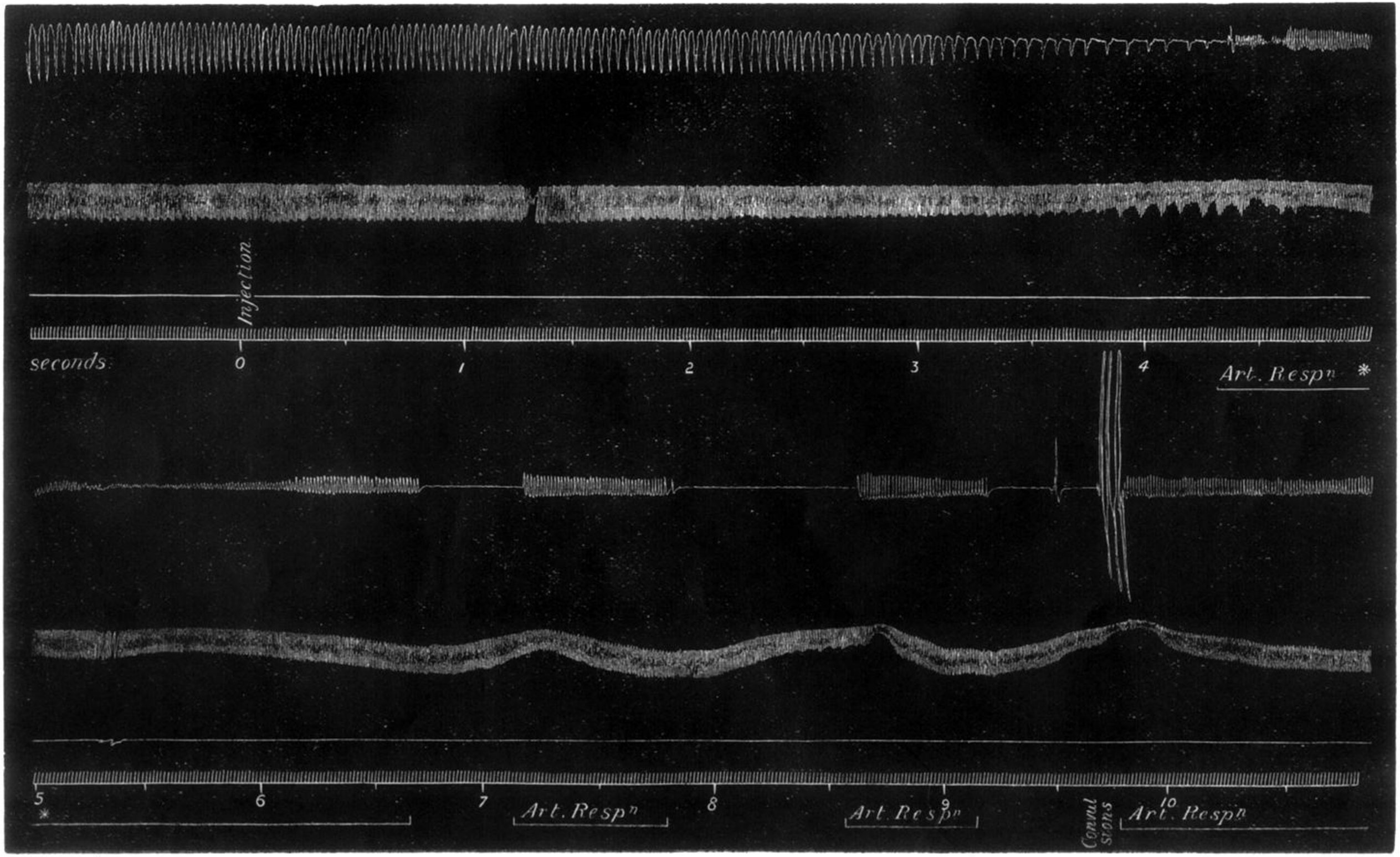
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