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A Thesis

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by

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A Contribution

to the

Pharmacology of Cobra Venom.

C O N T E N T S .

Preliminary remarks & Bibliography	pp 1 - 2
Methods; & M L D of the specimens of venom used	pp 3 & 3A
Perfusion of the vessels of the frog	pp 4 -6
Perfusion of the frog-heart	pp 7 -18
Perfusion of the Mammalian heart	pp 19 - 21
Summary of conclusions arrived at	p 22
Conclusion	p 23

Appendix 1 Tables of some experiments for the fixation of the M L D of specimens of the venom used.

Appendix 2 Charts showing graphically the results of a number of experiments in the perfusion of the vessels of the frog with cobra venom.

Illustrations etc

Plate I Schafer's Plethysmograph (modified)	to face p 7
Plate II Portion of a tracing to show accidental curves produced by a badly fitting piston.	to face p 7
Plate III Photographs of Frog-heart perfusion tracings (strong sol-ns of cobra venom)	to face p 13
Plate IV Do do do(stronger sol-ns)	to face p 15
Plate V Extract from a Frog-heart perfusion tracing (sol-n = 1/1,000,000)	to face p 16
Plate VI Photographs of Frog-heart perfusion tracings with sol-ns of Strophanthin	to face p 17
Plate VII Photographs of perfusion tracings from the mammalian heart	to face p 20
Plate VIII Do do do	to face p 21

The work dealt with in this paper has been carried out in the Materia Medica & Physiological laboratories of Edinburgh University, & I cannot begin without acknowledging my great indebtedness to Professor Sir Thomas Fraser & to Professor Schafer for their kindness in permitting me to use their laboratories, & for the ever-ready & valuable help they have given me.

At Sir Thomas Fraser's suggestion I commenced last May to study the pharmacological action of Indian Cobra-venom, & I made a number of blood-pressure & other tracings. The farther the work progressed & the more closely one analysed one's tracings, the more obvious did it become that one was dealing with a poison whose action was highly complex. In the production of a single familiar phenomenon, such for instance as the slowing of the heart, several influences were plainly at work, & some of these were apparently antagonistic to each other.

There are 3 features of a blood-pressure tracing taken from a cobra-poisoned animal which are especially striking, namely the failure of respiration after a preliminary stage of intermittent excitation, the slowing of the heart, & the maintained high level of blood-pressure. If however a rapidly fatal dose of the venom is administered, the phenomena are somewhat different. Blood-pressure & respiration fail suddenly, & though they may both be temporarily restored, a late steady fall quickly sets in, & soon heralds death. The slowing of the heart is likewise modified, for though it is a very evident feature at a late stage of the tracing, it is often masked or absent in the early stages.

When one comes to follow up one's previous work by the study of the changes in the force of the auricular & ventricular beats, with an open chest, & the use of heart levers, the results obtained appear conflicting, & are certainly most puzzling. Indeed, the more one varies one's methods of experiment, & the wider ground one covers, the more is one impressed with the complexity of the influences at work. There can be no doubt, for instance that all the medullary centres are in process of asphyxiation, as an outcome of respiratory failure; side by side with this, there appears to be evidence that these same centres are first stimulated & later more or less paralysed by the direct action of the circulating venom; nor can there be much doubt that certain nerve-ends receive their share of the paralysing effects of cobra poison. The vagus may be cited as a case in point. Add to all this that the venom has a direct & important action both on the cardiac tissue & also on the muscular coats of the vessels, & the difficulties of the problem to be solved are sufficiently obvious.

I had little difficulty in deciding that no real progress could be made till the direct influence of the venom on the heart & blood-vessels had been clearly ascertained;& the motive of the present thesis is to fill in this void in our knowledge,& thus to enable us to proceed later to a just appreciation of the complicated influences which mould the form of a blood-pressure tracing taken from a cobraised animal.

The thesis naturally falls into 4 heads,which will be dealt with in turn,& which may be thus summarised:--

(1) A study of the minimum lethal dose of the venom ~~used~~ for each different class of animal used in this research.

(2) A study of the direct influence of Cobra-venom on the walls of the frog's blood-vessels.

(3) A study of the influence of cobra -venom on the isolated frog-heart. &

(4) A study of the influence of the same poison on the isolated mammalian heart.

Before I enter on my own work,however,a few words must be said as to the bibliography of the subject.Before Professor Fraser & Professor Calmette published their epoch-marking work,the literature of snake venom research was comparatively limited. Since that time it has grown by leaps & bounds & the mere categorical repitition of titles & authors on this subject would alone fill pages.Amongst all this work there is however comparatively little on the pharmacological action of venoms.

For a long time Brunton & Fayrer,& Weir Mitchell & Reichert remained alone in the field. Since the renaissance of the subject in the last decade other aspects of the case ~~have~~ seemed to have proved more attractive to workers,& it was this which made Sir Thomas Fraser suggest to me that the thorough investigation of the pharmacological action of venom would be a fruitful & profitable line for research.

Martin's work on the subject invaluable as it is,is mainly directed & naturally so towards the Australian venoms,whilst I am equally naturally most interested in the Cobra.

Of Ragotzi's work I will have more to say in a subsequent communication, when I hope to be able to do more justice to this subject than is at all possible at present.

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AN ACCOUNT
OF
SOME RESEARCHES INTO THE NATURE AND ACTION OF SNAKE VENOM.

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[THE work dealt with in the following pages was carried out with the aid of a grant most generously made to the writer for the purpose by the Madras Government, and it seems fitting that this record should be prefaced with an acknowledgment of the author's indebtedness to that Government for the large-hearted policy which has enabled him to carry out these researches; he is all too conscious of how little has been done in return for their generosity.]

I.—PRELIMINARY REMARKS ON THE METHODS EMPLOYED.

Method of Collecting and Storing Venom, Bile, etc.

In order to collect and store venom, bile, etc., the snake is first chloroformed and nailed on to a deal table; the blood is then collected by manipulations to be described later on, and the gall bladder is removed by making a long median ventral incision over it, cutting out the bladder together with the surrounding fat, and then making an incision into the most dependent part of the sac with a sharp pair of scissors; the bile flows into an evaporating dish, which is at once removed to a water bath kept at a temperature of 100° F.; as soon as the contents of this dish have dried the vessel is placed under the receiver of an air-pump, over concentrated sulphuric acid, and the air is exhausted; after twelve hours in the receiver the dish is removed, and the now dry bile is easily separated from the bottom of the dish, with the aid of a spatula or knife; it is at once placed in a dry glass tube, and tightly corked till required for use.

After removing the gall bladder the poison sacs are next dissected out, and their contents squeezed into a dry watch-glass, which is placed in a cupboard for a few hours till the poison dries into scales; if the atmosphere is damp, as is the ease in the monsoon, the venom is dried by floating the watch-glass on a water bath at 100° F. for a couple of hours. The ease with which the poison separates from the glass when dry is remarkable; tapping the bottom of the glass is enough to separate the whole amount of venom from it in a few seconds. The dry poison is removed without delay into glass tubes previously dried at a high temperature, and allowed to cool before use; the tube is tightly corked as soon as filled. When undertaking a series, or a number of series of experi-

ments, the total stock of poison which it is intended to use is transferred to a glass mortar, finely pounded, and intimately mixed; it is then stored as before in a well-corked bottle and kept in the dark.

Every manipulation is carried on with the strictest attention to the prevention of the introduction of septic matter, all vessels being sterilised by heat beforehand, carbolic lotion 1-20 being used for such instruments as cannot be safely made hot; all corks used undergo a prolonged baking on a tin plate above the flame of a spirit lamp.

I have had frequent occasion to notice the correctness of a statement, made to me by Professor Fraser, that the poison of the Russel viper dries in long needle-shaped crystalline-looking masses unlike the shorter fracture of the dried poison of the cobra.

While working with live snakes a tourniquet of india-rubber tubing, a sharp knife, a cautery of some handy form, and a stock of crystals of potassium permanganate should always be kept handy. It is well, when possible, to have another medical man present; accidents occur when least expected, and the means to combat them should be ready at moment's notice. I should be wanting in gratitude if I failed here to acknowledge the debt I owe to Captain Samman, R.A.M.C., whose prompt action saved me from the very unpleasant consequences which might have followed a serious accident with which I met whilst handling a Russel viper. Captain Samman at once applied a firm ligature, and followed the fang puncture down to the bone with a knife; he then sucked the wound dry, and finally filled it with crystals of potassium permanganate. The result was that I escaped with nothing more than a painful sloughing wound. In the future a stock of Calmette's antivenene will always be kept at hand.

Standardisation of Solution and Mode of Administration.

When it is intended to perform a series of experiments, a rough calculation is first made of the total quantity of poison likely to be required, and a quantity in excess of this is carefully weighed out in decimals of a gram, on a scale showing $\frac{1}{10}$ mg. In order to minimise any possible error in weighing the writer never weighs out less than 20 mg. of poison at a time; each milligramme of venom is then dissolved in 1 or in 10 c.cm. of freshly boiled and cooled water,[1] according to the strength of solution required. It will be noticed that these two solutions are respectively of the strength of 0.001 gram and 0.0001 gram of venom per c.cm. of water; the weaker solution is only used when the doses to be given are so small as to render their calculation difficult otherwise.[2]

While conducting a series of experiments the solution is frequently stirred with a glass rod in order to ensure the evenness of the strength of solution. For each dose some solution is drawn up into a 10 c.cm. syringe, and the air having been expelled, the exact amount which it is required to inject is marked off by the aid of the revolving button on the piston axis; the dose is then given, either subcutaneously, or otherwise as may be desired. The syringe is sterilised by filling it several times in succession before operation from a beaker of rapidly boiling water.

Parallel series of experiments were conducted to ascertain whether the degree of dilution of the venom in the above-

(1) Ringer's fluid is now used instead of water to dissolve the venom, used for injection.

(2) A third strength of solution - viz: ·01 gramme = 1 cc (1%) - is now used for animals such as cats, which take a large dose of venom

RMR
NOV. 1903.

named two strengths influenced the lethal properties of the dose. The length of this article forbids the publication of these tables here; but the result showed most clearly that the influence of the 10-fold dilution was inappreciable. It will be noticed, however, that in the various series of experiments one or other strength has been rigidly adhered to.

Preparation of Animal.

The animal is prepared for the injection by cutting off fur or feathers, and carefully washing the skin with 1-20 carbolic lotion.

Before undertaking any series of experiments it is necessary that all preliminaries should be carefully arranged beforehand in order to avoid confusion. Each animal of the series is therefore first weighed in pounds, ounces, and quarter-ounces; the result is converted into kilogrammes.[3]

Means for subsequent identification are next taken; rabbits are branded in one ear with a serial number, care being taken to brand deep enough to destroy the fur and fur-bearing skin and no more; if less is done the mark wears out, while if more is done the result is sloughing, cicatrisation, and confusion; the animals scarcely seem to feel the branding. Fowls are marked by means of a wooden tally tied on under one wing. In all cases the colour, sex, etc., of the animal is noted.

The weight in kilos. being known, and the dose per kilo. having been decided on, a simple multiplication gives us the total dose the animal is to be given; and knowing the strength of our solution of venom, another easy calculation gives us the actual dose required in cubic centimetres and fractions of a centimetre.

The following columns in the experiment book can now be entered:

Serial No. of Experiment.	Date.	Hour.	Identifying Marks.	Weight in Kilos.	Dose per Kilo.	Actual Dose in c.cm.	Remarks.	Course of Events.

The first seven columns should be entered before starting to work on the series, the others subsequently.

The preparation of the mongoose requires a separate paragraph.[4] These animals are so vicious, so wonderfully agile in their movements, and so strong and active, that it is advisable to weigh them, calculate their dose of poison, and administer it at one and the same time; they were brought in cages by the men of a caste who spend their lives in such occupations; in order to catch them, a running noose was passed through a hole in an iron rod, and this was insinuated over the animal's neck through the slightly opened door of the cage, the noose was then quickly pulled tight, and the animal was dragged out, securely tied, weighed and dealt with at once. In spite of every precaution, both I and one of my assistants received severe bites; these were delivered down to the bone in the fraction of a second, and were extremely painful.

Calculation of Dose: Idiosyncrasy of Animals.

Before starting to work on any species of animal, a long series of experiments was first undertaken to ascertain the dose of the poison to be used which would be certainly fatal in a limited period of time. At first an effort was made to work with the minimum lethal dose per kilo., but it was soon

(3) All animals are now weighed directly in grammes.
(4) Cats also require careful handling.

RMR
Nov 1903.

found that this method introduced many difficulties. It was found, for example, that a dose of cobra venom corresponding to 0.0004 gram was the lowest dose per kilo. fatal to a rabbit; on the other hand, higher doses than this from time to time proved non-lethal, and rabbits occasionally recovered from doses as high as 0.0007 per kilo., though this was very rare.

