The action of some isoquinoline derivatives / by P.P. Laidlaw.

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Our knowledge of the fairly extensive series of iso-quinoline derivatives has recently been considerably augmented by Pyman.[1] He undertook the study of the oxidative decomposition of laudanosine with the object of producing 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride, which, in view of its close chemical relationships to hydrastinine and cotarnine, was expected to have a similar physiological action. The work was continued along lines opened up by the original investigation, and a number of other new substances were made. These new substances have been examined with a view to determining their pharmacological action, and although the examination cannot be considered as exhaustive, sufficient has been done to suggest an interesting relationship between chemical structure and physiological action. This relationship is still further emphasised when the structure and physiological action of closely related compounds, which have been worked out by other observers, is taken into consideration.

I desire, therefore, to bring forward this relationship in the first part of this paper, and to devote the second part of it to a consideration of the action of one of the new iso-quinoline derivatives which appears to possess features which may render it of use in therapeutics, namely, 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride.

Part I

The action which is characteristic of a large number of iso-quinoline alkaloids is that of producing a condition of increased reflex excitability. The degree of hyperexcitability induced by these substances varies within

wide limits. The effect is comparable in many instances with that of strychnine. It is this strychnine-like effect which appears to bear a definite relationship to chemical structure.

There are three series of compounds corresponding to three series of alkaloids which illustrate the relationship:—

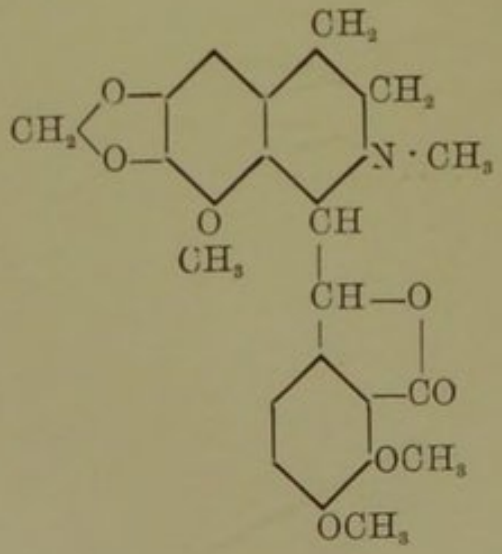
I. Narcotine and its derivatives.

II. Hydrastine and its derivatives.

III. Laudanosine and its derivatives. (This series may also be regarded as Papaverine derivatives.)

The first two have been worked out for the most part by other observers, and with one or two exceptions I have relied entirely on their statements. Where no name is quoted the observation is original, although not necessarily new.

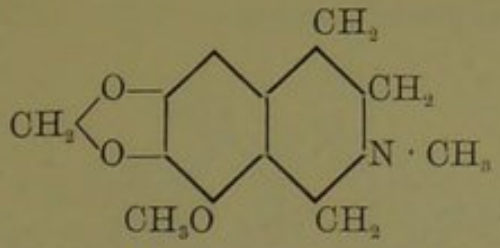
The animals used for my investigations were frogs and guinea-pigs. Both of these readily show a characteristic picture with pharmacological agents of this nature, and moreover, small doses are as a rule sufficient to elicit the characteristic effect in them. Narcotine, amongst other opium alkaloids, has long been known to produce a strychnine-like effect on the spinal cord (von Schroeder,[2] Stockmann and Dott,[4] etc.). It has the constitutional formula



and is the parent of the first series of alkaloids we will consider. It is readily decomposed into opianic acid and cotarnine, and from the latter a series of compounds have been made which produce convulsant effects when administered to animals.

Hydrocotarnine, which occurs in small quantity in opium, was shown by Falck,[3] Stockmann and Dott[4] to produce strychnine-like effects. It

is 8-methoxy 6 : 7-methylene-dioxy 2-methyl-tetrahydro-iso-quinoline.

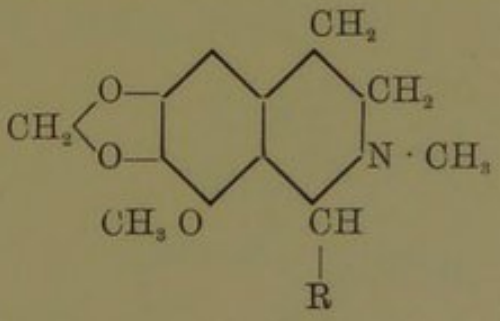


It has been shown by Heinz,[5] who worked with material prepared by Freund and Reitz, that the four following derivatives—

1-ethyl hydrocotarnine,
1-propyl hydrocotarnine,
1-benzyl hydrocotarnine,
1-phenyl hydrocotarnine,

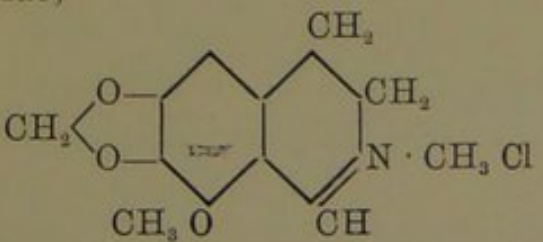
were convulsant poisons of the strychnine class. The ethyl and propyl derivatives are particularly potent in this respect.

This series of compounds which produce the characteristic state of hyperexcitability culminating in convulsions have the general formula



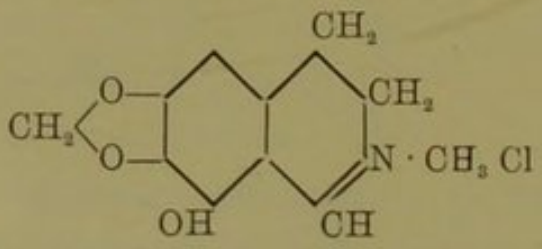
where R is an opianyl, benzyl, phenyl, propyl, ethyl group or merely hydrogen. Anhydrocotarnine acetone also produces a condition of greatly increased reflex excitability in frogs and guinea-pigs, which, in view of this generalisation, would indicate that it reacts as 1-acetonyl-hydrocotarnine.

If, now, R is removed and the substance is no longer a tetrahydro-iso-quinoline derivative, this strychnine-like action disappears, e.g., cotarnine, or 8-methoxy 6 : 7-methylene-dioxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride,



has a purely depressant effect on the central nervous system, and never produces increased reflex irritability. The same is true of the

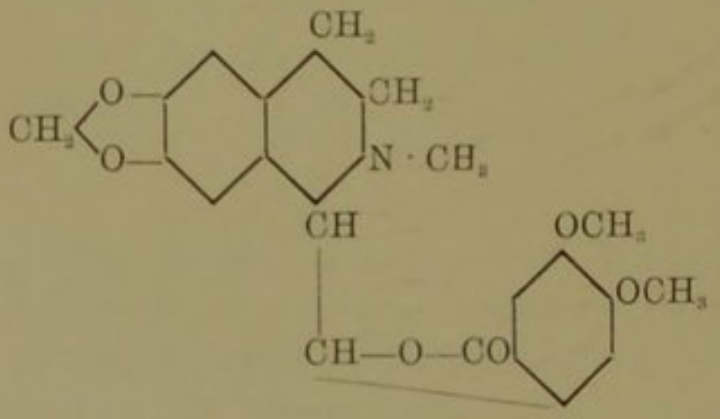
chloride of cotarnamic acid, 8-hydroxy 6:7-methylene-dioxy 2-methyl 3:4 dihydro-iso-quinolinium chloride



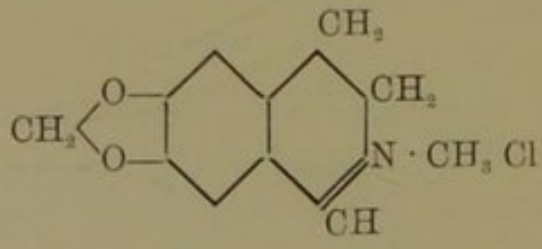
In the frog some stiffness in movements is noticeable after some hours, but convulsions do not supervene, and in the guinea-pig the effect is purely depressant.

Turning now to hydrastine, we find the same relationship, but there are not so many examples illustrative of it.

Hydrastine itself was shown by Falk,[6] Marfori,[7] Cerna,[8] Pembrey and Phillips,[9] to produce a strychnine-like effect. It is narcotine without a methoxy group in the 8 position of the iso-quinoline ring.

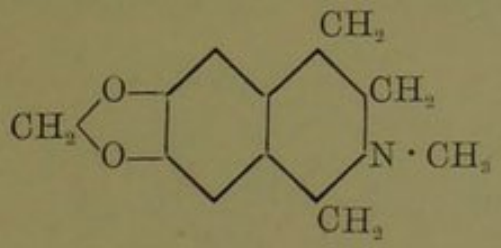


Hydrastinine chloride, the homologue of cotarnine chloride and prepared by analagous methods, has been shown to be devoid of a strychnine-like effect. (Marfori,[7] Falk,[6 & 12] etc.)

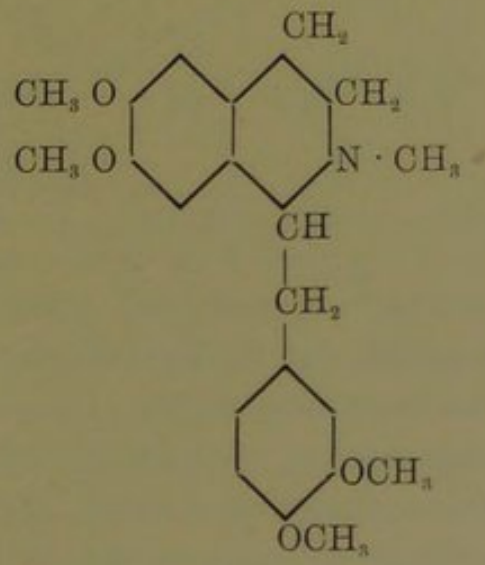


Like cotarnine, it has a purely depressant effect on the central nervous system, and causes death by respiratory failure. On the other hand,

hydro-hydrastinine (cf. hydrocotarnine) possesses a characteristic strychnine-like action in a high degree (Kramm[10]).

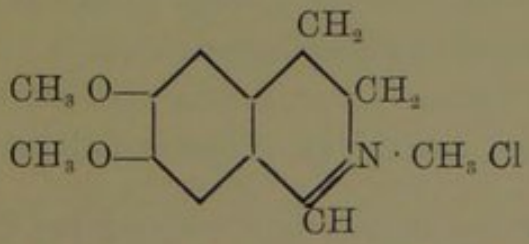


In the laudanosine series a similar relationship obtains. Laudanosine itself was shown by Babel[11] to produce a typical condition of hyperexcitability. Reference to the structural formulae will show its close relationship to narcotine and hydrastinine.



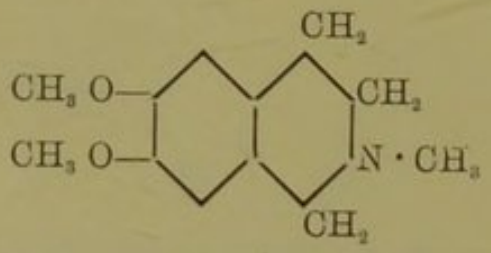
Pyman[1] showed that this alkaloid was susceptible of oxidative decomposition along lines analogous to those producing cotarnine and hydrastinine from narcotine and hydrastine respectively.