If one fact stood out in bolder relief than any other it was this: that the personal element (if one may be excused the term applied to animals) is a factor never to be forgotten. Granted that the greatest care is taken to weigh the animals under the same conditions, that the poison is from the same stock, and most carefully weighed and apportioned, that every effort is made to inject the venom into the same tissues in all experiments, and to keep all known conditions as even as possible, granted all this, there still remains an element of uncertainty as to the fate of the subject which I can only attribute to "the personal factor." In order to eliminate this factor as far as possible, the dose chosen has always been one capable of certainly causing death, or at least of producing serious symptoms of snake poisoning.

In order to impose a further check on results, no series of experiments was undertaken without putting aside one or more animals as control snake-poison subjects. By this means the standardising of the lethal dose was kept as accurate as possible. This was the more necessary, because it was noticed that certain batches of animals presented a greater resistance than others, possibly due to the method of feeding, etc.

Lethal Dose for Various Animals.

Rabbits will often appear to get over the first effects of the venom, and will look as if on the way to recovery, but loss of weight continues, and after a longer or shorter period animals will succumb which at first sight one hoped would survive; their lower resistance to these secondary effects of venom poisoning renders them very inferior to fowls as subjects; the latter make a better fight to begin with, and do not suffer to anything like the same extent from remote sequelæ. Rabbits in the hills of India are also very susceptible to the influences of the frequent and trying changes of climate which prevail.

Rabbits.

All doses of cobra venom below, and including 0.0003 gram per kilo. of body weight proved non-lethal; doses of 0.0004, 0.0005, and 0.0006 gram per kilo. were lethal in most cases, but could not be certainly relied on; even 0.0007 gram per kilo. occasionally failed to kill, but it never failed to produce grave and well-marked signs of cobraism; doses of 0.0008 and 0.0009 were fatal in a very few hours. A dose of 0.0007 gram per kilo. killed in from six to eighteen hours as a rule, but life might be prolonged for days; it was accordingly selected as the dose for most of the experiments. The above conclusions appear to harmonise well with Professor Calmette's observations on dosage in his lecture delivered in the laboratories of the Royal Colleges on July 27th, 1896.

Daboia venom in doses of 0.0004 up to 0.0009 took a fortnight or more to kill; even so high a dose as 0.001 took twelve days to kill, and one of 0.002 took seven days; a dose of 0.003, however, killed with very fair uniformity in under twelve hours; 0.004 killed in four hours and a-half. The dose of 0.003 gram per kilo. was, therefore, chosen for daboia poison experiments.

Fowls.

Cobra venom in doses up to and under 0.001 proved non-lethal; 0.0015 gram per kilo. killed in fifty hours; 0.002 and 0.0025 killed in over twelve hours, and 0.003 in between six and twelve hours; these latter doses were therefore selected.

Daboia venom in doses of and above 0.003 gram per kilo. of body weight proved fatal; 0.003 gram killed in about twelve hours; all higher doses also proved lethal.

The Determination of

The Minimum Lethal Dose of the samples of venom used, for various animals submitted to its action, in the course of this research.

This section of my paper must be prefaced by some account of my methods of obtaining snake poison, & of the general principles governing my work. I have taken the liberty of attaching hereon the preliminary paper of the series I published in the B M Journal in 1900, & have noted threupon a few remarks showing any changes of procedure etc I have since been led to adopt.

The stock of poison with which this series of experiments was started proved to be of relatively low lethality, & for the later work a fresh stock was substituted. The latter proved to be very much the same in strength as the samples with which my Indian work was done, & it is of interest to notice that it is decidedly weaker than the venom with which Sir Thomas Fraser's classic work was done. It is possible that the method I employ for obtaining my samples has something to do with the question, & Sir Thomas Fraser has suggested to me that possibly more mucus is squeezed out by my method than by the more usual medes of obtaining the poison. In favour of my method it is to be remembered that it yields an aseptic product & that it introduces no error into one's calculations, provided that one has carefully ascertained the minimum lethal dose for the sample used. I always collect a large supply of venom, pound it all up together aseptically in a mortar, & then mix it by thorough shaking in its flask, which is carefully labelled at once.

A reference to Appendix 1 will show the results of this preliminary work for the 2 specimens of venom, which will be henceforth alluded to as "the old stock of venom" & "the new stock of venom.

A short summary of results will save time & trouble.

The M L D for rabbits of the old stock of venom = ·004 grme per kilo
do of the new stock of venom = ·0006 grme per kilo
The M L D for rats of the old stock do = ·004 grme per kilo
do of the new stock do = ·0005 grme per kilo.
The M L D for frogs of the old stock do = ·004 grme per kilo
do of the new stock do is below ·0007 grme per kilo, but is not yet fixed definitely.

Perfusion of the vessels ,in the frog.

The apparatus used for this purpose is that in common use in Sir Thomas Fraser's laboratory,& has the great virtue of simplicity.There are 2 rigid stands each of which is provided with a platform.One of these platforms supports an arrangement of Marriotte's flasks & the other the animal which is the subject of experiment. A pressure of 6½" of fluid was used in these experiments;needless to say the arrangement of the instrument permits great latitude in the pressure selected.A simple arrangement of tubes enables one to draw at will either on the normal liquid or on that under course of observation,without in any way disturbing the progress of the experiment.A pointed tongue,at the lower end of the platform which supports the frog, facilitates ~~the~~ & directs the escape of fluids into the graduated cc-measures destined to receive them

The cannula is inserted with the usual precautions into one or other aorta of a prepared frog,& is tied securely in place,the opposite vessel being then ligatured.It is essential to avoid the presence of air bubbles,however small,in the cannula,& this can readily be done by inserting the latter whilst there is a free flow of fluid,into its vessel through the cut apex of the ventricle. A wide opening into the auricles permits the ready escape of of liquid which has traversed the vessels.

A continuous record is taken of the number of cc flowing away at the end of each successive minute. The fluid used to obtain a normal in all my earlier experiments was a 0.6% solution of sodium chloride dissolved in Edinburgh tap water;this served also for the exhibition of the venom.It was found that with this solution slight but steady dilatation of the vessels took place after prolonged perfusion,& I now always employ a modification of Ringer's solution suggested by Rusch,the formula for which is given later in this paper(vide section on heart-perfusion).

The pithing of the frog requires to be very thoroughly done,& it is most necessary to allow an interval of ½hour to elapse between this operation & the commencement of perfusion.

A reference to the charts composing Appendix 2 will show the results of a number of perfusions with cobra venom of the weaker stock. It will be

observed that ~~observed~~ ~~that~~ a solution of one part of cobra venom in 50,000 parts of the salt solution reduced the flow from about 1.3 cc per minute to 0.3 cc per minute in a little over one hour. A 1/100,000 solution was tried next followed in turn by 1/300,000, 1/1000,000, 1/2,500,000, & 1/5,000,000. In each case the constriction of the arterioles under the stimulus of the venom was plainly discerned. At a strength of 1/10,000,000 a new phenomenon was observed, viz a preliminary decrease of the flow follow-ed by a late increase, which even overpassed the original normal. On now reducing the strength of venom to 1/15,000,000, no preliminary contraction of the vessels was indicated, but on the contrary there was evidence of vascular dilatation from the first. The same result was repeated with a 1/20,000,000 solution, & it was evident that one was practically working with saline solution, the venom no longer being present in sufficient concentration to produce any marked effect.

It is to be borne in mind that for some reason which is not apparent, the particular specimen of snake poison under observation was of rather low lethal power, the minimum lethal dose for a rabbit being about ~~twice~~ six or seven times that of the specimens I have usually worked with. If one may assume (I do not venture to say definitely that one can) that the power of constricting the vessels is lost pari passu with the lethal power of the venom, then it is clear that even weaker solutions would produce an appreciable effect.

It remains to consider the probable influence of cobra venom, circulating in blood, on the walls of the vessels, under the conditions which prevail in the body of a snake bitten man.

Calmette has calculated that the larger venomous snakes such as the Cobra, yield on an average about 50 mgrmes of venom (weighed in the dry) at each bite. He thinks this a liberal estimate, & he infers that the fatal dose for a man is about 10 mgrmes. (Vide Calmette's note to the article on Venom in Allbutt's System of Medicine).

Sir Thomas R Fraser has calculated an average of .255 gramme of Cobra Venom for each bite, but thinks an average of .195gramme probably a truer estimate. Calculating on the cat-basis he places the minimum lethal dose at .317 gramme. Again he thinks a lower estimate more correct, & gives

.0317 grms as nearer the true mark.* It is not possible for me within the limits of this communication to discuss this question, but I believe from some work I have done on the subject that Fraser's estimate is the more correct; indeed it is probably remarkably near the true figure. We shall however err on the side of safety if we assume that a fatal dose for an average man is about 30 mgrmes. This figure is covered by the estimates of an average bite arrived at by both authorities.

We may assume the weight of an average man to be about 70 kilos, & may put the weight of his blood at 1/7th of this figure or 10 kilos.

If the above assumptions are accepted, & if it is supposed that the total quantity of venom injected is absorbed into the blood & circulates therein, we find that poison will be at a concentration of about 1/333,333.

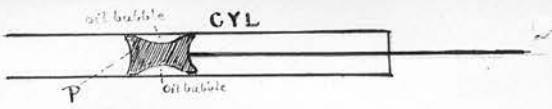
Even should the above calculations be considered over-liberal, or should it be objected that one cannot safely assume more than a fraction of the total dose of venom to be actually in circulation at one time, we have still a very wide margin between 1/333,333 as above arrived at, & 1/10,000,000 which has been shown capable of profoundly affecting the blood vessels in so short a period as 35 minutes. Nor must it be forgotten that in the usual duration of life after snake-bite is reckoned not in minutes but in hours.

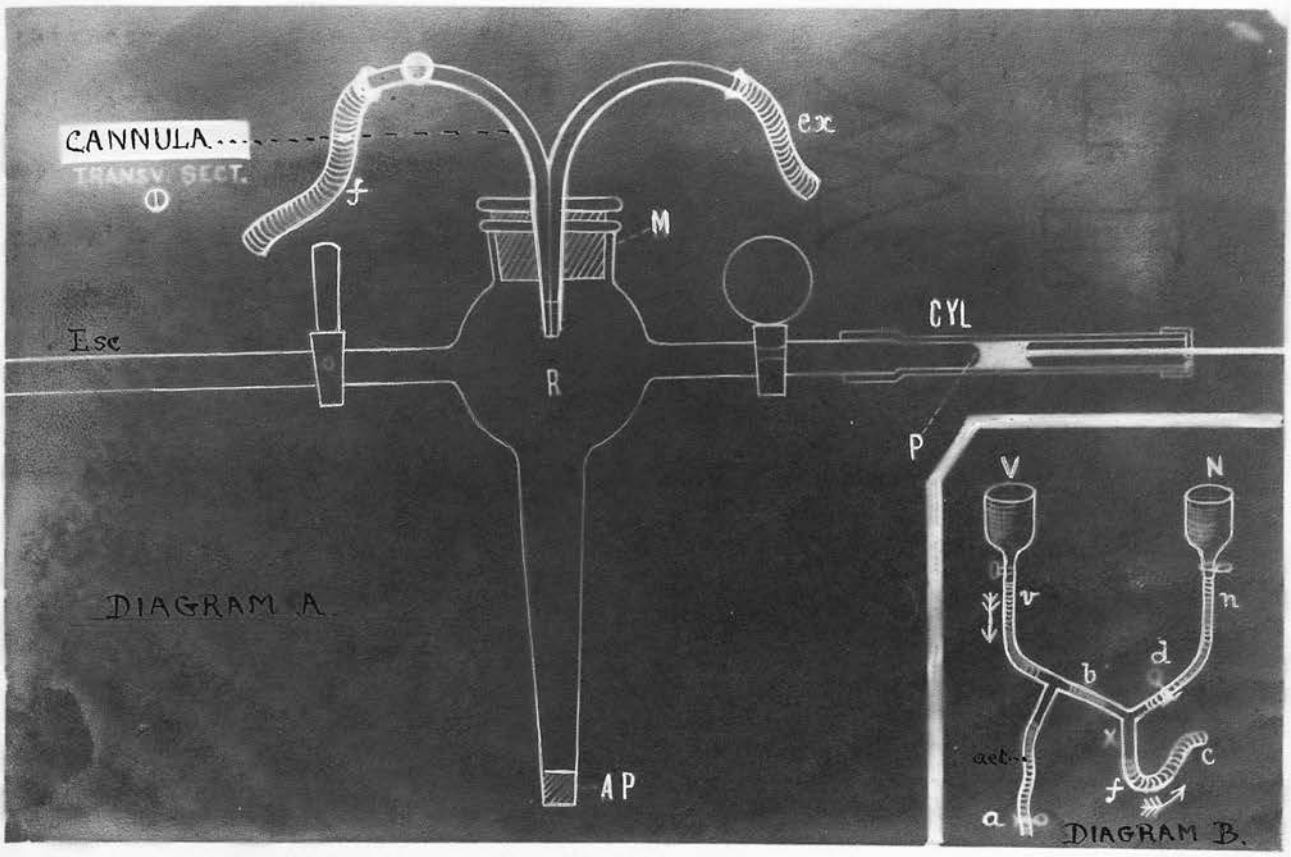
It is clear therefore that the sustained high pressure, which is so marked a feature of blood-pressure tracings in slow death from cobra-poison, is explainable without going any farther than the direct action of the venom on the walls of the vessels. I purposely avoid any discussion of the exact mode of such action, as being beyond the present position of physiological knowledge.

* Foot-note :- Fraser on Immunisation against Serpents' Venom, etc before The Royal Institution of Great Britain. March 20th 1896

PLATE. I.

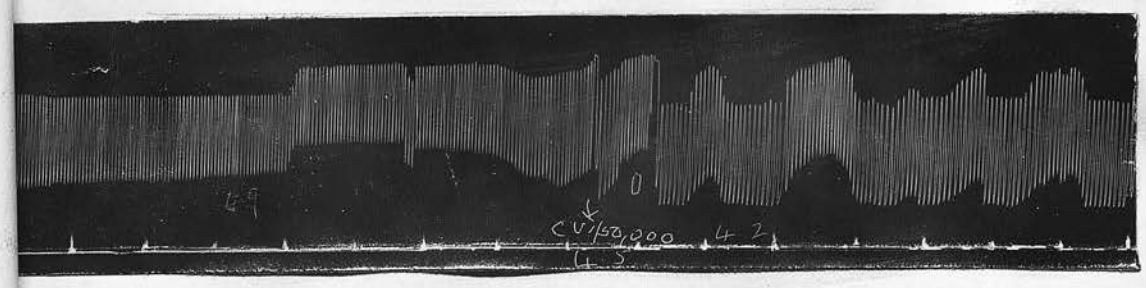
DIAGRAM. C.





Schäfer's Plethysmograph.
(Modified)

PLATE II



To show the adventitious curves produced by a badly fitting piston, and altering the level of the base line of the tracing. Such alterations are never observed while using a piston working in a well-turned metal cylinder.

Perfusion of the Frog-heart.

This was carried out by means of Professor Schäfer's plethysmograph. At first very considerable difficulties were met with, as the heart either worked very irregularly or refused to work altogether, in spite of changes in the nutrient fluid, in the method of stimulation, etc. These preliminary difficulties seem to justify a detailed description of the method one is now adopting, since it is found that this method easily yields uniform & excellent results.

I will first deal with the construction & fittings of the instrument, then with the fluids used, & finally with the steps of the operation.

The instrument:--

This is, with a few modifications, the same as that in use in Professor Schafer's laboratory, & is shown in Plate I (attached), to which all letters in the text refer.

1. The mouth (M) should be sufficiently wide to allow of the heart being easily passed into the receiver (R) without risk of abrading its surface whilst so doing. If this is not attended to, or if the endothelial layer of the visceral pericardium is in any way damaged, I believe that troublesome leakage may be the result. Many of the instruments are sent out with necks which are too narrow.

2. It is of advantage to have the limb of the cylinder (Cyl) in which the piston works, made of brass throughout that portion of its length traversed by the piston. The objection to glass tubing is that it can seldom be obtained with a sufficiently uniform diameter. Consequently the piston does not work uniformly in it, & at different times during the experiment bubbles of air are drawn behind the piston or oil is driven in front of it, thus altering the level of the base line of our tracing, & introducing a set of curves which are purely accidental, & which have no bearing on the experiment. (Vide portion of a tracing attached; Plate II)

3. In choosing the material for the piston, it is necessary to consider the question of friction; thus it will not do to have a brass working on brass. An aluminium piston works comparatively well in a brass cylinder, so does a vulcanite one.

4. The exact size of the tube is a matter of some importance, inasmuch as a definite amount of fluid displaced by the heart movements will obviously cause a greater excursus of the piston in a narrow than in a wide tube. Other factors have however to be considered, such as the greater power of the heart over a wide piston, on the one hand, & the lessened friction-area presented by a narrow piston on the other. The diagram shows the size of tube which has been used with advantage in my experiments (diagram c of Plate I).

5. It is advisable to have as light a piston as possible. Aluminium & vulcan-ite are therefore indicated. For the same reason a hollow piston, or one scooped out in the middle (vide diagram c of Plate I.) is better for our purpose. Attention to these details is of more importance since the piston must be of a certain length to prevent the point of the indicator-needle from falling when the former is far out to the end of the cylinder.

6. My personal preference is strongly in favour of a metal cannula. It is not so easily broken as glass; it can be firmly sealed into the stopper, without the intervention of a cork; it admits of a screw-clamp being attached to one of its limbs, whereby unipolar stimulation can be applied to the heart; it can easily be made without lateral grooves; & lastly & most important of all it can be made with comparatively thin walls, & with tubes of relatively large bore.

The glass tubes ordinarily supplied with this instrument have clumsy walls, an almost capillary bore, & a well-marked groove on each side; they are also very brittle. A tiny clot of blood will block a tube effectually & so spoil what might otherwise have been a successfull experiment; even small particles of suspended debris will do the same. If a glass cannula be used, it is necessary to have a pair of electrodes introduced through the cork which closes the cleaning aperture(Ap). There are many objections to this method. It is hard to stimulate the outer surface of a heart without doing damage to its endothelium, & so causing a leakage. After a while the tube ~~carrying~~ carrying the electrodes works stiffly in the cork, & with every care may move with a jerk during manipulation & wound the heart. Even if it works smoothly, as it often does at first, & even if the wires are bent backwards to avoid scratching edges, the heart is far from safe. My own opinion is that electrical stimulation of the heart is to be avoided if possible, & that if unipolar stimulation & patience fail to make the heart go, it is better to put on a fresh heart at once; & I speak after a good deal of experience, bought by many failures & disappointments.

7. Marriotte's flask arrangment for maintaining a constant level of the fluid perfused is theoretically preferable to the ordinary funnel arrangment, & for accurate work should certainly be employed. The effect of change of level is not however nearly so easily apparent as one would have expected. The variation in level can of course be met by conducting the exit tube back to the feeding funnel, but as this must increase the work the heart has to do, it is not a proceeding which recommends itself for a long experiment. It further requires a valve in the escape tube.

8. The diagram shows the arrangment of tubes used (vide diag B. Plate I). It is, I believe, that used in Professor Schafer's laboratory, or at least closely resembles it. Two objects are to be kept before us, whatever the exact disposition of tubing made use of. Firstly the tubes should be as short as is consistent with efficient working; and secondly the turning off of one solution & the admission of another must be so ordered as to entail a minimum of disturbance of the apparatus, & so as to ensure that as little delay as possible occurs before the new fluid reaches the heart. The reasons for the above postulates are obvious enough.

9 A Palmer's drum was used horizontally, & travelling about 1 cm in 10".

The Fluids Used.

In the earilier stages of my experiments, I met with a large number of failures, owing to my overlooking the influence of the oil used in the body of the instrument. I shall return to that point later, but mention it now to because it led me to try a number of different fluids, wrongly thinking that it was the nutrient medium which was to blame.

Almost every experimenter has a fluid of his own or a modification of some one else's fluid. This leads one naturally to suppose that the exact composition of the fluid is not so important as one supposed. I have yet to find however, a fluid which gives the same prompt, regular & maintained action that one gets from a blood mixture.

The fluid I have used for perfusion is composed of 1 part of blood shaken up with 2 parts of Ringer's solution. The latter was made according to Rusch's prescription (vide Pfluger's Archives Vol 73 p 546), & is as follows.

Na H C O3	.1 gramme,
Ca Cl2	.1 gramme
K Cl	.075 Gramme
Na Cl	6.0 gramme
Distilled water	1000 grammes.

Rusch lays stress on the necessity of dissolving the salts in the order above given, but does not clearly explain wherein the importance of so doing lies. To avoid any possibility of error, I have followed his directions in this respect. If his order is adopted the fluid remains clear; otherwise a precipitate forms.

As I shall presently have occasion to explain, I fill the body of the instrument with Ringer's fluid, made as above, & thus keep the heart throughout the experiment floating in a nutrient medium.

The Steps of the Operation.

1) Large frogs must be used, the German frogs being very suitable.

2) All preparation of the instrument etc should be made beforehand, in order to avoid loss of time after the heart is removed from the body. It seems to work both quicker & better, if it is transferred direct from the frog into the instrument.

3) Fill the piston cylinder with oil up to its tap, which should be kept shut, & then pass the piston into place by gentle pressure, taking care that no bubbles of air are left behind it. Next fill the body of the instrument with Ringer's fluid, & shut the tap of the escape-tube (Esc), after seeing that it is free of air. Be careful that the instrument is filled with fluid right up to its brim.

4) Fill the system of rubber tubes with their appropriate fluids in the following way. Put on clips at a, b, & c. (Plate I. diag B) Fill N with the normal fluid for perfusion, & V with the solution of venom or other liquid under examination. Open the stop-cock of N & remove clips a & b, at the same time raising the top of the tube a e t to allow all air to escape. Now close the clip b, & the stop-cock of N, & open the stopcock of V, to expel all air from this system of tubes also. It is generally necesaary to pinch the tubes v & n with a sharp repeated movement, in order to expel any air from them through the open stopcocks of V & N. Close both stopcocks & adjust the 3 clips in place & then remove the tubing f, & attach it to one limb of the cannula. The heart must now be prepared; but before doing so, a second short rubber tube (ex) is to be attached to the cannula by its second limb, & the whole (cannula & tubes) is to be filled with Ringer's fluid; & the ends of the 2 tubes are now to be clipped. We are thus saved from introducing air into the heart at a later stage of our operation. This is very important, for a frog-heart will not contract firmly on air which it cannot expel.

5) Expose the heart in the usual way, taking care however to open through the shoulder joints instead of cutting through bone, as rough bone edges are very apt to tear the heart during the subsequent manipulations. Having cut through the fraenum, & made deep cuts in the abdominal wall to allow of a free escape of blood, remove the 2 lobes of the liver, & lay the frog on its back on a folded cloth, with its head dependent. This manouvre causes the heart to fall backwards, & so greatly facilitates the single-handed intro-

duction of the cannula.Cut freely in the horizontal direction into the Sinus, just ~~below~~ distally to its junction with the auricles,& wash the heart free of blood by means of a stream of Ringer's fluid,taking care that no bubbles of air enter the bhambers of the organ.It is convenient to have a funnel mounted on a stand always on the table for the above purpose;it should be provided with a clip or stopcock at the lower end, just above the glass nozzle with which it is fitted.
Next pass the cannula up through the slit in the sinus ,right into the ventricle,after previously cutting through the auricular septum with a pair of fine scissors introduced through the same aperture.Allow the cannula to rest in place by its own weight,& slip a loop of string over the ventricle.If this falls into good position at once,draw the string tight & complete the knot . It is however far safer,to first cut the heart free from all attachments,taking care to cut each structure as far from the ventricle as possible;then with 2 forefingers on the auricles, one on each side work the heart into the desired position,& ask an assistant to tie it.The ventricle must be put straight on the cannula,with the point of the latter well inside its cavity but not pressed too far therein.The ligature should consist of a single loop of string,& should be placed as near the auriculo-ventricular groove as possible. If it infringes on the ventricle,there will not be room for full dilatation of the latter's cavity,& the stroke of the piston will therefore be a short one. If,on the other hand,it encircles the auricle above the groove,the auricular walls belly upwards at each stroke,& any chance bubbles of air become imprisoned there,& cannot escape.As a result contraction is imperfect,& irregular,& the air may be easily seen in the bulging auricular cavity. Now slip the tube f of the cannula on to the free end x of the Y-piece,after opening the stop-cock of N.This method ensures safety from air bubbles.Remove both clamps & the flow through the heart will at once begin.If it does not, the great probability is that there is air in the heart or that the cannula is blocked.Reverse the in-flow & out-flow tubes attached to the cannula;failing this suck the tubes, or pass a wire down. Electrical stimulation may also be tried.If the heart does not work at once,it may be given a little time. My experience is that if the above precautions have been carefully observed,nearly every single heart works almost at once & works well. If it does not do so,it is better to

put on another at once, rather than to try & doctor up a feeble one.