The substance 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride analogous to cotarnine and hydrastinine is incapable of producing exaggeration of reflexes.

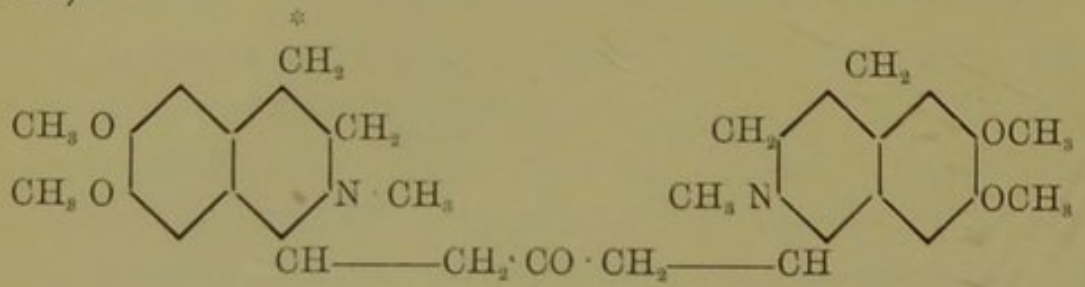


Its general effect is depressant to the central nervous system, and it is strictly comparable in this respect to its allies cotarnine and hydrastinine. The corresponding 2-ethyl compound is precisely similar in action to the

2-methyl compound. On the other hand, 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline induces a typical strychnine-like effect. (Compare with hydro-hydrastinine and hydrocotarnine.)



The only other tetrahydro-iso-quinoline compound in this series that I have tested is acetonyl bis (6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline).

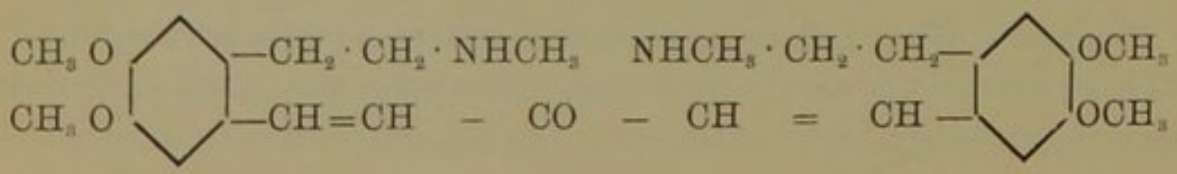


This substance also produces a condition resembling mild strychnine poisoning in frogs and guinea-pigs. This effect is not nearly so striking as in the case of anhydrocotarnine acetone.

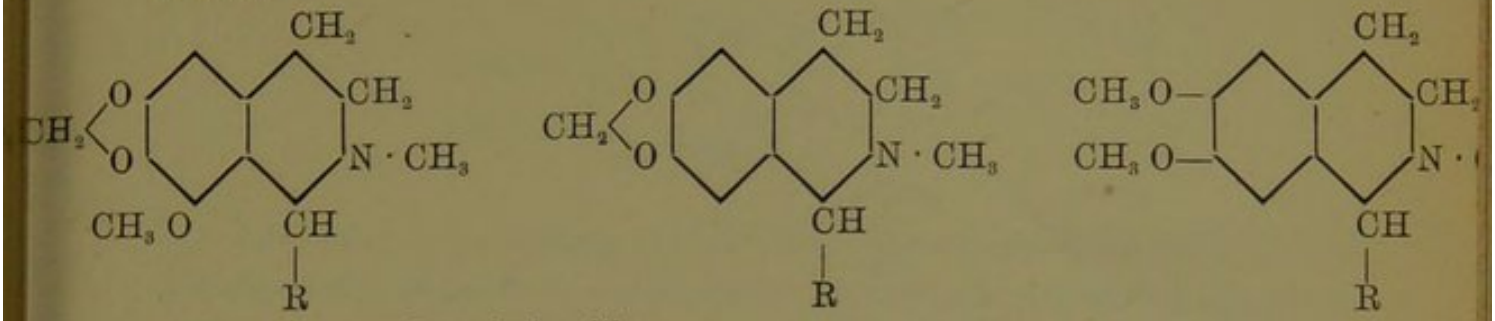
The degree of exaggeration of reflexes induced by these various tetrahydro-iso-quinoline compounds varies considerably. It would require careful comparison of the whole series to prove whether their potency bore any relationship to their composition. It is worth while noting, however, that hydrocotarnine, hydro-hydrastinine and 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline are intense convulsants. Anhydrocotarnine acetone is very potent, and the 1-ethyl and 1-propyl hydrocotarnines are reputed by Heinz to be very active in this respect. The smaller substituting group in the 1 position appears to increase this activity. The larger substituting groups seems to be more depressant to the higher centres (narcotine, laudanosine).

Summing up for these iso-quinoline derivatives, we may say that N-methyl-iso-quinoline derivatives substituted in the 6:7:8 positions

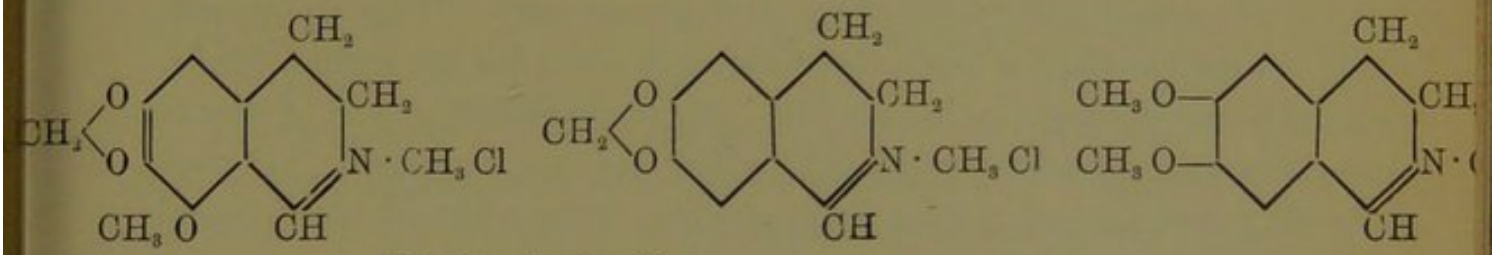
* It is equally possible that the formula of this compound is



with methoxy or methylene-dioxy groups, have or have not strychnine-like actions according as they are tetrahydro-iso-quinoline derivatives or not.

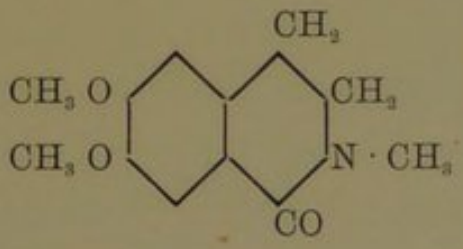


Strychnine-like.

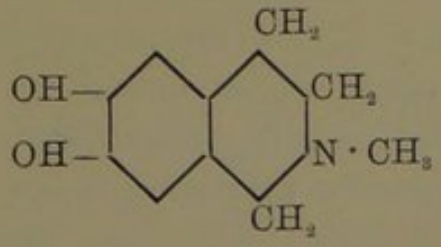


Not Strychnine-like.

A number of other compounds belonging to the laudanosine series have been investigated in connection with their power of inducing hyperexcitability. The introduction of a keto group to the 1 position abolishes the strychnine-like effect in the case of 1-keto 6 : 7-dimethoxy 2-methyl-tetrahydro-iso-quinoline. Although some stiffness of movement is noticeable in the frog, after large doses, similar to early strychnine poisoning, in the guinea-pig the effect is purely depressant.

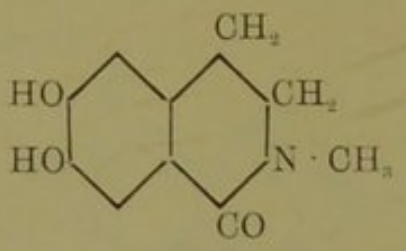


Replacement of the methoxy groups in 6 : 7-dimethoxy 2-methyl-tetrahydro-iso-quinoline by hydroxyl groups abolishes the power of inducing hyperexcitability.

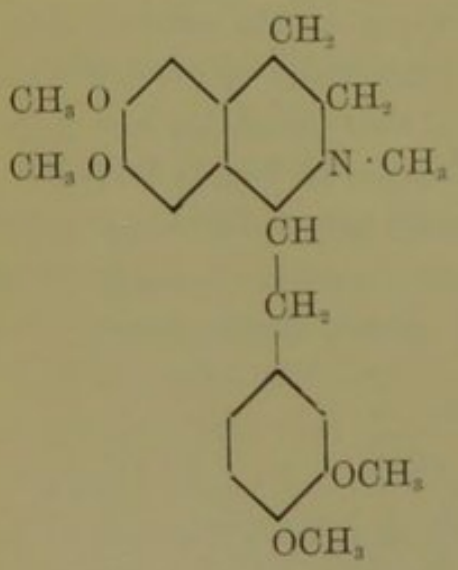


6 : 7-dihydroxy 1-keto 2-methyl-tetrahydro-iso-quinoline is also inactive in

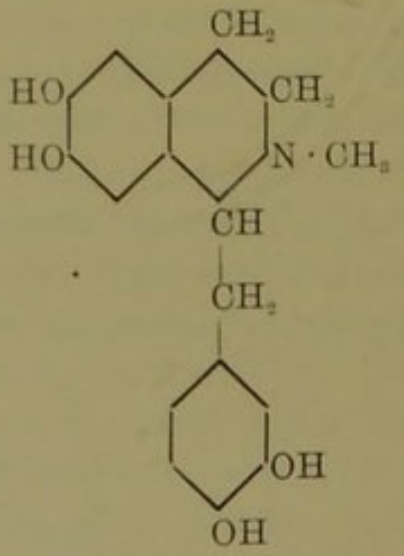
this respect. (Some stiffness of movement is noticeable in the frog.)



Replacement of methoxy groups by hydroxyl groups again abolishes the strychnine-like effect in the case of laudanosine or N-methyl-tetrahydro-papaverine, N-methyl-tetrahydro-papaveroline being devoid of this effect when tested upon both frogs and guinea-pigs. This compound, which has not previously been described, was prepared by Pyman by the action of hydrochloric acid at 170° on laudanosine. The hydroxy compounds are more susceptible of oxidation, and in alkaline solution are readily decomposed. This may to some extent explain the different behaviour of these substances.



N-methyl-tetrahydro-papaverine.
Convulsant

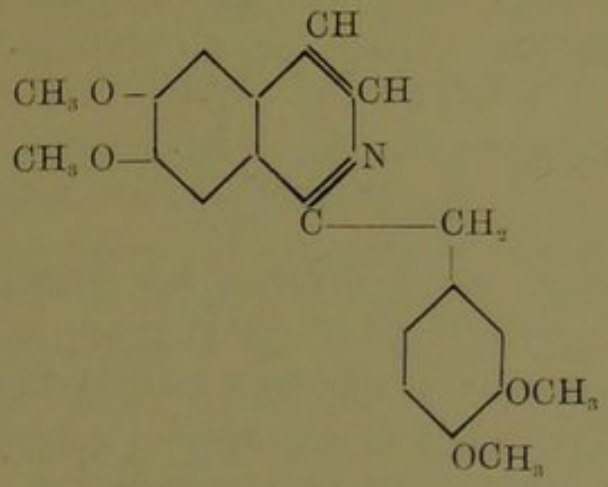


N-methyl-tetrahydro-papaveroline.
Not convulsant.

The previous series of compounds are all substituted N-methyl-iso-quinoline derivatives. There are a number of other convulsant poisons which also possess an iso-quinoline nucleus; for example, papaverine (von Schroeder, Babel). These have not been studied in such detail, as the number of known substances is not so large.

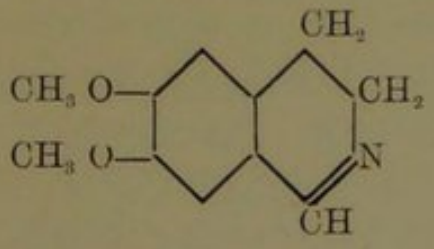
Comparison of those which have come under my notice suggests again a relationship between chemical structure and pharmacological

action. Papaverine produces a stage of reflex hyperexcitability followed by depression in the mammal and coma in the frog.

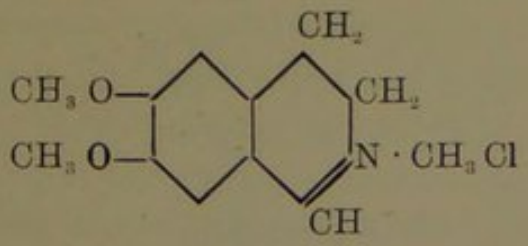


The introduction of a methyl group in relation to the nitrogen abolishes this effect. Papaverine metho-chloride produces a few muscular twitches and then coma in the frog. It produces depression and a few muscular twitches in the guinea-pig. At no stage during onset or recovery from the action of the drug is there any sign of a strychnine-like effect. It thus falls into line with our law with regard to 6 : 7-dimethoxy 2-methyl-iso-quinoline derivatives (see above). When this is reduced to the tetrahydro derivative (laudanosine) the strychnine-like action reappears.

A similar relationship obtains in the simpler compound 6 : 7-dimethoxy 3 : 4-dihydro-iso-quinoline. This substance resembles hydrocotarnine in its strychnine-like action.

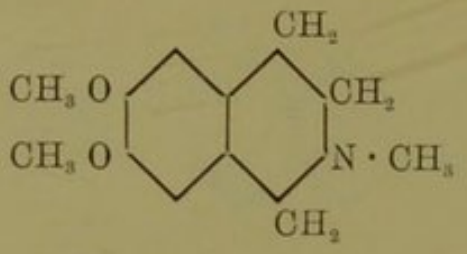


Methylate the nitrogen and this property disappears.



6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride.

Reduce this to 6 : 7-dimethoxy 2-methyl-tetrahydro-iso-quinoline and the strychnine-like effect reappears in full strength.

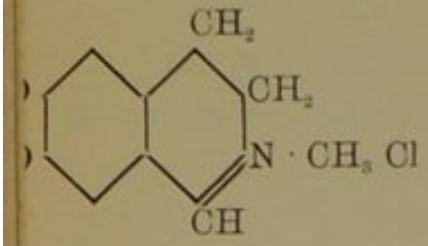


It is thus evident that a different law prevails in the case of iso-quinoline derivatives without a methyl group on the nitrogen, but sufficient examples are not available for a generalisation.

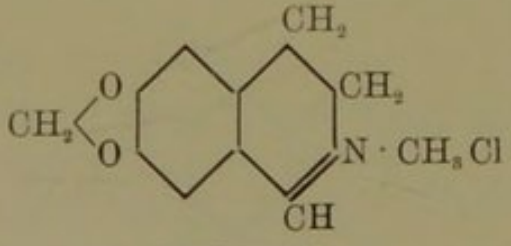
It seems clear, however, if the compounds containing hydroxy groups or keto groups are excepted, that one generalisation will cover all these iso-quinoline alkaloids. Iso-quinoline compounds substituted in 6 : 7 : 8 positions with methoxy or methylene-dioxy groups are convulsant when the nitrogen is trivalent, and are devoid of this effect when the nitrogen is pentavalent.

Part II

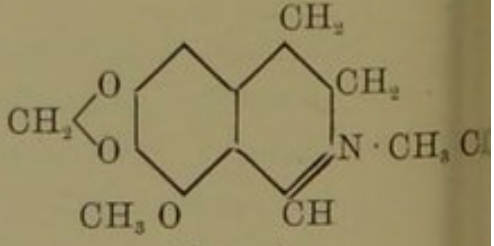
The alkaloid which forms the subject of this part of the paper is 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride. As will be seen from the structural formulae below, it has close chemical relationships with hydrastinine and cotarnine. These two alkaloids have for long enjoyed a reputation in therapeutics as haemostatics, particularly in abnormal uterine conditions. The similarity in chemical constitution suggests a similarity in physiological action. This would seem to be the more probable when the similar action of the parent alkaloids and other derivatives from them is taken into account. Reference to the earlier part of this paper will make this point clearer. The close relationship between the new alkaloid and hydrastinine and cotarnine is well shown in their structural formulae.



diamethoxy 2 methyl dihydro iso-quinolinium chloride.



Hydrastinine chloride.



Cotarnine chloride.

It was therefore considered advisable to undertake a comparative investigation of the three substances. This appeared to be the more desirable since it was soon clear that differences in action between these three compounds were for the most part differences of degree and not of kind.

Previous investigators have unfortunately obtained very contradictory results when working with hydrastinine or cotarnine. This necessitated the conduction of a number of experiments with a view to ascertaining their mode of action with greater certainty, and if possible reconciling the divergent views of earlier workers. A number of experiments were also undertaken to determine the action of 6:7-dimethoxy 2-ethyl 3:4-dihydro-iso-quinolinium chloride. It was found that in most respects its action did not differ materially from that of the corresponding 2-methyl compound. But it is considerably more toxic.

6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride was given hypodermically in varying doses to frogs and guinea-pigs. Very little can be made out by this means of investigation beyond the two facts that it is depressant to the nervous system and that the cause of death in the mammal is failure of the respiration. When 50 mgm. doses of the alkaloid are given to guinea-pigs (about 700 grams) no abnormal symptoms are noticeable for the first twenty or thirty minutes. At the end of this time there is usually observable a few signs of depression, such as disinclination to move, cessation of feeding, etc. When the animal is disturbed, however, the movements are well co-ordinated and apparently normal.

With larger doses, 100 mgm. for a guinea-pig of about the same weight, the depression becomes much more obvious, and towards the end of the first hour succeeding the administration of the alkaloid the respiration becomes slower and is slightly laboured. This dose is sometimes fatal, and it is noticeable that the respiration becomes gradually slower and more laboured and then intermittent. The heart continues to beat vigorously until the respiration is failing. With the failure of respiration the heart beat becomes weaker, and a few convulsive movements ensue and the animal dies.

A guinea-pig survived three subcutaneous doses of 75 mgm. of the alkaloid in twenty-four hours. It is therefore either readily destroyed or rapidly excreted.

The descriptions of the action of cotarnine or hydrastinine on the

intact mammal, as given by various authors (Falk, Marfori, von Bunge, Ronsse, etc.) are much the same as the above. A few experiments were made with cotarnine, and essentially the same clinical picture was seen with toxic doses; but cotarnine appeared to be less toxic. Doses of 150 mgm. of cotarnine were found to be fatal for guinea-pigs of about 700 grams, but 100 mgm. doses were not. The heart beat also did not appear to be quite as well maintained during the later stages of poisoning by this substance. On the central nervous system these three alkaloids exert a depressant action, and cause death by respiratory failure. The failure of the respiration is of central origin, since there is no evidence of any well-marked peripheral action by these three substances (see later).

The vascular system. A series of experiments were performed with a view to determine the action of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride on the vascular system. These were for the most part performed upon pithed cats, but a number were also performed upon anaesthetised animals. Chloroform, ether, paraldehyde and ether, and urethane, were the anaesthetics employed. The main features of the results obtained upon cats were confirmed upon rabbits.

When a dose of 10 mgm. of the new alkaloid is injected into the blood stream of a pithed cat, and the blood pressure is recorded by a mercury manometer from the carotid artery, there is noticeable a rise of blood pressure. At the same time as the blood pressure rises the heart beat becomes slower and the oscillations of the mercury manometer increase in size. With larger doses, 20 mgm., the same action is observable, but both are slightly more marked. Considerable variation is met with in the height of the rise of blood pressure. It is never very large, and rarely exceeds 40 mm. of mercury with doses of 10-20 mgm. Fig. 1 shows one of the smaller rises of blood pressure; others will be seen in other figures. The rise in blood pressure is the result of two factors—(1) slight vaso constriction, (2) increased cardiac output.

The rise of blood pressure being partly accounted for by increased cardiac activity, and being never very large, it will be obvious that the vaso-constriction must be slight. It was found, indeed, that the vaso-constriction was not easy to demonstrate.

Inspection of the viscera during the administration of a full dose of the alkaloid appeared to show that some constriction of the arterioles occurred, but it was never incontestable except in the case of the highly vascular organs, such as the uterus of a virgin rabbit. In this instance

slight pallor occurred as the result of the administration of the drug, but the experiment is complicated by the fact that the alkaloid causes a contraction of the uterine muscle. In oncometric observations a change of organ volume is sometimes seen, but as a rule the volume remains practically unchanged. It would seem that the rise of blood pressure compensates for the vaso-constriction, and the blood content of the viscus is unaltered. The intestines and kidneys were the viscera employed in making oncometric observations. (See fig. 16.)

In order to eliminate the effect of variations in blood pressure, a number of perfusion experiments were performed upon isolated organs. Slight slowing of the venous outflow was noticeable as the result of injecting 6:7-dimethoxy 2-methyl 3:4 dihydro-iso-quinolinium chloride into the perfusion cannula. Fig. 2 is a drop record of the venous outflow from a rabbit's kidney under the influence of the alkaloid. The effect is small and of short duration. A number of experiments were performed with a perfusion apparatus similar to that employed by Brodie and Dixon[23] when studying the innervation and action of drugs on the vessels of the lungs. Here the venous outflow is collected in a small air-tight receiver, from which it is sucked away by a pump which is capable of very fine adjustment so as to balance exactly the venous outflow. The volume of the fluid in the receiver is recorded by a Brodie bellows recorder. Variations in venous outflow cause variations in the amount of fluid in the receiver, and hence in the record traced by the bellows recorder. In all cases 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride caused a slowing of the venous outflow.

The vaso-constriction is the result of an action of the alkaloid upon the plain muscle of the arterioles, and cannot be attributed to an action on nerve endings or the vaso-motor centre. For the rise of blood pressure is no greater in an anaesthetised animal with the vaso-motor centre intact than in a pithed animal with the vaso-motor centre destroyed; indeed, it is usually less in the former case than in the latter. The effect of the anaesthetic might account in part for this, but the response to the alkaloid was no greater in a high-pithed animal (respiratory and vaso-motor centres intact) than in a low-pithed animal. Moreover, after sufficient ergotoxine had been given to cause complete reversal of the adrenine response (see Dale[21 & 22]), the rise of blood pressure was unimpaired.