When the heart is beating satisfactorily transfer it at once to the plethysmograph, opening the tap of the escape tube as the cannula is being inserted, & shutting it sharply at once to prevent loss of fluid in the receiver. Screw the ground glass stopper well home, & at once open the stopcock leading to the piston.

It is most necessary to put a thin coating of lard on the stopcocks, & on the stopper before commencing work, as they then work easily & safely.

There is a point to which I have more than once alluded, but which I have purposely left till last for discussion, as it is of paramount importance. It concerns the fluid used in the body of the receiver to suspend the heart in.

Oil very soon becomes rancid, & has then a strong acid reaction. This I have proved for myself on a specimen which looked clear & smelt good. A heart placed in this acid liquid very soon passes into systole & refuses to beat. Sometimes when removed from the oil it begins beating again, whilst an active heart speedily becomes quiescent after a few moments of immersion.

Indeed it was the observation of these facts which led me to substitute Ringer's fluid for oil as the suspending medium. From the first time I did so I passed from almost unbroken failure to uniform success with heart perfusions. Hence the stress I now lay on this detail of the experiment.

PLATE III.

Photographs of Frog-heart Perfusion Tracings

luded to in the text. All the tracings are to be read from left to right, and systole is upwards.

e time is taken in seconds. All details can be made out by the aid of a lens.

(1) Perfusion with a 1/10,000 solution of Cobra Venom.

(2) Perfusion with a 1/20,000 solution of Cobra Venom.

(3) Perfusion with a 1/50,000 solution of Cobra Venom.

(4) At the end of 6 hours' perfusion with blood mixture, a 1/5,000 solution of Venom was run in, causing death in less than 2 minutes.

lead the tracing from below upwards.

The Results of Perfusion of the Frog-heart with Solutions of Cobra Venom of various strengths.

In dealing with strong solutions of snake poison,& by this term one means any thing below about 1 in 50,000,the duration of a perfusion experiment is to be reckoned in seconds or at the outside in minutes.The heart is killed, or at least is started on the way to death,almost at once.Under such circumstances it is obvious that a free flow through the instrument will materially affect the length of time the organ lives.Naturally a fast stream of fluid passing through an actively beating heart will reach the organ & commence its lethal influence quicker than a slow or trickling flow.

The outcome of all this is that it is not possible to compare with mathematical accuracy one's results from a number of perfusions made with different strength solutions of snake venom.Nor must it be forgotten that the individual resistance of different frog hearts varies greatly.Such influences as the breeding season,the duration of captivity,& the existence of parasitic & other diseases have all to be taken into account,& one can not do much work with the frog heart without finding that these factors have to be reckoned with & allowed for.

The main results of these (with strong venom) perfusions are however always the same.The beat of the heart becomes more frequent & the organ tends to pass into a condition of systolic tone,in which it dies.An analysis of the action of venom in some of the experiments with solutions of from 1 in 5000 to 1 in 50,000,will now be given. Those results have been selected which were obtained from strongly & regularly beating hearts through which a good flow was taking place. (Vide Plate III.)

(1) On 29 10 03 a heart was perfused with a solution of 1/10,000

Before the poison was run in,there were 4 beats in 20";
1 minute after poison was started there were 7 do ;
2 minutes do do 9.5 do ; &
3 minutes do the heart was in tight systolic position.

28 10 03 A heart was perfused with a solution of 1/20,000.

Before the poison was run in,there were 4 beats in 20";
1 min after poison was started,there were 5 do do ;
2 do do do 6 do do ;
2½ do do ,the heart was in tight systolic position.

(3)31 10 03 A heart was perfused with a solution of 1/50,000.

Before the poison was run in,there were 4 beats in 20";
2 min after the poison was started there were 5 do do ;
4 min do do do 6½ do do ;
5 min do do ,the heart was in tight systolic position

The next result is not strictly comparable with the previous ones as it was taken from a heart which had been beating for nearly 6 hours. The beat was however strong & regular, & to close the experiment a solution of venom of a strength of 1 in 5000 was perfused.

11 11 03 A heart was perfused with 1 in 5000 solution of cobra venom.
Before the poison was run in, there were 5 beats in 20";
1 min after venom was started, there were 9 beats in 20", &
2 min do do , the heart had passed into a tight systolic position.

In all the above experiments a contraction of the excursus took place very early, thus in experiment (1) above it had begun in 30"; in experiment (2) in 13"; in experiment (3) in 100"; & in experiment (4) in 40".

In every case the shortening took place at the expense of the diastolic end of the excursus, & never at that of the systolic end. The tops of the curve in the tracings represent the amount of systole, & it will be observed that they are in a straight line, whilst the diastolic ends of the excursus slope gradually upwards till firm systole is reached. (See Plate III attached).

We pass next to a consideration of the effects of venom in much weaker solutions, in those in fact below a strength of 1 in 50,000, & we are now confronted with a new difficulty. It is a very easy matter to prepare a heart which will beat regularly for an hour, especially if blood-mixture (1pt blood, & 2 pts Ringer's fluid) is used; again it is not difficult to make a heart beat for many hours with blood-mixture, or even , though with less certainty, with Ringer's fluid, but in experiments which last 3,4 or more hours it is essential to an interpretation of the tracing that we should be able to distinguish clearly between the effects of fatigue & thos of envenomation. Fortunately this has not proved difficult, after a sufficient number of experiments had been made.

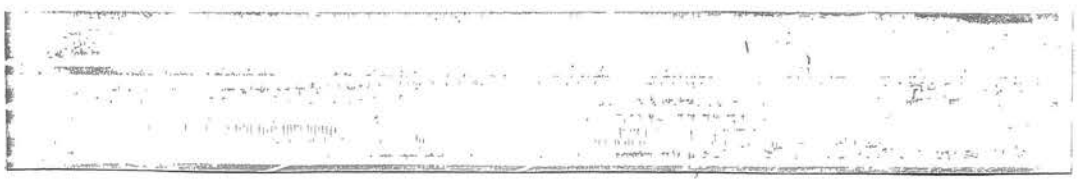
A heart through which blood-mixture, pure & simple, has been perfused for several hours shows the following changes in its graphic record:--
(a) the excursus of the beat very gradually diminishes;
(b) the rate undergoes a slight augmentation on the whole, but oscillations thereof are found to occur; finally it slows steadily down with a tendency towards long diastolic pauses.
(c) the tracing moves gradually but surely & steadily down towards diastole & never in the reverse (systolic) direction.

An observation of the heart shows that the explanation of this is that the organ is slowly passing into the diastolic state, doubtless as a result of fatigue.

PLATE IV.

Photographs of Frog-heart Perfusion Tracings.
(Instructions as in Plate III).

Perfusion with a 1/100,000 solution of Cobra Venom.



(7) Perfusion with a 1/250,000 solution of Cobra Venom. It was a feeble heart to begin with. The first effect of Venom in steadying the heart is worthy of note.

It will be advantageous now to give an analysis of a few of the tracings which show the effect of the more dilute solutions of cobra venom;--

(5) 31 10 03 A heart was perfused with a solution of 1 in 100,000.

Before the venom was run in there were 3 beats in 20"

5 minutes after the venom was started there were 5 do

15 do do do 4 do

30 do do do 3.75 do

32 do do then heart was in a position of strong systole. The excursus began to diminish within a minute of the venom being perfused.This diminution was altogether at the expense of the diastolic end of the curve,the line of systole actually rising the while. The measured heights of the excursus taken at intervals were as follows;-- beforehand =16mm;5' after=13 mm;15' after=4.5mm;30' after=0.5mm.

(6) 3 11 03 A heart was perfused with a sol-n of 1 in 250,000.

	Beats in 20"	Height of exc-s
Before venom was perfused	4	7mm
7' after do (diastolic end of curve began to shorten)		
10' do	5.0	5.75mm
20' do	5.75	2.75mm
1 hr do	5.5	1mm
3 hrs do	the heart was dead in moderate systolic position	

(7) 14 11 03 A heart was perfused with a sol-n of 1 in 250,000.

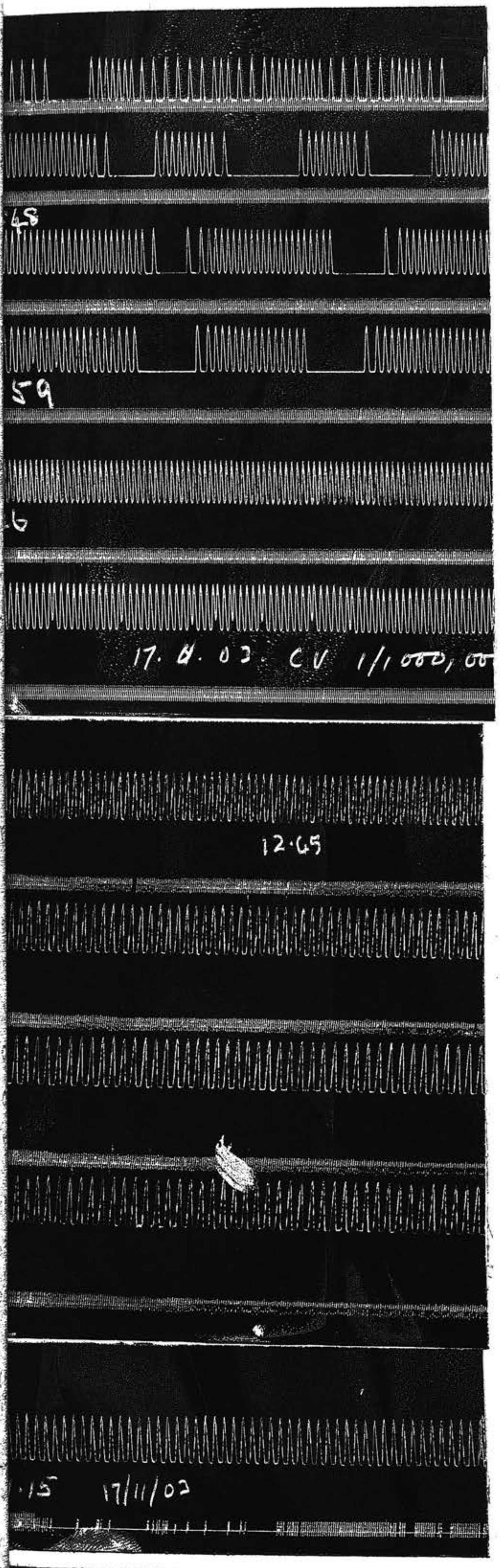
	Beats in 20"	Height of excursus
Before venom was perfused	5	5.5mm
2' after do	6	5mm
5' after do	7	5.5mm
10' after do	7.25	3.25mm
20' after do	7	1mm
27.5'after do	heart was dead in a position of systole. The	

diastole commenced to shorten 1½' after the venom was first run in.The shortening of the excursus was all at the expense of the diastolic end of the beats,the systolic ends keeping practically on the same level.

(8) 6 11 03 A heart was perfused with a solution of 1 in 500,000. It died in systole after 6 hours of tracing.The rate increased steadily from 2.75 in 20"at first to nearly 6 in the same time after between 2 & 3 hours.The shortening of the diastolebecame very obvious about this time &

Plate V.

Extracts from a Heart-perfusion Tracing (Cobra-Venom Solution 1 in 1000,000).



226' after Venom perfusion began.

202' after Venom perfusion began.

177' after Venom perfusion began

153' after Venom perfusion began

130' after Venom perfusion began.

106' after Venom perfusion began.

79' after Venom perfusion began.

57' after Venom perfusion began.

35' after Venom perfusion began.

13' after Venom perfusion began.

Normal before Venom was perfused

Remarks. The time is marked in seconds, the drum is travelling slightly faster in the upper six tracings, completing the round in 24' instead of in 22'. Due allowance being made for this fact, it will be observed that the rate of heart beat increases steadily from the 2nd to the 6th line (counting upwards); it then slows again. The quickening is a venom effect. The late slowing, the movement of the tracing down towards the base line, & the long periods of diastole, all noticed in the upper four lines are evidences of fatigue. The other features of the chart are obvious. It is read from left to right, & systole is upwards.

N.B. This tracing is not alluded to in the text.

The excursus from this time forward lay in the systolic portion of the curve, though there was evidence of fatigue to be clearly noticed.

(9) 12 11 03 A heart was perfused with 1 in 1,000,000 sol-n of venom. The rate of beat before =4, & the excursus =6.5; 30' later the figures are 8 for rate & 7 for excursus; An hour after the venom was run in they are the same as last count. 81' after the commencemeet of venom perfusion the heart suddenly passed into systole, with very small beats of the rate of 10 per second. A certain amount of recovery slowly took place, but within 4 hrs from the venom perfusion starting, the organ was passing into a condition of fatigue. Through this curve one can clearly trace the ~~ddddd~~ influence of the venom & of fatigue pulling in opposite directions, the one towards systole, the other towards diastole.