In this respect it appeared at first that 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride differed from hydrastinine, for most

observers attribute the vaso-constriction it induces, in part at any rate, to an effect on the vaso-motor centre. Thus Falk[6 & 12] found that hydrastinine produced a constriction of the arterioles, which he attributed in part to an action on the vaso-motor centre, since chloral in large doses diminished but did not abolish the rise in blood pressure induced by this substance. Marfori[7] found that section of the cord in the cervical region in a dog abolished the usual rise of blood pressure induced by hydrastinine. But the protocol of the experiment illustrating this point shows that his animal was in very poor condition after the operation. Ronsse[24] found that chloral had practically no effect on the rise of blood pressure induced by hydrastinine. Pick[13] found a diminution of venous outflow from the femoral and mesenteric veins in a dog as a result of the administration of hydrastinine.

Kurdinowski[18] found no variation in the rate of outflow from a perfused isolated uterus, and therefore attributed its specific constriction effect to a central action. von Bunge,[14] on the other hand, found an increased outflow from the veins of isolated perfused kidneys and diminished outflow from the veins of perfused hind limbs and spleen. He attributed the effect of hydrastinine to an action on a peripheral nervous mechanism and not on the muscle, because he found that the venous outflow from an organ which was removed from the body and perfused at once, was diminished when hydrastinine was added to the perfusion fluid, while the venous outflow from the organ which was perfused some hours after death of the animal, was increased when hydrastinine was administered. In his view the nerve or nerve-ending died before the muscle, and this explained the difference in the result of the two cases. In this position he stands alone, and the evidence in his favour is not of the best.

In view of this conflicting evidence, a number of experiments were made with hydrastinine, and it was found that its action on the vessels is almost identical with that of 6:7-dimethoxy 2-methyl 3-4-dihydro-iso-quinolinium chloride.

It produces a rise of blood-pressure of about the same height as that of the new alkaloid. As will be seen later, this is partly explained by increased cardiac output. It produces a slight vaso constriction and diminished outflow from the kidney vessels (fig. 3). It produces a similar degree of diminished venous outflow from the hind limbs of a cat perfused by the Brodie-Dixon method. The rise of blood-pressure is not greater in animals under anaesthesia with the vaso motor-centre intact than in

pithed animals. A high-pithed animal does not show a better response than a low-pithed one. Finally, after sufficient ergotoxine had been given to reverse completely the adrenine response, the rise of blood-pressure induced as the result of the administration of hydrastinine is unimpaired (fig. 4). The evidence in favour of a central action is not good, and depends for the most part on a supposed selective depressant action of chloral on the vaso motor-centre, and is disproved by the evidence adduced above. The action of hydrastinine is due to a direct effect on the muscle of the arterioles, and is at best a weak one, very similar to that of 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride. Cotarnine is usually stated to have no effect on the arterioles, (Falk,[15] Marfori,[16] Ronsse,[24]) and this is in agreement with the results of my experiments. When a dose of this substance is injected intravenously into a pithed cat there is produced a sudden fall of blood-pressure with a slow return to the normal.

Kehrer found in some few cases a slight rise after the primary fall, and he attributes this to a central effect. The amount of the rise appears to have been trivial, and was probably due to some other factor, since it was variable in his experiments and absent in my own. The colour of the viscera remains unchanged under full doses of the substance or in some instances there is a transient flush observable, but this effect is slight. Fig. 5 shows the result of adding cotarnine to the perfusion fluid, which was employed to perfuse the hind limbs of a rabbit. There is practically no alteration in venous outflow. In some instances a slight increase in venous outflow was obtained.

Action on the Heart. The second factor in the rise of blood-pressure is increased cardiac output. When 6 : 7-dimethoxy 2-methyl 3-4-dihydro-iso-quinolinium chloride is injected into the perfusion cannula which is being employed to perfuse an isolated heart after the Locke-Langendorff method, there is noticeable a slowing of the heart beat accompanied by an increase in force. When cardiometer records are taken of the cardiac output, the results are very similar. Fig. 6 shows such a record from a pithed cat. It is obvious from inspection that the output is increased, and that the rate is perceptibly diminished. Measurement shows that (1) the average output per beat is 41 v. (where v. is a constant depending on the bellows recorder); (2) the heart rate is 35 beats in 10 seconds before administration of the drug. At the point where the maximal effect of the drug occurs—

The average output per beat is 56 v.
The rate is 31 beats in 10 seconds.

Before administration of the alkaloid the output in 10 seconds = 1,431 v., and afterwards the output in 10 seconds = 1,736 v.

In other words, the output per minute is increased by a little more than one-fifth.

Figs. 7 and 8 show the effect of hydrastinine on the isolated heart and on the cardiac output respectively. Measurements from the cardiometer record show :—

Rate before drug	42 beats in 10 seconds.
Average output per beat	14 v.
Rate after drug	37 beats in 10 seconds.
Average output per beat	19 v.
Output per 10 seconds before drug ...	588 v.
Output per 10 seconds after drug	703 v.

In other words, the output per minute is increased by a little less than one-fifth.

In this respect also hydrastinine has a very similar action to that of the new alkaloid.

Cotarnine, on the other hand, does not produce the same effect. The heart becomes slower, but the output remains the same, or may even be diminished (marked decrease in force of heart beat or output per beat is unusual). Fig. 9 shows a fairly typical result. In this tracing before the administration of the drug the average rate of heart per 10 seconds was 32, the average output per beat 12 v.—hence output per 10 seconds = 384 v.

At the maximal effect of the drug (20 mgm. intravenously, 2½ kilos. cat)—

The average rate of heart per 10 seconds was 29·5.
The average output per beat was 11 v.
Hence output per 10 seconds = 324 v.

In other words, the output from the heart under the influence of cotarnine is diminished by about one-fifth.

It is clear that the fall in blood-pressure induced by cotarnine is of cardiac origin. Vaso dilatation may play a part, but if so it must be very slight. The fall of blood-pressure induced by cotarnine preparations is recorded by Heinz[5] to be of cardiac origin. Kehrer also attributed the fall to diminished cardiac activity. Neither appears to have demonstrated the point successfully, however. The effects of hydrastinine and cotarnine

on the vascular system are not of such a kind as would seem to render them valuable therapeutic agents in haemorrhage.

Since the effects of these three substances on the vascular system cannot explain their reputation as haemostatics, it appeared to be possible that they might aid the process of blood coagulation. A number of experiments were therefore conducted to determine the influence of these substances on the clotting of blood-plasma and fibrinogen solutions.

None of them can replace fibrin ferment. In small doses they did not affect the rate of clotting, or, as far as could be determined, the consistency of the clot. In large quantity they delay the rate of clotting. 10 c.c. of oxalate plasma 0·5 c.c. calcium chloride solution, and 4 drops fibrin ferment clotted in ten minutes. An exactly similar test mixture to which sufficient 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride was added to make a 1 in 1,000 solution took thirteen minutes.

Similar results were obtained with hydrastinine and cotarnine. Marfori's[16] statement that cotarnine is unable to hasten the process of blood coagulation is thus confirmed and extended to the two other alkaloids. There still remained the possibility that these substances might have an effect on the rate of blood coagulation when injected into an animal. It was possible that their presence in the circulation might, although incapable of influencing the rate of blood coagulation directly, bring about some difference in the normal equilibrium point indirectly. That is, produce an effect comparable to that produced by peptone, but in the opposite direction. To test this point an anaesthetised rabbit was bled from one carotid artery at intervals into a series of small test-tubes. The coagulation time was determined by timing the point at which the test-tube could be inverted without spilling the contents. This method of determining the coagulation is, of course, crude, but the results are as good as those with more complicated apparatus. It was found that the coagulation time was reduced after the administration of 20 mgms. 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride, from about 6 min. to 1 min. 25 secs. As, however, six small bleedings (2 c.c.) had been made in the fifteen minutes, it was doubtful how far this result is due to the drug and how far to the influence of bleeding, for bleedings shorten coagulation time. A similar series of bleedings was therefore made upon a second rabbit as a control, and it was found that after the first three bleedings from the carotid artery the coagulation times became very irregular, and some were very short, e.g. 1 min. 45 secs. It is very probable that the short times of later bleedings are due to

small quantities of fibrin formed at the cut end of the carotid artery which were swept into the test-tube on collecting the later samples. The method is therefore worthless for making a series of comparative coagulation times.

A method for timing the rate of blood coagulation on small samples was then employed, and blood taken from the ear vein of a rabbit. Here the results were again very irregular in the normal animal. Thus coagulation times as low as fifty-eight seconds are obtained if the blood be taken from a large ear vein and pressure is employed along the vein to aid the expulsion. On the other hand, if the bleeding is taken from a very small superficial vein near the tip of the ear the coagulation time may be nearly four minutes. The results obtained depend on the amount of kinase picked up by the blood during its passage from the vessel to the surface, and this depends on (1) the depth at which the vein lies, (2) whether pressure is used to aid the expulsion of the drop of blood. This method was therefore abandoned since the results obtained are incapable of accurate interpretation. Attempts were also made to measure coagulation times by bleeding a series of samples through a paraffin-coated cannula from the carotid artery. In this case also, clotting started in the cannula at the end of twenty minutes, and the coagulation times became very irregular. These methods having failed to demonstrate any action of the series of alkaloids on the coagulation of the blood, the point was not further investigated. It is clear that none of these substances have a profound influence on the coagulation time of blood since they never cause intravascular clotting even when given in heroic doses

So far the haemostatic effect of these substances is unexplained, but, as will be seen below, these drugs have a definite effect on the uterine musculature, and it is this effect which is probably the source of their reputation in therapeutics. Their rational application appears to be limited to uterine haemorrhage.

Action on the Uterus. On the isolated uterus of cat, rabbit, or guinea-pig, the effect of a few milligrams of 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride is to produce a well-marked increase in tonus. The degree of contracture varies considerably with different organs, but the response is always motor.

Fig. 10 shows the effect of 10 mgms. of the new alkaloid on an isolated virgin cat's uterus suspended in 250 c.c. of warm oxygenated

Ringer solution. On this particular uterus a dose of 3 mgms. produced an almost maximal response. Occasionally a poor response is met with, as the result of a dose of 10 mgm. a slight rise of tonus without any alteration in the rhythm may be the only observable effect.

Similar results are seen when similar quantities of hydrastinine are given under these conditions (fig. 13). Kurdinowski[18] found identical results in the case of perfused isolated rabbits' uteri. Cotarnine with small doses (3 mgms.) does not give quite such a good response as the other two; but, in view of the variability in the degree of the response, no great weight can be attached to this point. The effect of 10 mgms. is shown in fig. 12. It is in all essentials similar to the other two. Kehrer[17] found similar effects with cotarnine in the case of the cat's uterus.

When the movements of the uterus are studied in the body the results depend on the animal used. The response is different for example in the pregnant cat from that in the non-pregnant. In the non-pregnant cat an injection of 10 mgms. of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolium chloride causes a relaxation of the uterus and inhibition of rhythm (fig. 13). This effect persists after section of the hypogastrics. It is evident, since the isolated uterus gives a definite motor response (fig. 10), that the effect of this alkaloid upon the muscle of the uterus is overcome by an inhibitor effect generated in the cells of the post-ganglionic neurones of the sympathetic system. The dominant influence of these cells is in the non-pregnant cat inhibitor (Dale[21]).