(10) 13 11 03 A heart was perfused with a sol-n of 1 in 2,500,000. The result was very similar to that found in the last case, the beat nearly doubling in rate, with an almost inappreciable shortening of excursus. At the same time the heart's action ~~ddddd~~ continued very regular. Later the organ showed a strong tendency to pass into the systolic phase, but air was accidentally admitted & the conclusion of the experiment was unsatisfactory.

A number of other tracings were taken, but enough has been said to show the action of this venom on the heart of the frog. At present weaker solutions still are being employed. ?

We may now summarise the lessons learnt from these perfusions.

(1) In concentrated solutions cobra venom kills the heart in a systolic position, & does so more quickly than any other poison with which I am acquainted.

(2) In dilute solutions the venom increases the activity of the heart, both quickening its rate & steadying its rhythm. At the same time it is true that there is no amplification of the beat, provided that the heart is contracting actively & freely before the venom is introduced. The same remark however, holds good for drugs like strophanthin. If the heart is working with a short beat beforehand, any one of these substances will amplify the excursus. This is due to the conditions of the experiment, & need not be farther discussed here.

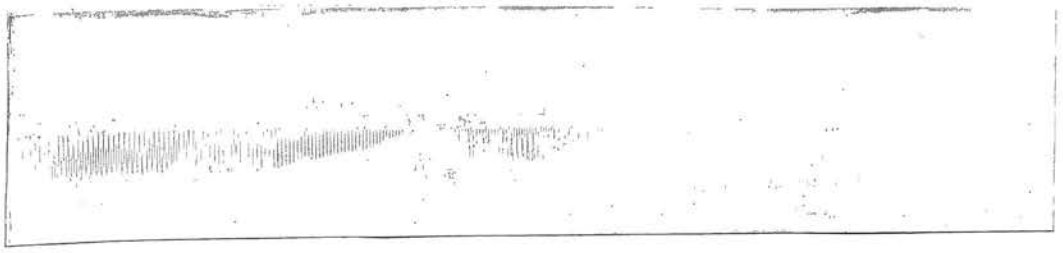
(3) Even when using dilute solutions one finds, after a time which varies with the strength of venom used, that there is a shortening of the beat, &

?: Post script. A solution of 1/5,000,000 shows decided & unmistakeable quickening whilst in one of 1/10,000,000 there is still slight evidence of the action of the venom. RHK.

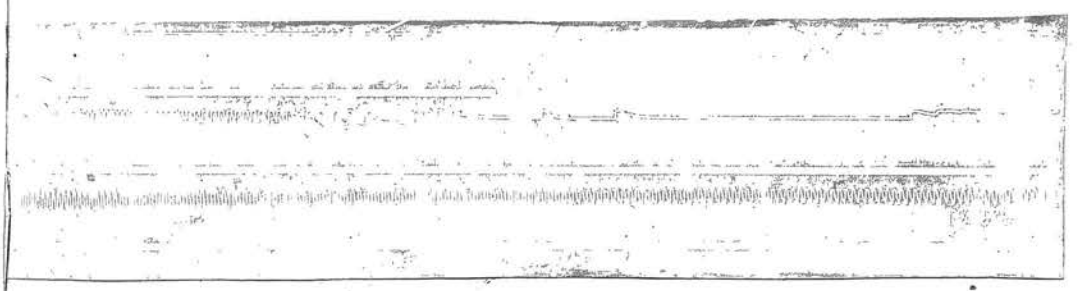
PLATE. VI.

Photographs of Frog-heart Perfusion Tracings, to obtain which Solutions of Strophanthin were used.

(Instructions as in Plate III).



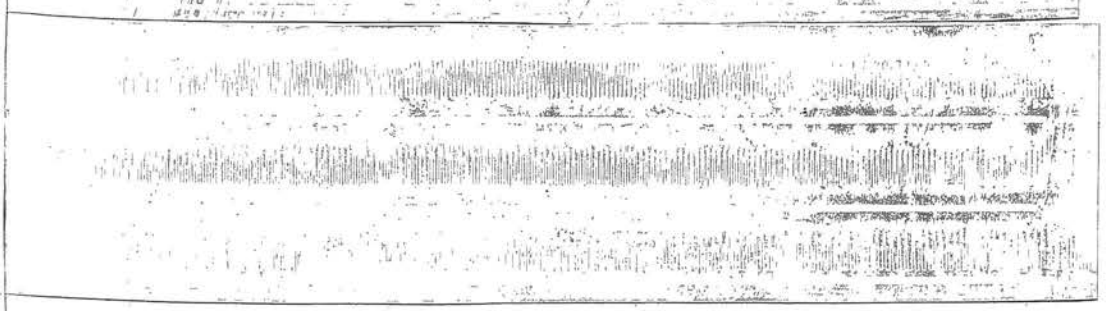
Perfusion with a 1/100,000 solution of Strophanthin.



Perfusion with a 1/10,000 solution of Strophanthin.

(N.B. This tracing should have come first).

Perfusion with a 1/1,000,000 solution of Strophanthin.



Foot-note. Read the tracings from below upward.

that this shortening is always at the expense of the diastolic portion of the tracing. In other words the heart, under the stimulus of cobra poison, tends constantly to pass into a condition of systolic tone.

(4) Lastly, & on this point I lay the strongest emphasis, the ultimate tendency of solutions even so weak as 1/2,500,000 is to kill the heart in systole.

Having ascertained the above facts, it naturally occurred to one to take tracings of the heart's action under the influence of members of the strophanthin group, whilst keeping all the conditions of experiment as much alike those of the preceding series as possible.

By Sir Thomas Fraser's kindness I was able to obtain very pure specimens of Strophanthin & of Acokantherin, & some of the tracings taken from the former drug are here reproduced for comparison with the cobra poison tracings. These speak for themselves, but one may be permitted to draw attention to 2 points viz., that when compared in solutions of the same strength, the action of strophanthin is much slower than that of cobra venom & secondly that under such conditions the former allows of a short rally & a temporary return to diastole after the heart has once passed into a strong systolic position. Such a phenomenon never occurs with cobra venom, which in strong solutions kills once & for all in a state of firm systolic contraction. (See Plate VI)

The same remarks apply to Acokantherin which, in the specimen worked with, appeared to be a weaker poison than Strophanthin.

Lastly I made some experiments with sulphate of atropia. This drug has been recommended for the treatment of cases of cobra bite in India. Some time back I tried it in various doses on rats rabbits & frogs which had received low lethal doses of venom. The result was entirely negative.

Theoretically it should be of value, by virtue of its action on the vagus nerve ends in the heart, for blood pressure tracings show clearly that the heart is slowed by means of stmuli which reach it through the vagi, when cobra venom is circulating in the blood.

Practically one found that, while atropine in the early stages of an experiment has a slight beneficial action on the heart, the opposite is the case later on. Indeed Dr Prentice & I found that even the careful administration of the drug at such a stage was liable to cause sudden failure of blood-pressure & death. This result is readily explained by the light of my recent work; for I find that whereas the sulphate of atropia in strong solutions (ca gr 1/1000) causes death of the heart in diastole, the opposite result holds for weaker strengths. Thus a 1/5000 or a 1/10,000 solution greatly quickens the heart & causes it to pass into a condition of systolic tone, in which the excursus is greatly shortened at the expense of the diastolic portion of the curve.

With still weaker solutions, ex gr 1/50,000, the only observable effect of the drug is to quicken the heart.

It is obvious that if the heart is already under the influence of a poison whose tendency is to kill it in a position of systole, we must be careful how we introduce a drug which has, even in a lesser degree, the same tendency, lest the accumulated influence of the two should produce the very disaster we are trying to avert.

The accompanying plates of photographs from some of my ~~~~ tracings needs little explanation. In all cases the same conditions, as far as possible prevail. Blood mixture was used for the perfusing fluid throughout. The tracings are to be read from left to right, & the systolic end of the stroke is invariably upwards. The white lines below the traces are a record of the time in seconds. Each chart is clearly marked with the nature of the experiment; the letters C V mean cobra venom, & the figures following them show the strength of the solution in which the poison was used. If a lens be used, the details of the tracings can be made out almost if not quite as clearly as in the originals. I am greatly indebted to Mr Burnett BA Lond for his valuable assistance in helping me to photograph my records, also for his excellent drawings to be found on Plate I

Perfusion of the Mammalian Heart.

The number of experiments of this kind so far performed has been limited, but some very clear indications of the mode of action of cobra venom have been obtained. Considerable difficulty has been experienced in getting a cat's heart to beat regularly & steadily, & even rabbit hearts have been far from satisfactory.

By the kindness of Professor Sherrington & Miss Sowton, I was able to do a few experiments with their apparatus in Liverpool, & operating there on cats I had no diifficulty in obtaining good results every time. I used the appara-tus they have fully described in the Report of the Chloroform Committee, which is printed as a supplement to the B M Journal of July 18th 1903. The solution used was as follows:-- Na Cl 0.9%, Ca Cl2 0.024%, K Cl 0.042%, Na H C O3 0.01%, Dextrose 0.1%.

The following notes give a brief summary of the results obtained.

* Exp-t No 1. A cat's heart was perfused with a solution of 1/10,000 of Cobra Venom.

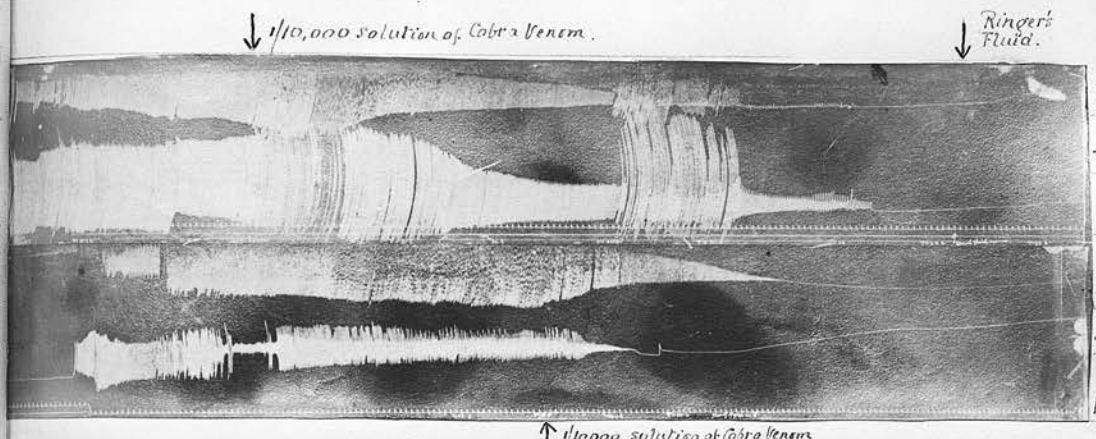
In less than 5 secs the auricle & ventricle passed into a stage of violent excitement, which lasted nearly 175"; they then steadied down & beat with great regularity for 140"; a second period of excitement came on & lasted for 105"; the excursus of the beats then rapidly diminished & the heart had ceased pulsating 125" later. 85" later perfusion with normal fluid was resumed, & after another 200" the ventricle began to beat again, the auricle having commenced nearly a minute earlier. The singular phenomenon was then observed of the occurrence of 2 ventricular beats for each beat of the auricles. This did not last long, but is of more than passing interest as I have noticed the same phenomenon in the body of an animal on which a cobra raised blood-pressure tracing was being taken. The apparent explanation is that there is an obstruction to the onward passage of a wave of contraction, & that this obstacle lies somewhere betwwen the auricle & the ventricle, being of such a nature that the wave is broken up & enters the ventricle at 2 spots, an interval occurring between the 2. The result is that 2 waves of contraction invade the ventricle for each auricular beat. After the heart had been beating for 7 minutes, & had settled down to fair regularity, the venom solution was again run in, with the result that the excursus of both auricle & ventricle rapidly diminished. The ventricle had ceased in 1½min, & the auricle in 4 minutes, thus closing the experiment. The tendency of both the auricle & ventricle to pass into a systolic phase was obvious in both stages of the experiment. The flow through the coronary vessels decreased rapidly & at the close had almost ceased. This was undoubtedly due to the direct constrictor action of the venom on the vessels of the heart.

* Please see Plate III for photographic reproductions of these tracings.

PLATE VII.

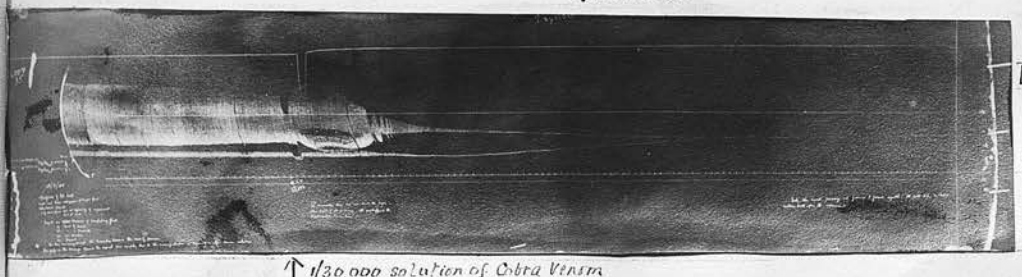
Photographs of Perfusion-tracings from the Mammalian Heart.

[a]t all tracings from left to right, & from above downwards. Systole is upward, diastole [d]ownward. Time is marked in 10 secs. Arrowheads show where venom &c were introduced.



Experiment No 1. Two sheets of tracing shown. The upper shows the 1st stage of the Experiment, the lower the 2nd

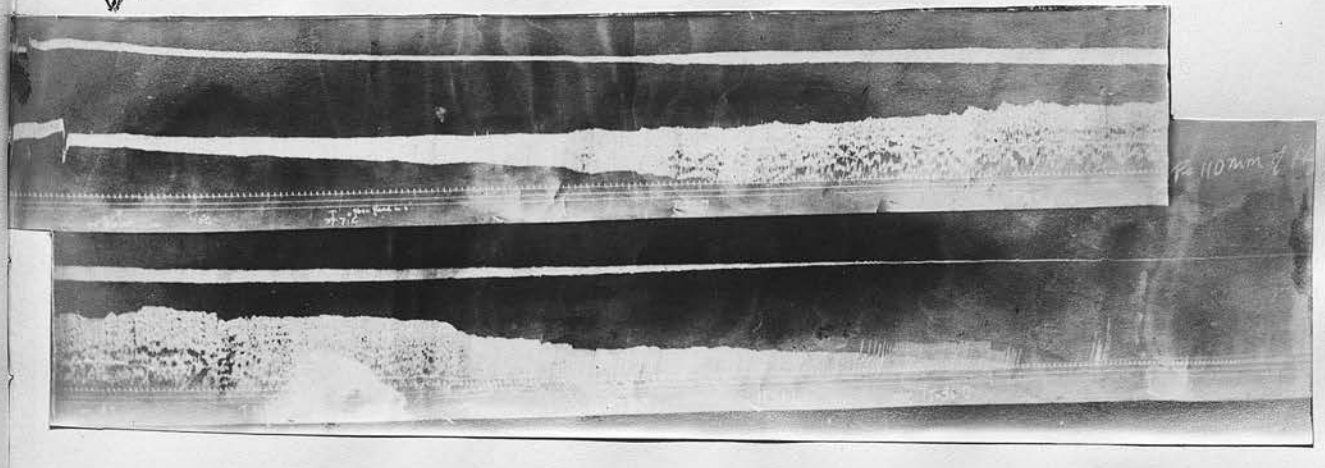
1/10,000 solution of Cobra Venom again ran in.



Experiment not mentioned in the text. Rabbit's heart used.

1/30,000 solution of Cobra Venom

1/50,000 solution of Cobra Venom.



Experiment No 2. Two sheets of the tracing are shown, the first overlapping the second in the photograph.

To the same cause we must attribute another feature of all these 4 experiments, namely the fall in temperature of the perfused fluid, & consequently also of the heart. Anything which slows the flow & so delays the fluid on its way to the heart from the warm bath will in cold climates obviously lower the temperature of the fluids. Professor Sherrington & Miss Sowton have had very frequent occasion to observe that cooling of the fluid perfused slows down the heart. This will explain why in this & the other tracings the rate of heart beat undergoes little if any quickening at first, & soon slows down very markedly. Before one can argue as to the effect of venom on the rate of the mammalian heart beat, it will be necessary to improvise a warm chamber for the organ, & this I hope shortly to do. The fact that a slight quickening is at first observed in some of the tracings instead of an immediate slowing argues that the effect produced is the same as in the frog heart, & is merely masked by the causes already dealt with.

It only remains to add that the excitation of the heart in this experiment was so great that it was not possible to keep the levers on the drum: they rode over the edges of the paper.

Exp-t No 2. A cat's heart was perfused with a solution of 1 in 50,000 of Cobra-venom.

The amplitude of the ventricular excursus began to increase almost at once, reaching its maximum between the 11th & 20th minutes. It then decreased rapidly, & had practically ceased beating 7 minutes later. The auricle was much later in first becoming affected, & also continued beating about 1 minute after the ventricle had ceased. Phases of excitement with remissions are observable throughout both the auricular & ventricular tracings, but are much more marked in the latter than in the former. Both tracings show a decided upward tendency towards systole. The same phenomena, dependent on constriction of the coronary circulation again appear on this tracing.

Exp-t No 3. A cat's heart was perfused with a solution of 1 in 100,000 of venom (cobra).

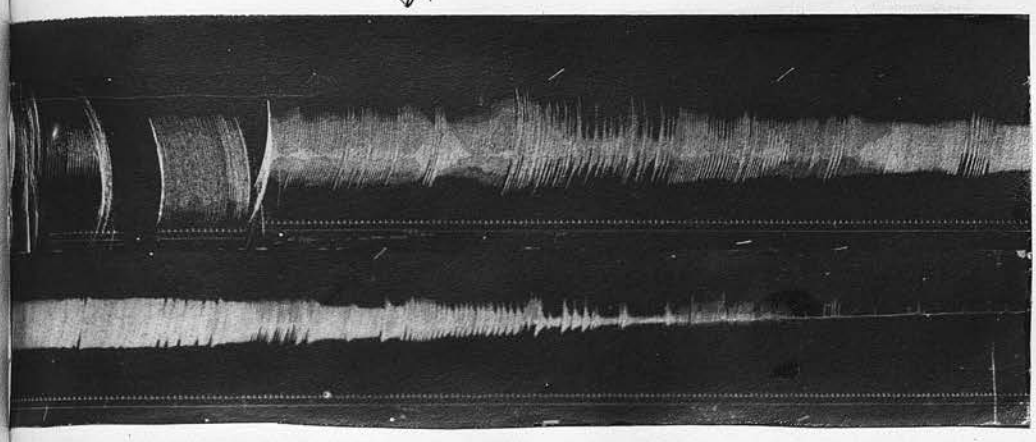
The heart showed a tendency to beat in groups when perfused with the normal fluid. One minute after the venom solution was first run in, the auricular excursus began to show signs of excitement; this stage lasted 7 minutes, & was succeeded by an intermediate phase in which the beat became much more regular than it had been up to date. This lasted for about 7 minutes. The heart then became more irregular than ever, & ceased to beat about 9 minutes later. The auricle alone was recorded. Its tendency towards systole was most marked.

PLATE VIII.

Photographs of Perfusion-tracings from the Mammalian Heart.

(Instructions as in Plate VII)

↓ 1/100,000 solution of Cobra Venom.



Experiment No 3. Auricle alone recorded. Two sheets of the tracing shown.

↓ 1/500,000 solution of Cobra Venom

Experiment No 4.

Four sheets of tracing shown, from above downwards. The irregularity of the rhythm is well shown in the beginning of the second sheet.

Exp-t No 4. A cat's heart was perfused with a 1/500,000 solution of cobra-venom.

Within 2 minutes of the venom reaching the heart ,both beats commenced to increase in volume,displaying a decided rhythm in so doing.4½ minutes later the ventricle became suddenly & violently excited,this stage lasting 11 minutes,& exhibiting an increase of the rhythmical variations of the strokes.At the same time the auricle was passing through a similar but less pronounced phase of excitement.It is of interest to notice that the periods of rhythmical excitation & ~~int~~remission do not synchronise in the tracings of the 2 beats. The heart at first showed a slight quickening,but this was soon masked. For over an hour and a half a steady regular & even beat was maintained.Any changes in rate seemed to follow corresponding alterations in the temperature of the perfused fluid.On the whole the excursions diminished,& the experiment was brought to a close by the venom solu-tion giving out.

When allowance is made for the influence of the coronary circulation this tracing reminds one strongly of the results yielded by frog-hearts. ~~Hee~~ Here the organ is first excited,then steadied,& finally slowly poisoned.It is important to remember that a strength of 1/500,000 of venom might possibly be present in the circulation of a snake bitten man.

A number of other tracings are available to show the effect of cobra venom in strong solutions (1/10,000 up to 1/50,000). In every one of them the tendency of the auricle & ventricle to pass into firm systole are clearly seen,whilst most of them show a distinct quickening of the heart very soon after it first feels the venom. It hardly seems worth while to quote details of these tracings at length,though they are here before me.

One is now in a position to formulate certain conclusions which have been drawn from this work on the mammalian heart:--

(1) The action of strong cobra-venom solutions on the heart of the cat & rabbit is to kill the organ in a position of systole in a very short space of time,both cavities being similarly affected.

(2) The above action is preceded by a phase of violent excitement,during which the movements of the heart are greatly exaggerated.

(3) During the stage of excitation it appears certain that the rate of beat is increased,though this is obscured by a (instrumental) fall in the temperature of the perfused fluid,this fall being due to obvious causes which will require elimination in the future.

(4) The powerful constriction of the Coronary vessels must have a ~~ø~~ very decided effect on the circulation through the tissues of the heart.

(5) Less strong solutions of cobra-venom appear to have a steadying & stimulating effect on the heart.The ultimate tendency of the strengths hitherto ~~her~~ used is to eventually kill the heart,but unfortunately it has not been possible so far to work with any solution below 1/500,000,which however is probably beyond the limit usually found in the blood of a cobra's victim.

In closing this section of my subject, I desire to express my great indebtedness to Professor Schäfer, to Professor Sherrington & to Miss Sowton for their valuable & ready help.

Summary of Conclusions.

Cobra Venom is one of the most powerful poisons known, since it has a distinct action both on the isolated heart & also on the muscular tissue of the blood-vessels of the frog, when it is employed even in solutions of a strength of 1 in 10,000,000.

In the case of the heart & the vessels alike, it appears to act by increasing the irritability of the muscular tissue & so favouring the explosion of motor impulses.

Under its influence the heart beats faster, & tends to pass into a systolic phase.

The experiments with the mammalian heart have seemed to show that the auricle is less easily affected than the ventricle: but this is probably merely due to their relative rates of circulation under these abnormal circumstances of these experiments.

In strong solutions, the action of this venom is to kill an isolated heart in systolic position in a very short space of time, whilst in very weak solutions it appears to act as a stimulant & a tonic, closely resembling stophanthin in this respect.

In all strengths up to 1 in 10,000,000 the effect of cobra venom on the arterioles is the same in kind. It causes spasmodic contraction of the muscular fibre of the vessels, & so lessens the flow of blood through them & raises the arterial blood-pressure.

The increased force & frequency of the heart-beat, which results from the administration of low doses of venom, & which ~~occurs~~ are observed in the early stages of action of even fatal heart-doses, must help to raise the blood-pressure, & thus exaggerate the effect of the arterial spasm. Large doses of venom, which when injected into a vein lower the blood pressure suddenly, act directly on the heart, but probably also on its nervous apparatus. The slow fall of blood pressure which precedes death in experiments with cobra venom is likewise partly due to direct poisoning of the heart muscle.

It is obvious that the present research deals merely with a part of the question, & that in estimating the factors which determine death, one must never lose sight of the inhibition of the heart through the vagus nerves. Whether such inhibition is brought about by the direct poisoning of the centres of those nerves, by the venom, or whether the nerve ends are the parts attacked one cannot at present discuss, for such questions are outside the scope of this paper. Still less can one enter on the influence which an asphyxiated condition of the circulating blood exerts on all centres alike & not least on those of the vagi. These matters will be dealt with in a later communication elsewhere.

In conclusion I desire to express my gratitude fo Sir Thomas Fraser, to Professor Schafer, to their assistants & to all others who have so kindly assisted me in one way or another in the conduct of this research.

J W Elliot.

MB, BS Lond, etc Captain I M S

Appendice I.

Tables of some Experiments for the Fixation of the Minimum Lethal Dose of Specimens of the Venom used.

To ascertain the Minimum Lethal Dose of Cobra Venom (old stock) for Rats.