That this is the explanation of the relaxation is probable, since when large doses of nicotine have been given, and these cells are greatly depressed, the response to the alkaloid becomes motor.

Moreover, in the pregnant cat, where the dominant sympathetic supply has become motor (Dale), the effect of this substance is to produce contraction.

Again, in the rabbit, which has a dominant motor supply in non-pregnant and pregnant conditions, the response to the alkaloid is a well-marked powerful contraction (fig. 14).

Hydrastinine gives precisely similar results. There is something peculiar with regard to the effect of cotarnine on the rabbit's uterus, for when its movements are studied *in situ* there is no contraction observable when doses as large as 25 mgms. are given intravenously (fig. 15). Yet on the excised organ it exercises a marked effect (guinea-pigs, rabbits and cats). I can offer no explanation of the anomaly.

On the uterus then there is a twofold action by these substances—

(1) direct on the plain muscle;
(2) through the sympathetic nerve cells.

The result of these two actions may be a pure motor response, as in the rabbit and pregnant cat, or an inhibitor response as in the non-pregnant and virgin cat. That this series of compounds possesses an action on the ganglion cells or the sympathetic system, supplying the uterus, is unfortunately incapable of further proof. Owing to the peripheral locality of these cells it is impossible to conduct degeneration experiments which would place the above conclusion beyond all doubt.

One feature of the action of these substances on the uterus is worthy of mention. Repeated administration of large doses for some time produces a greatly increased amplitude in the rhythm of the organ. And this persists after removal of the drug. This probably is the result of an increased excitability of the uterine muscle, induced by this series of iso-quinoline derivatives. Kurdinowski[18] was able to show increased excitability as a result of perfusing the isolated rabbit's uterus with Locke's solution containing hydrastinine in small quantity. The explanation in the one case probably holds for the other two.

Another contradiction in the pharmacology of these alkaloids is met with at this point. Kehrer found the same results as those described above with hydrastinine and cotarnine on the isolated uterus of cats and rabbits; but he describes and figures tracings which show that both these alkaloids produce contraction of the uterine muscle in the body. He states that pregnant and non-pregnant cats respond in the same manner. I am quite unable to reconcile his results with those described above, and am convinced that there must have been some source of error in his experiments which I am quite unable to indicate.

It might be thought that other cells of the sympathetic system would be affected in a similar manner. This, however, does not appear to be the case, since direct application of the new alkaloid to the superior cervical ganglion of a cat in weak and strong concentration did not cause any variation in pupil and nictitating membrane. Moreover, an excellent response could be obtained by stimulating the cervical sympathetic with a weak tetanising current (coil 23-25) both before and after application of the drug to the ganglion.

As mentioned above, the rise of blood-pressure is unchanged after full doses of ergotoxine, consequently hydrastinine and 6:7-dimethoxy

2-methyl 3:4-dihydro-iso-quinolinium chloride do not appear to have any action on the sympathetic nerve cells innervating the plain muscle of the vessels.

The conclusion is thus arrived at that this action on sympathetic nerve cells or postganglionic nerve fibre is limited to those cells or fibres innervating the uterine musculature. This strictly limited effect of this series of alkaloids on sympathetic nerve cells is somewhat unusual, and yet is the only one which will explain the facts.

On the urine flow the new alkaloid has practically no action. In one experiment a transient diminution of the urine flow was noticed after 20 mgms. of this substance had been given intravenously, accompanied by a small transient diminution in kidney volume. Fig. 16 shows the result of another experiment. The rise of blood-pressure counterbalances the vaso constriction, kidney volume is practically unaltered, and the urine flow remains unchanged. It is noteworthy that a diminution in urine flow was noticed by Falk[12] as the result of the administration of hydrastinine, while other observers could detect no difference. My experiments lead me to the conclusion that such effects as are produced by these drugs on the urine flow are so slight as to be negligible.

Excretion. It appeared probable from the fact that von Bunge[14] detected hydrastinine in urine that the path of excretion would be the same in the case of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride. 0·2 gram. of this substance was given in a gelatine capsule by mouth to a dog. The urine was collected for the next twenty-four hours, and worked up in the following manner:—After a preliminary filtration the urine was made strongly alkaline with caustic soda, and shaken out four times with chloroform. The chloroform extracts were joined together and washed with a small quantity of water. The chloroform extract was then shaken with dilute hydrochloric acid. The aqueous layer, which was of a primrose-yellow colour, was made strongly alkaline with caustic soda and the extraction with chloroform repeated. The chloroform extract was washed with water and taken to small bulk on the water bath after the addition of a few drops of hydrochloric acid. A yellow residue remained. This was crystallised from alcohol and ether and again from chloroform. The product consisted of clusters of fine primrose-yellow needles, but was contaminated by traces of amorphous brown pigment. Yield 0·065 mgms. or 32 per cent. of theoretical. The product melted at 53·5-55° C., and when mixed with its own bulk of the drug originally administered it melted at 56-57° C. The melting point

of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride is 61-62° C. (corr.). The physical properties of the product were the same as the original product, and, moreover, the physiological action was identical; it produced a rise of blood-pressure accompanied by some slowing of the heart beat (fig. 17), and had the characteristic effect on the isolated uterus.

There can be no doubt, then, that the method of excretion of this iso-quinoline derivative is by the urine. The yield obtained was not very large, but it is very probable that if larger doses were given and the urine collected for a longer period after the administration that this would be greatly increased.

This series of iso-quinoline derivatives is without action on intestinal movements. Isolated rabbit's jejunum suspended in a Ringer bath continues its rhythmic movements, unaltered in time, extent or tonus, when any one of this series of alkaloids is added to the bath.

Bladder. The tone of the bladder is very slightly increased by 6:7-dimethoxy 1-methyl 3:4-dihydro-iso-quinolinium chloride, and this was also found to be the effect of hydrastinine. Cotarnine, on the other hand, was without effect.

Respiration. As was noticed in the earlier part of this paper, 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride and hydrastinine and cotarnine have an effect on the respiratory centre. In large doses their depressant action on the centre is sufficient to cause cessation of respiration and death.

Slowing of the respiration without significant alteration in depth was noticed when doses of 20 mgms. of any one of the three iso-quinoline derivatives was administered intravenously and the respiratory movements recorded above the blood-pressure. Cotarnine has a slightly more marked effect than the other two.

6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride has practically no effect on striped muscle. A nerve-muscle preparation soaked in 1 in 1,000 solution of this alkaloid in Ringer solution for two hours responded quite well to electrical stimulation of its nerve, or of the muscle direct. This concentration is considerably above that which can occur in the body. Santesson[19] found that there was a slight diminution in sensitiveness to stimulation through the nerve when large doses of hydrastinine were given to frogs. He compares this result with those he obtained with iso-quinoline and N-methyl-iso-quinoline.[20] The methylation of the nitrogen producing a mild curare effect. The effect

is not nearly so profound as that produced by methylation of nitrogen to quaternary bases and can only be of secondary importance.

In conclusion, I desire to express my indebtedness and to thank Dr. F. Pyman for correcting the chemical formulae of this paper, and for supplying me with the new alkaloids he had prepared, also to acknowledge the kind assistance and advice of Dr. H. H. Dale throughout the work which is the foundation of this paper.

Summary

1. A review of the action of a number of iso-quinoline alkaloids is made, and their action is compared with their chemical constitution.

A law of relationship for one series is put forward.

2. The new alkaloid 2-methyl 3:4-dihydro 6:7-dimethoxy-iso-quinolinium chloride has an action very similar to that of hydrastinine.

3. The generally accepted view of the action of hydrastinine on the functions of the body is erroneous in several particulars.

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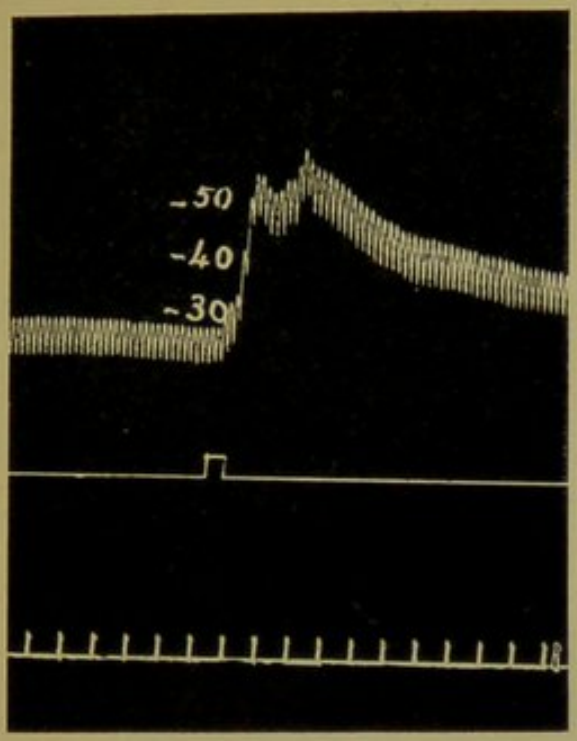


FIG. 1. Cat, decerebrate. Blood pressure, base line and signal. Time in 10″. Effect of 10 mgs. 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

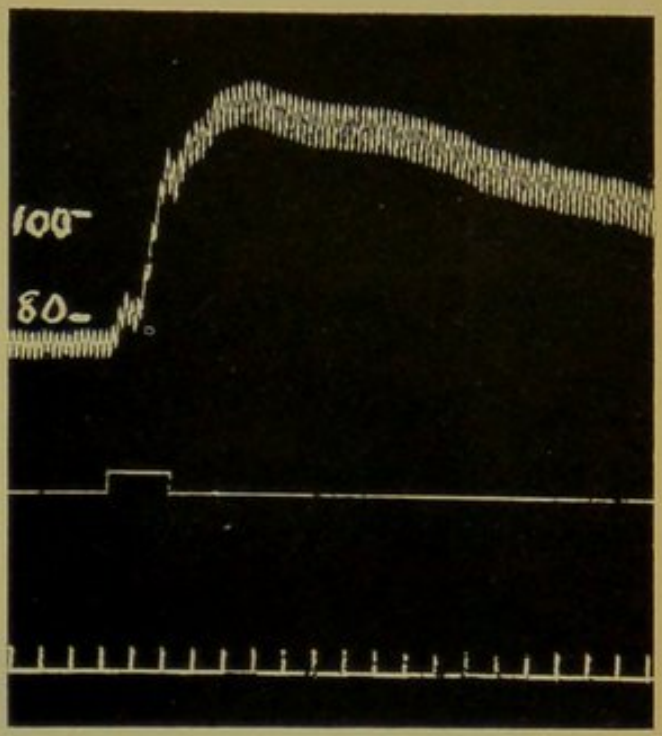


FIG. 4. Cat, decerebrate. Blood pressure, base line and signal. Time in 10″. Sufficient ergotoxine had been given previously to reverse completely the adrenine response. Effect of 20 mgrs. hydrastinine.

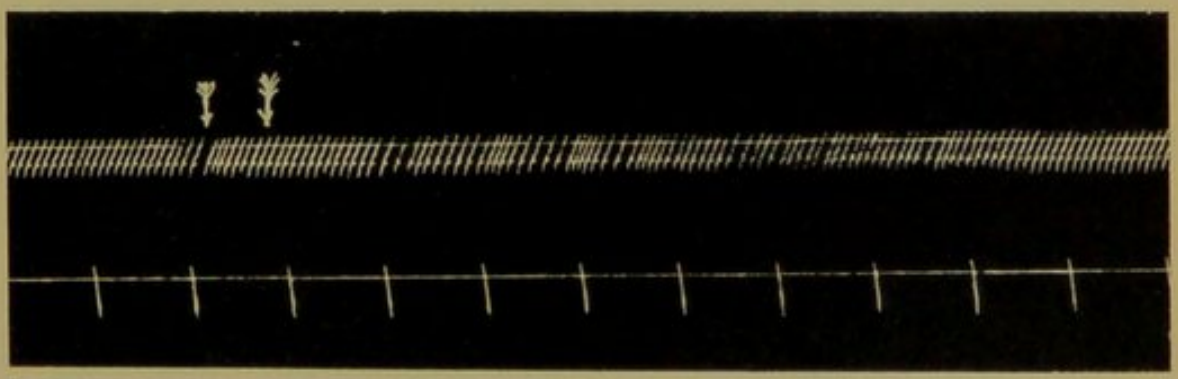


FIG. 2. Perfused rabbit's kidney. Drop record of venous outflow. Time in 10″. At arrow, 5 mgrs. 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride injected into the perfusion cannula.

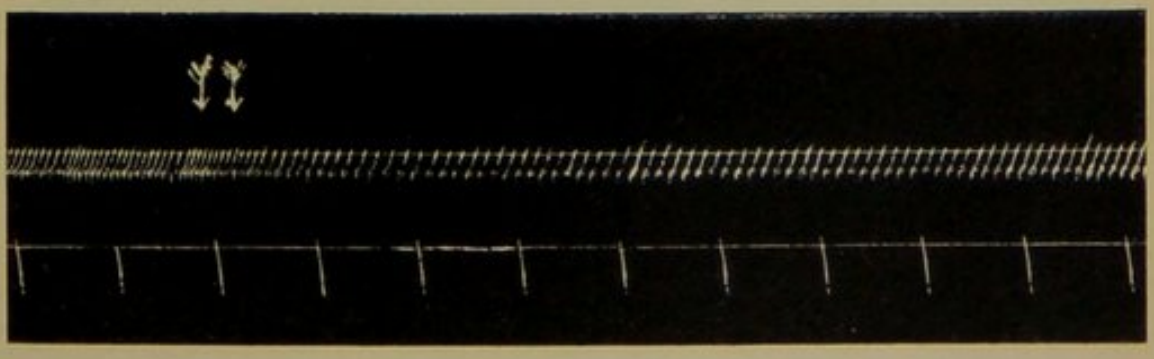


FIG. 3. Same experiment as above. Effect of 5 mgrs. hydrastinine.

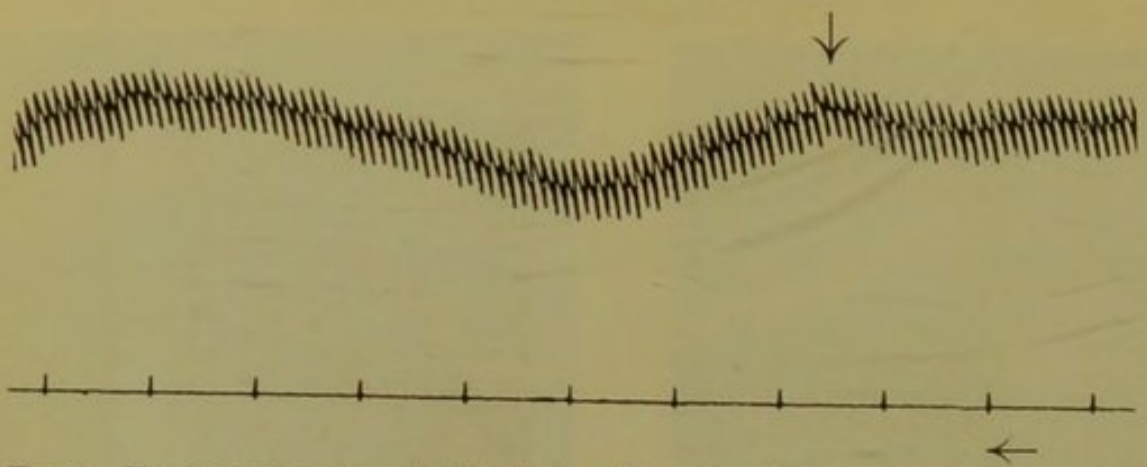


FIG. 5. Venous outflow from hind limbs of rabbit. Brodie-Dixon method. Time in 10″. Absence of effect on injecting 10 mgrs. cotarnine into perfusion cannula. To be read from right to left.

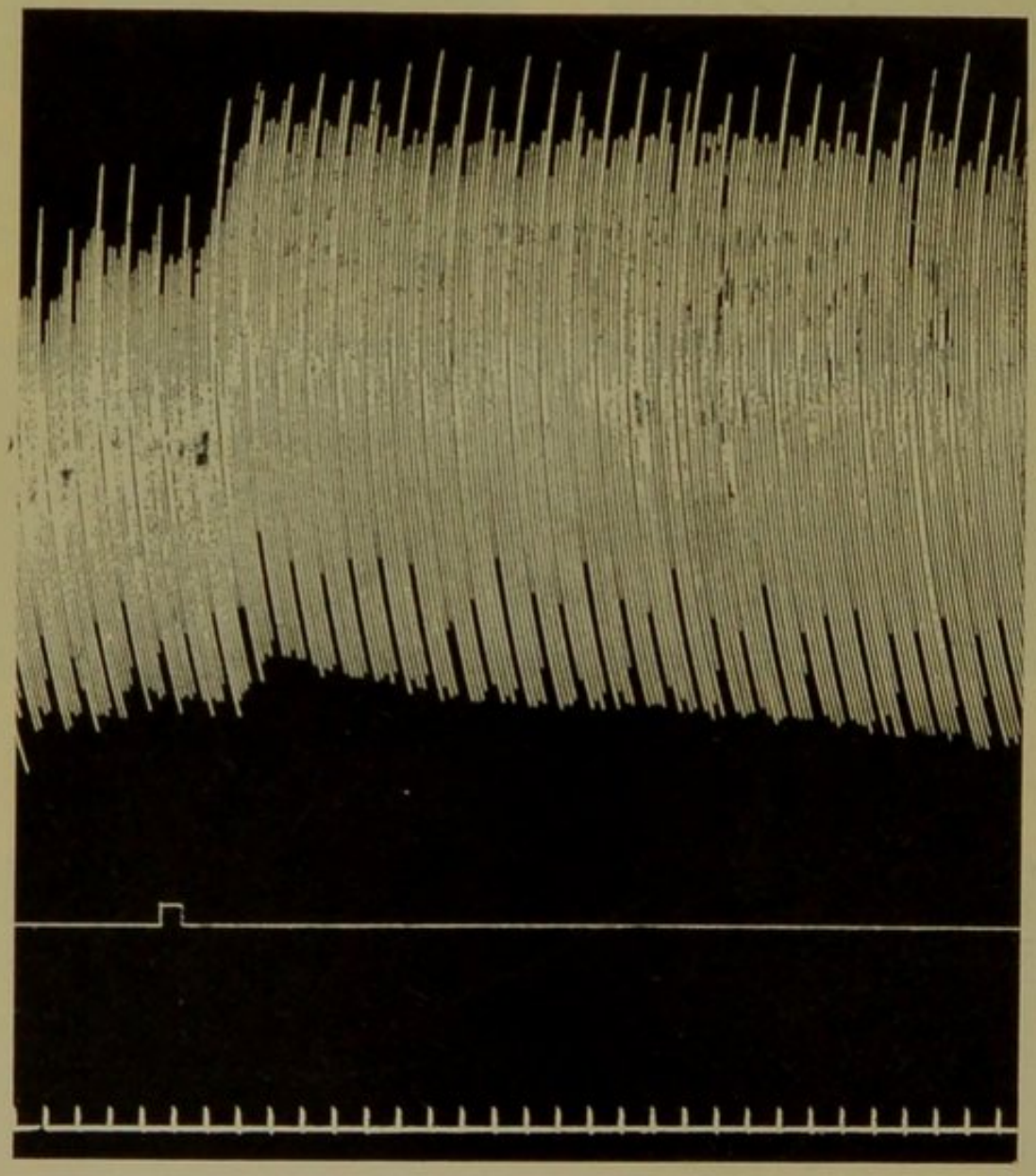


FIG. 6. Cardiometer record from cat's heart. Decerebrate cat. Effect of 50 mgrs. of 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