Date	Weight of Rat.	Dose per kilo.	Result.
1.6.03	·185 kilo	·00025 gramme	Recovered.
do.	·200 kilo.	·0005 gramme.	do.
do.	·185 kilo	·0006 gramme	do.
do.	·180 kilo	·0007 gramme	do.
1.6.03	·179 kilo.	·0009 gramme.	Died on 8th day. Death probably due to Septicaemia.
do.	·172 kilo	·001 gramme	Recovered.
1.6.03.	·180 kilo	·0012 gramme	do.
do.	·165 kilo.	·0014 gramme	do.
do.	·168 kilo.	·0016 gramme	do.
do.	·138 kilo.	·0018 gramme	do.
6.11.03.	·227 kilo	·0018 gramme	Never seriously ill. Recovered.
do	·144 kilo	·002 gramme	Unwell at first. Recovered.
do	·186 kilo	·0025 gramme	Ill at first. Recovered.
do	·235 kilo	·003 gramme	Very ill for two days. Recovered.
do	·157 kilo	·0035 gramme	Very ill indeed for two days. Recovered.
do	·217 kilo.	·004 gramme.	Very ill from the first. Dead in 14 hours

To ascertain the Minimum Lethal Dose of Cobra Venom (new stock) for Rats.

Date	Weight of Rat.	Dose per kilo.	Result.	
5.10.03.	·184 kilo.	·01 gramme	Dead in $1\frac{3}{4}$ hrs.	The Cobra Venom was dissolved in Ringer's fluid (·001 = 1cc of Sol^{n})
do	·157 kilo.	·008 gramme.	Dead in 2 hrs 53'.	
do	·219 kilo.	·006 gramme.	Dead in 2 hrs 15'.	
do	·171 kilo	·005 gramme	Dead in 2 hrs 15'.	
do.	·256 kilo.	·004 gramme.	Found dead in less than $2\frac{1}{2}$ hrs after the injection.	
do	·288 kilo	·0035 gramme		
do	·169 kilo.	·003 gramme		
do	·265 kilo.	·0025 gramme.	Dead in 3 hrs 40'.	
do	·177 kilo.	·002 gramme	Dead in 4 hrs 11'	
do	·197 kilo.	·001 gramme.	Dead in 4 hrs 30'	
6.10.03	·202 kilo.	·0018 gramme	Dead in 4 hrs 43'	
do	·112 kilo.	·0009 gramme	Dead in 4 hrs 38'	
do	·217 kilo.	·0008 gramme.	Dead in 10 hrs 30'.	
do	·219 kilo.	·0007 gramme	Found dead in 21 hrs	
do	·232 kilo.	·0006 gramme	do do do	
do.	·174 kilo.	·0005 gramme	do do do.	
do	·212 kilo.	·0004 gramme	Very ill for 2 days. Appeared to be dying on 2nd. Apparently well on 3rd day.	
do	·193 kilo.	·0003 gramme	Very ill for 1 day. Apparently well on 2nd day	
do	·204 kilo.	·0002 gramme.	do do do do.	

To ascertain Minimum Lethal Dose of

Cobra Venom (old stock) for Rabbit.

Date	Weight in kilos.	Dose per kilo.	Result.
5.6.03	2·200.	·0005 gramme.	T up to 102 next day. R 24. Ht 216. Did not seem ill. Recovered.
7.6.03.	2·100.	·0005 gramme.	T next day 102. R 112. Ht 162. Did not seem ill. Recovered.
do.	1·980.	·001 gramme.	T next day 103. R 132. Ht 300. Quiet. Coat rough & staring. Well on 3rd day. Recovered.
9.6.03.	1·580.	·00125 "	Recovered.
do.	1·790.	·0015 "	Recovered.
do.	1·540.	·00175 "	Recovered.
5.6.03.	1·475.	·0025 "	Ill. Recovered.
do.	1·400	·0025 "	Ill. Recovered.
1.11.03.	1·900	·0025 "	Ill. Recovered.
do.	1·795.	·003 "	Ill. Recovered.
5.11.03.	2·054	·0035	Ill. Recovered.
do	1·650.	·004.	Dead in 5 hours
do.	1·385	·0045	Dead in less than 12 hours.
do	1·790.	·005	Dead in 1¼ hours.

To ascertain the Minimum Lethal Dose of Cobra Venom (new stock) for Rabbits.

November 1903.

Date	Weight in Kilos	Dose per kilo.	Remarks.
	1·625	·001 gramme	Dead in 2 hrs & 50'
	1·610	·0009 "	Dead in $4\frac{3}{4}$ hrs.
	1·860.	·0008 "	Dead in $2\frac{3}{4}$ hrs.
	1·940.	·0007 "	Dead in 76 hrs. (It is doubtful if this animal received its full dose).
	1·885	·0006 "	Dead in $3\frac{3}{4}$ hrs.
	1·800.	·0007 "	Dead in $8\frac{1}{2}$ hrs.
	1·415	·0005 "	Was very deeply cobraised, & seemed on the point of death 24 hrs after injection, but rallied & recovered.
	2·210.	·0004 "	Was very ill, & apparently moribund on the 2nd day, but recovered.
	1·895	·0003 "	Was ill for about 24 hrs, then recovered.

June 1903

To Ascertain the Minimum Lethal Dose of Cobra Venom (old stock) for Frogs.

Weight in kilos.	Dose per kilo.	Result.
·026	·0008 gramme	Recovered.
·0295.	·0009 "	Recovered.
·023	·001 "	Appeared to have recovered. Found dead on the sixth day.
·030	·002 "	Appeared to have recovered. Found dead on the ninth day.
·024	·003 "	Appeared to have recovered. Found dead on the Eighth day.
·022	·004 "	Signs of Cobraism deepened steadily. Found dead on the fifth day.
·024	·005 "	Dead in 9 hrs 20'
·024	·01 "	Dead in 27½ hrs.
·019.	·015 "	Dead in 17½ hrs.
·024	·020 "	Dead in 25½ hrs.
·021	·025 "	Dead in 6 hrs
·0205	·030 "	Dead in 4 hrs 18'

The dose fatal to frogs within 24 hrs is relatively large, as they are independent to a great extent of their pulmonary respiration. It is difficult to feed them in Captivity, & this adds an element of uncertainty to Experiments on them. I do not feel sure that any deaths below the dose of ·004 gramme per kilo can be safely attributed to the poison, as the other animals which died, appeared to have recovered in the interval. Further control Experiments gave almost the same results.

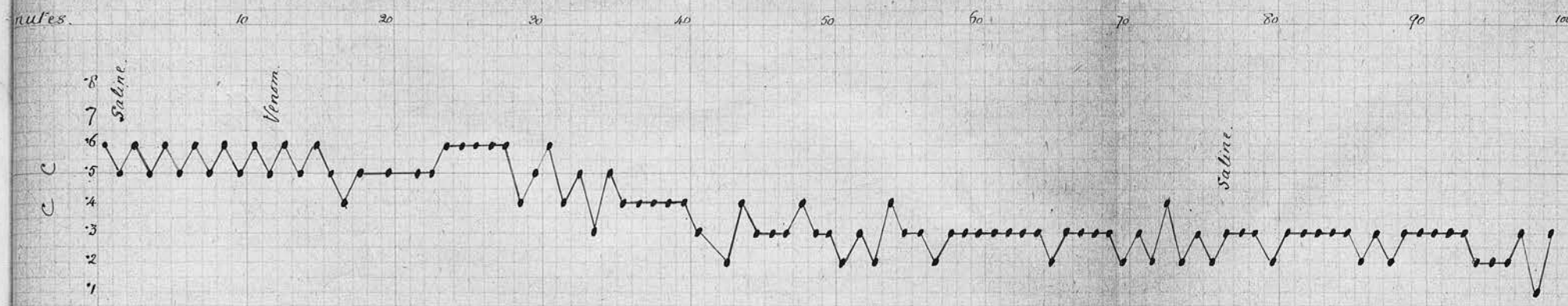
The dose of the new Venom for frogs has not been yet definitely fixed, but all doses of & above ·0007 gramme per kilo have proved fatal. In all cases, signs of Cobraism appeared early & deepened steadily till death supervened. The duration of life after injection was, however, always a matter of days.

Appendix 2

Charts showing results of Perfusion of the Vessels of the Frog with various strengths of Solutions of Cobra Venom.

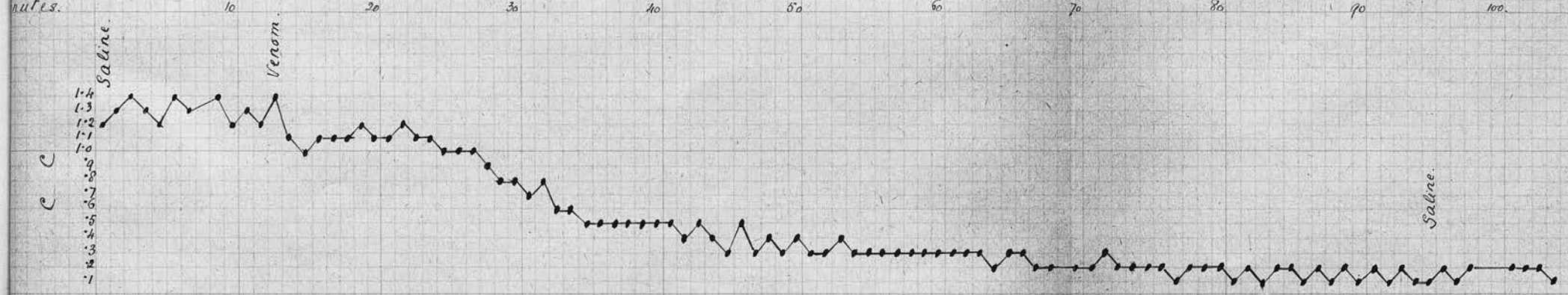
June 6th 1903.

Frog = 21 grmes. Gain in Wt = 2 grmes. Cobra Venom sol.n 1/50,000.



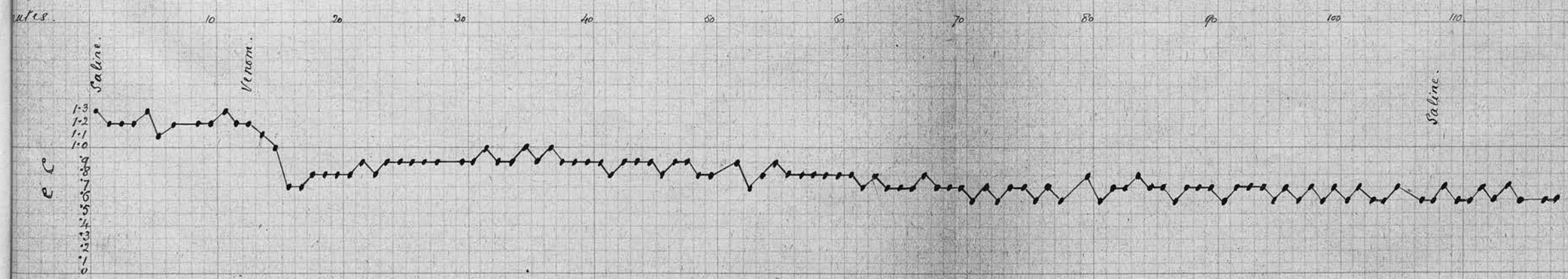
June 7th 1903.

Frog = 15grmes. Gain in Wt = 4 grmes. T = 76°F. Cobra Venom sol.n 1/50,000.



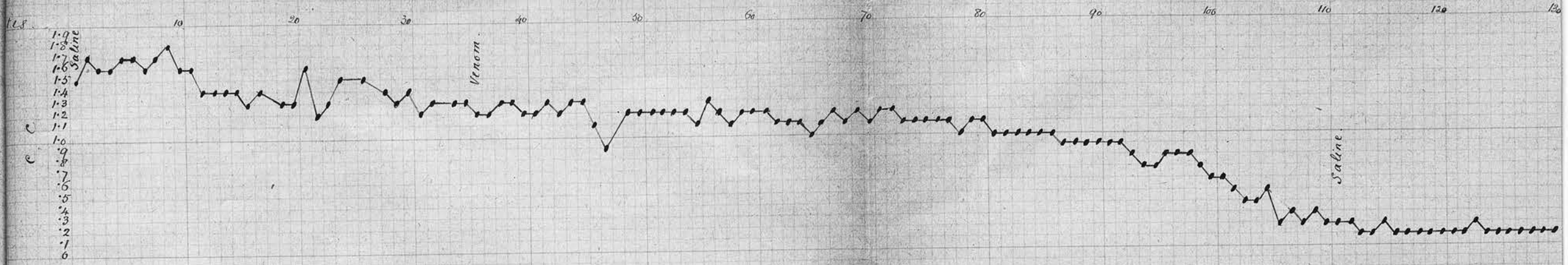
June 8th 1903.