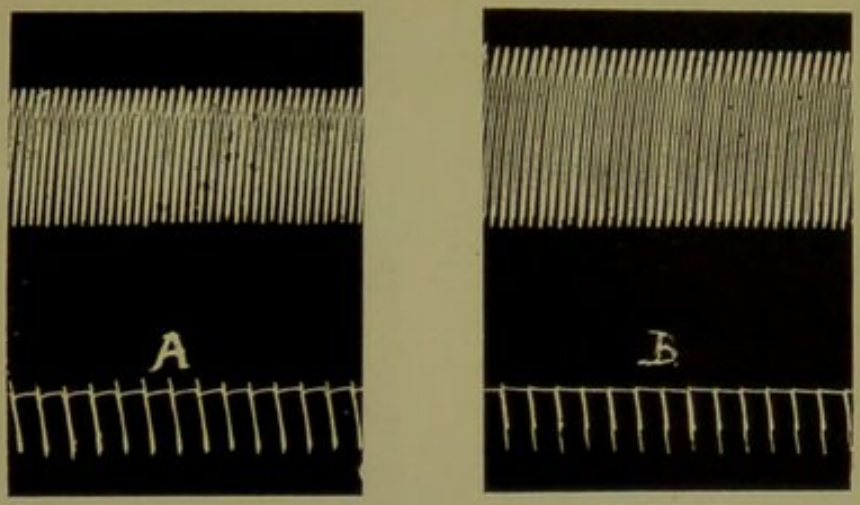
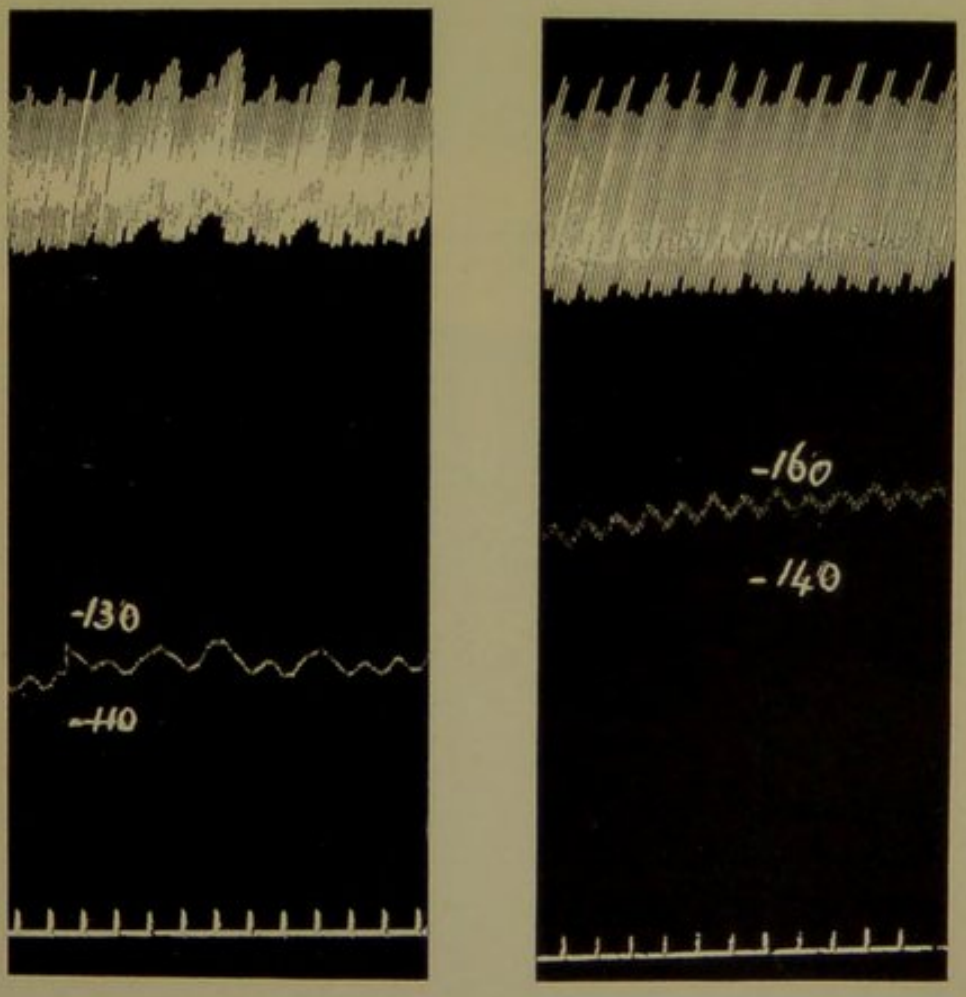
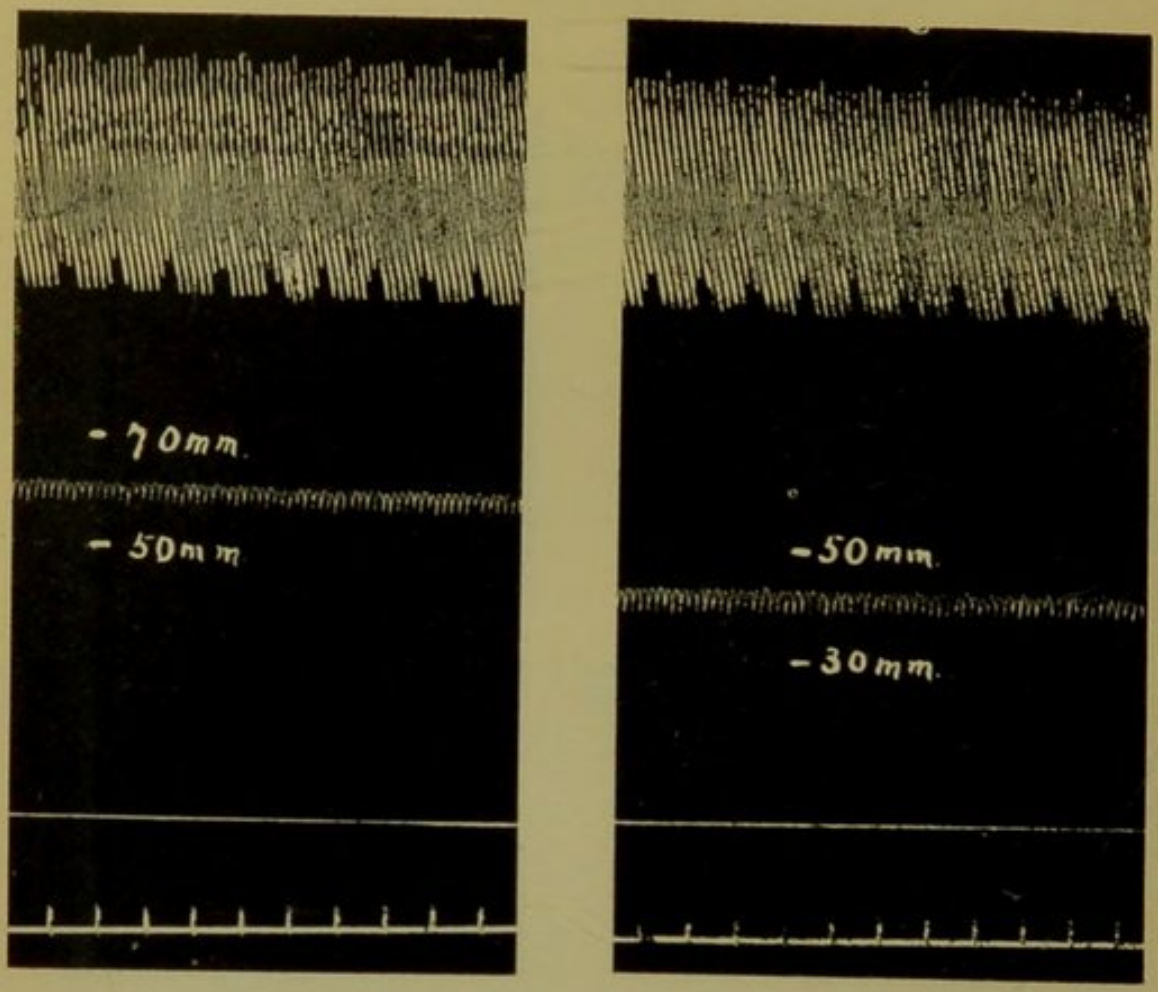


FIG. 7. Isolated rabbit's heart. Locke-Langendorf method. Time in seconds. A normal, B after 10 mgrs. hydrastinine had been injected into perfusion cannula.



A B

FIG. 8. Cat, paraldehyde and ether. Cardiometer blood pressure. Time in 2". A normal; B maximal effect after 20 mgrs. of hydrastinine.



A B

FIG. 9. Cat paraldehyde and ether. Cardiometer blood pressure. Time in 2″. A. Normal tracing. B. Maximal effect after 20 mgrs. cotarnine.

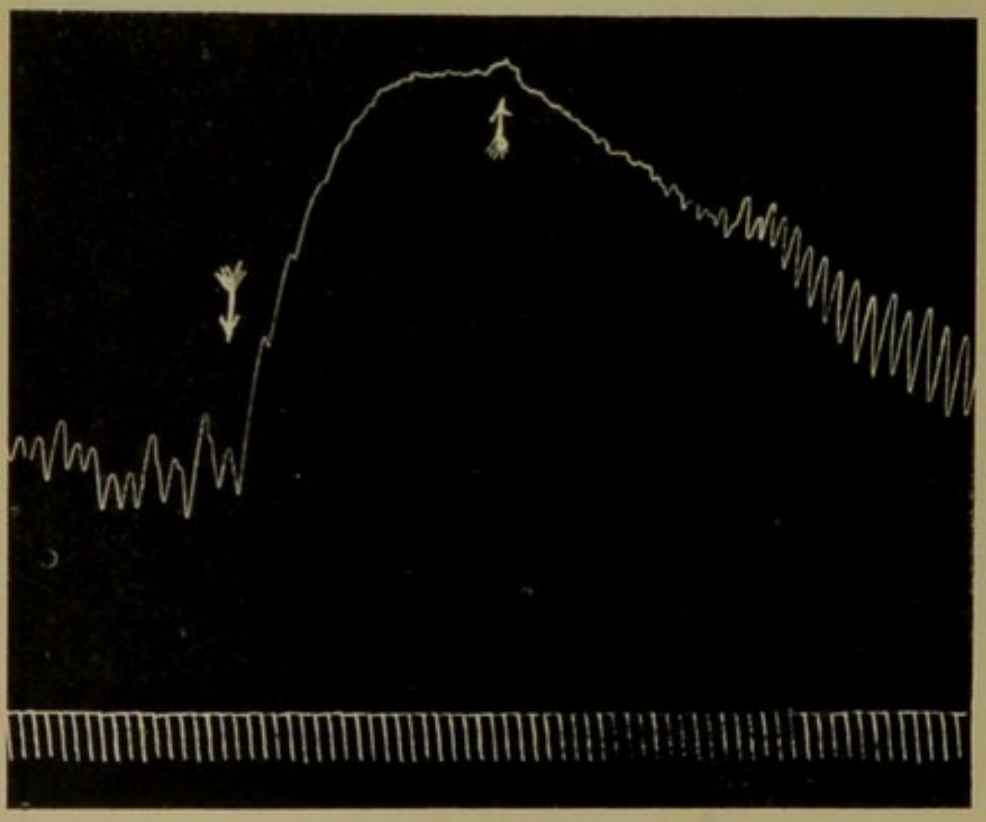


FIG. 10. Virgin cat, isolated uterus in 250 c.c. warm oxygenated Ringer suspension method. Time in 10″. At ↓ 10 mgrs. 6 : 7 dimethoxy. 2 methyl 3 : 4 dihydro-*iso*-quinolinium chloride added to bath at ↑ a change to fresh Ringer. Upstroke =

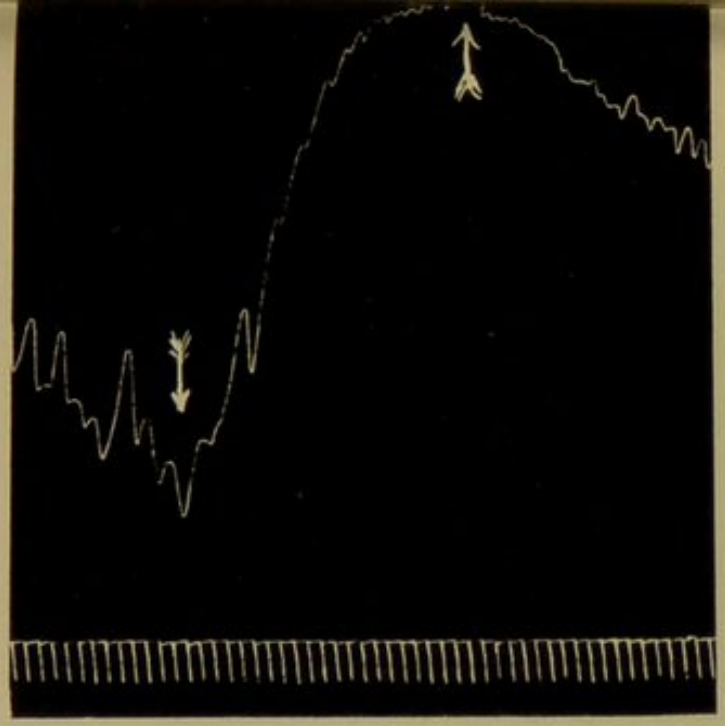


FIG. 11. From same experiment as Fig. 10. Effect of 10 mgrs. hydrastinine.