Frog = 18 grmes. Gain in Wt = 13 grmes. T = 60°F. Cobra Venom sol.n 1/100,000. (prepared 24 hours)



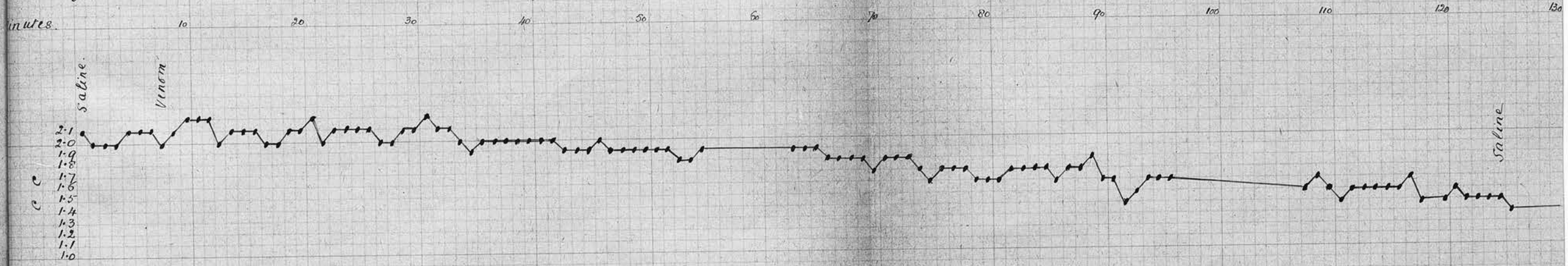
June 9th 1903.

Frog = 20 grmes. Gain in Wt. = 4 grmes. T = 60° F. Cobra Venom Soln 1/300,000 (prepared 48 hrs)



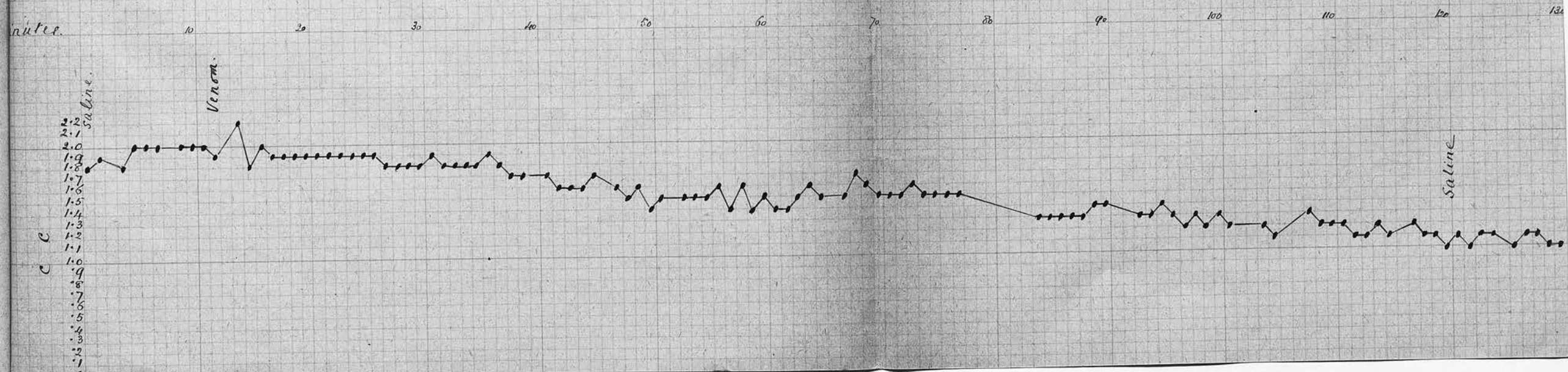
June 10th 1903.

of Frog = 27 grmes. T = 63° F. Cobra Venom Soln 1/1,000,000. (prepared 3 days)



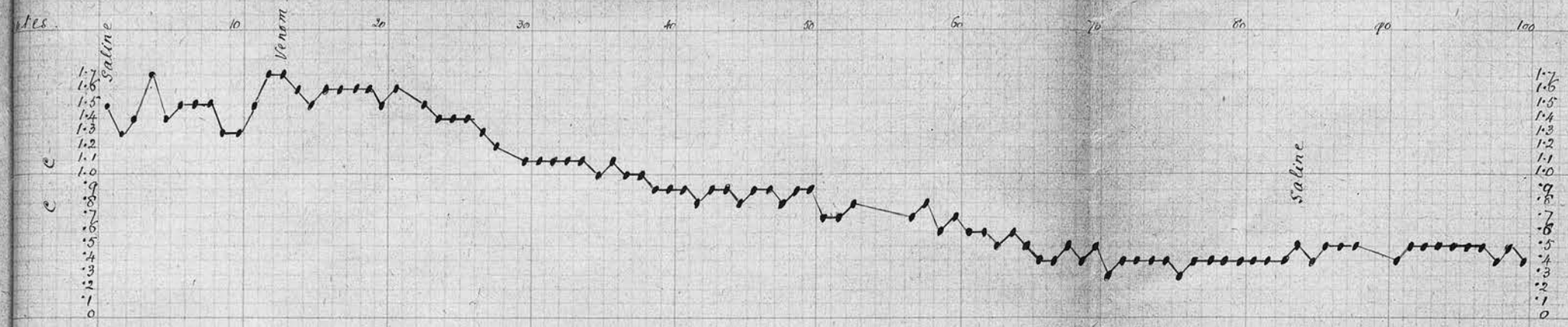
June 12th 1903.

of Frog = 19 grammes. Gain in Wt = 8 grmes. T = 60° F. Cobra Venom solution. 1/2,500,000. (freshly prepared)



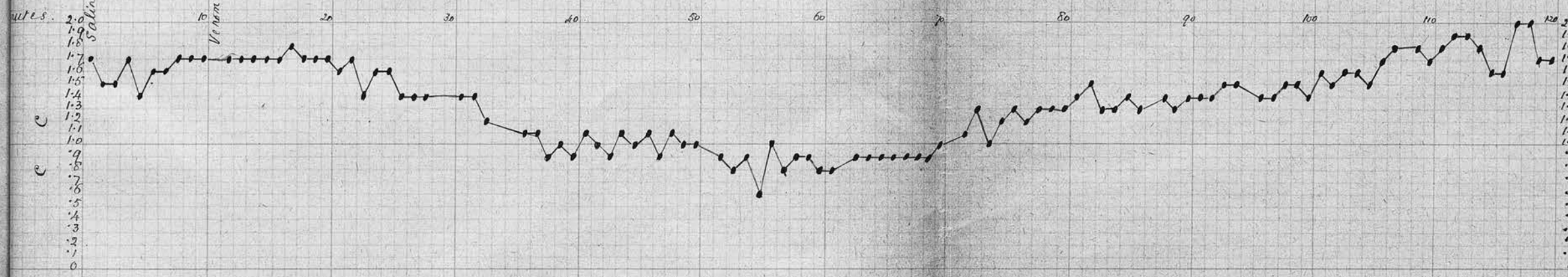
June 13th 1903.

Frog = 28 grms. Gain in Wt = 5 grms. Cobra Venom Solution 1/5,000,000. (prepared 2 days)



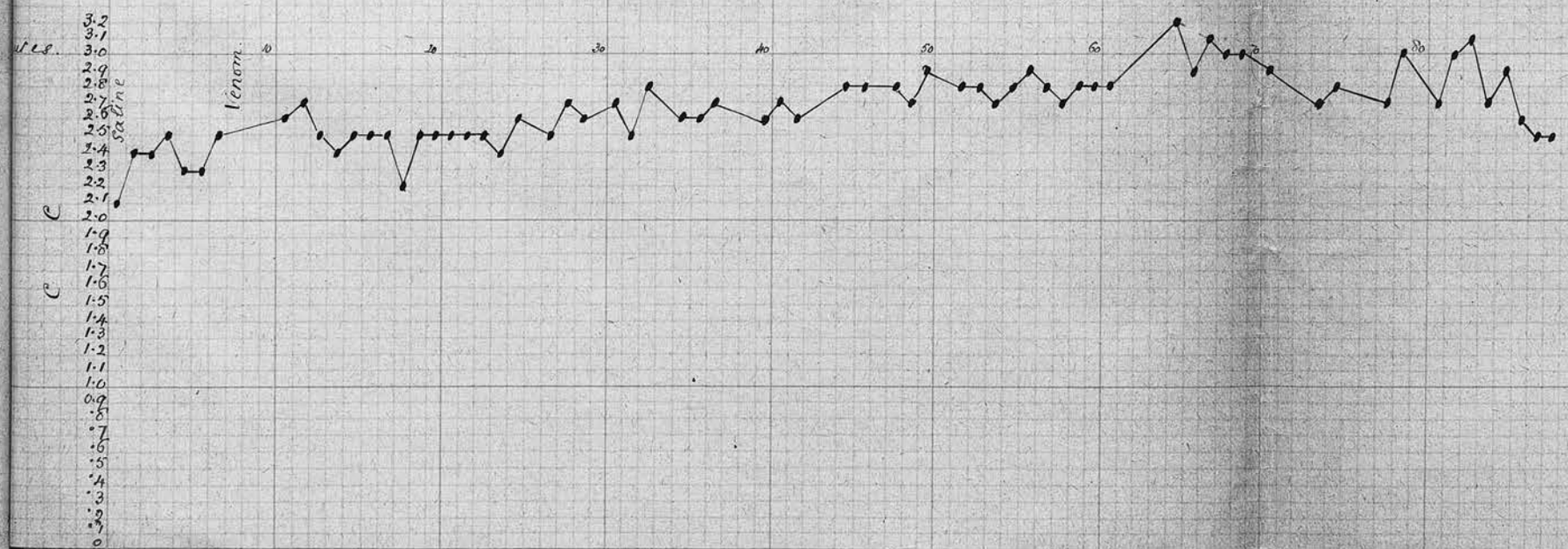
June 13th 1903.

of Frog = 15 grms. T = 62° F. Cobra Venom Solution 1/10,000,000.



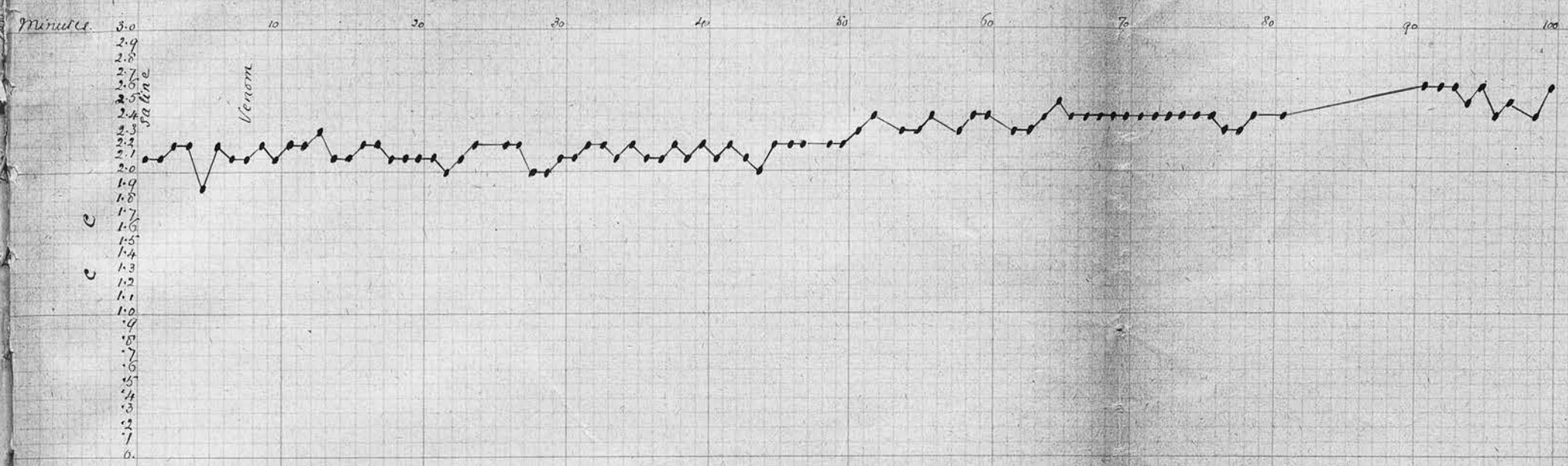
June 14th 1903.

of Frog = 19 grms. Gain in weight = 10 grms. T = 57° F. Cobra Venom solution 1/15,000,000.



June 14th 1903.

Wt. of Frog = 15 grms. Gain in Wt = 8 grms. Cobra Venom Solution 1/20,000,000.



June 10th 1903

Wt. of Frog = 12 grms. T of room = 60° F. Control. Normal Saline solution.