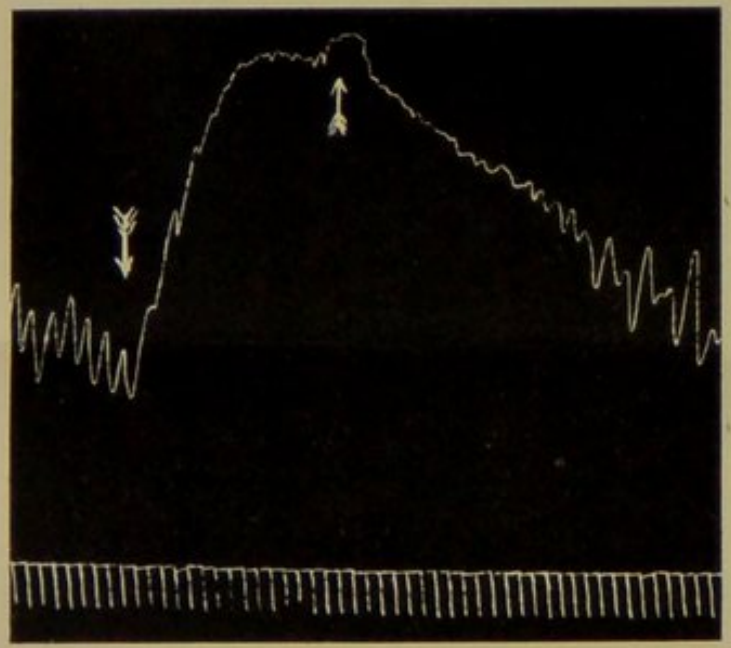


FIG. 12. From same experiment as Figs. 10 and 11. Effect of 10 mgrs. of cotarnine.

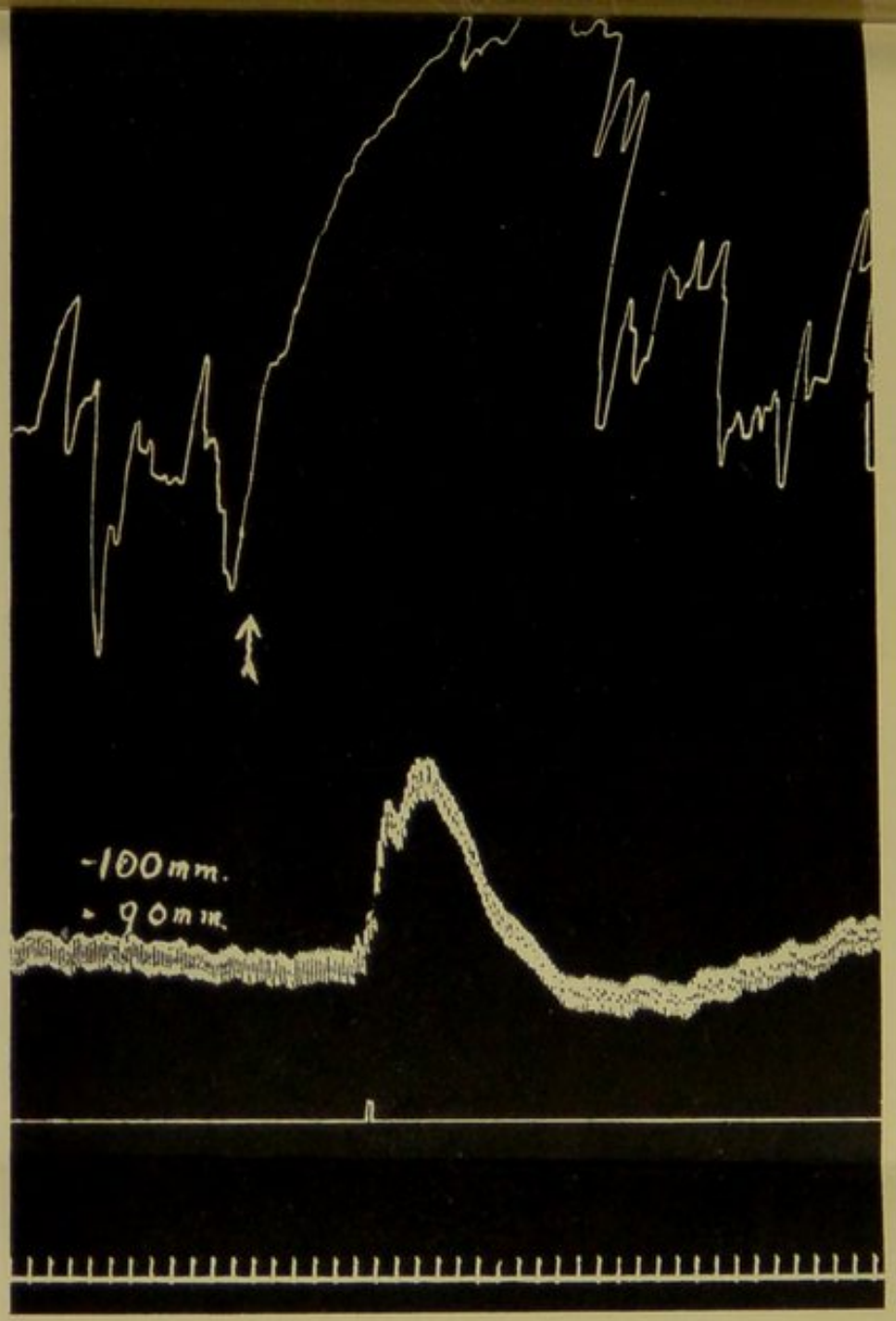


FIG. 13. Virgin cat, decerebrate. Movements of uterus, thread-pulley method, downstroke = contraction. Blood pressure base line and signal time = 10″. Effect of 20 mgrs. 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

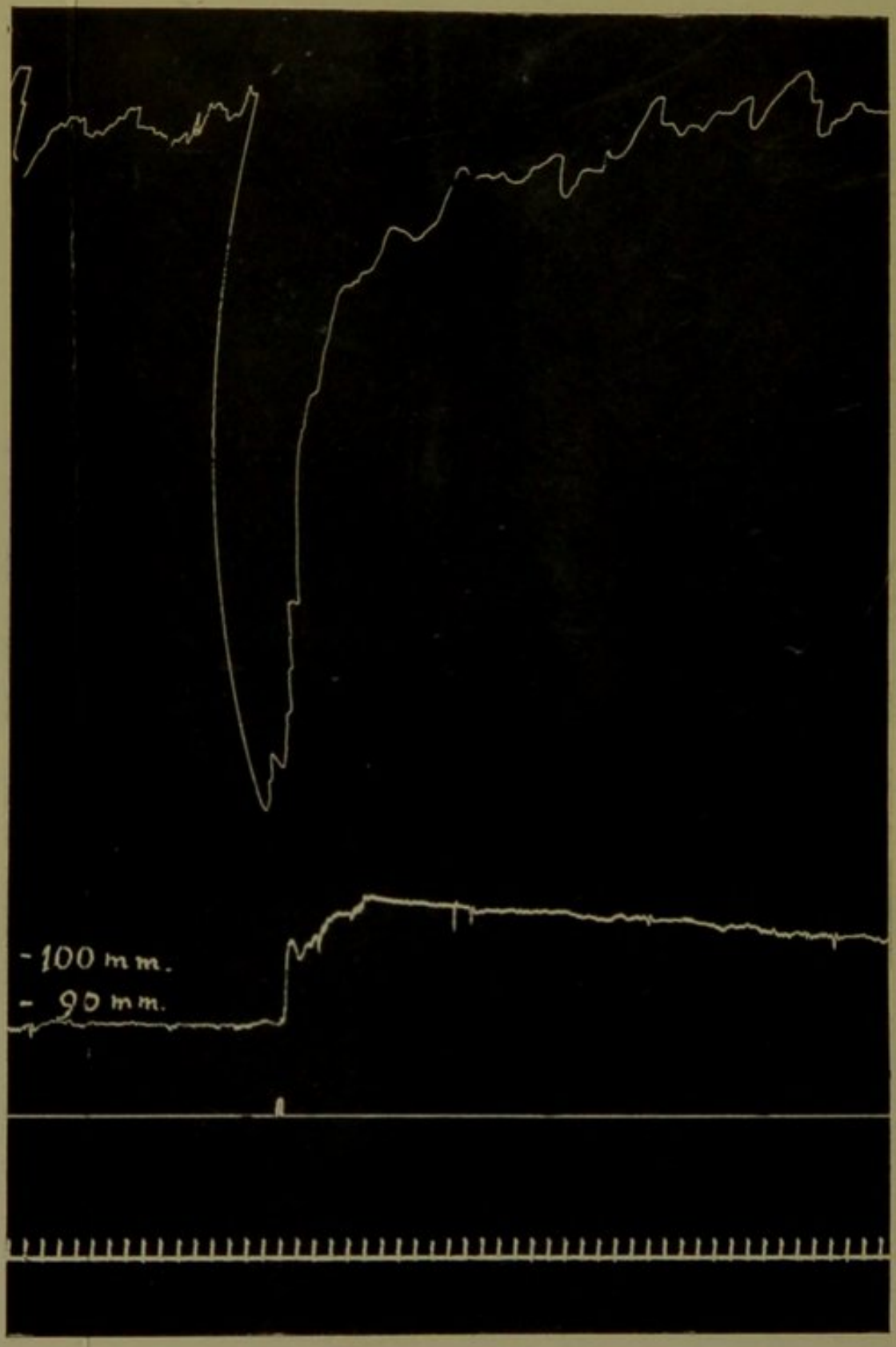


FIG. 14. Rabbit urethane. Movements of uterus, Cushny recorder, downstroke = contraction. Effect of 20 mgrs. 6 : 7 dimethoxy, 2 methyl, 3 : 4 dihydro *iso* quinolinium chloride.

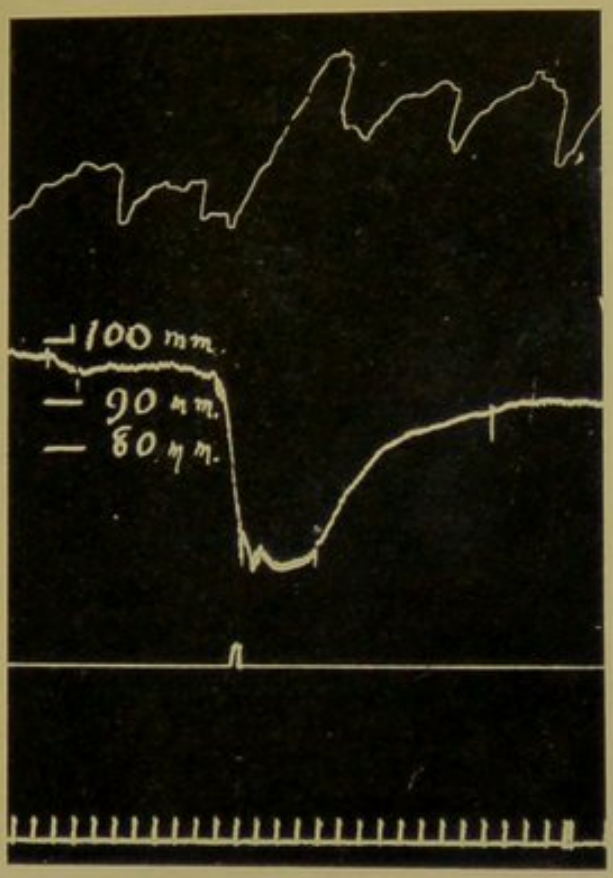


FIG. 15. Same experiment as above, effect of 20 mgrs. cotarnine.

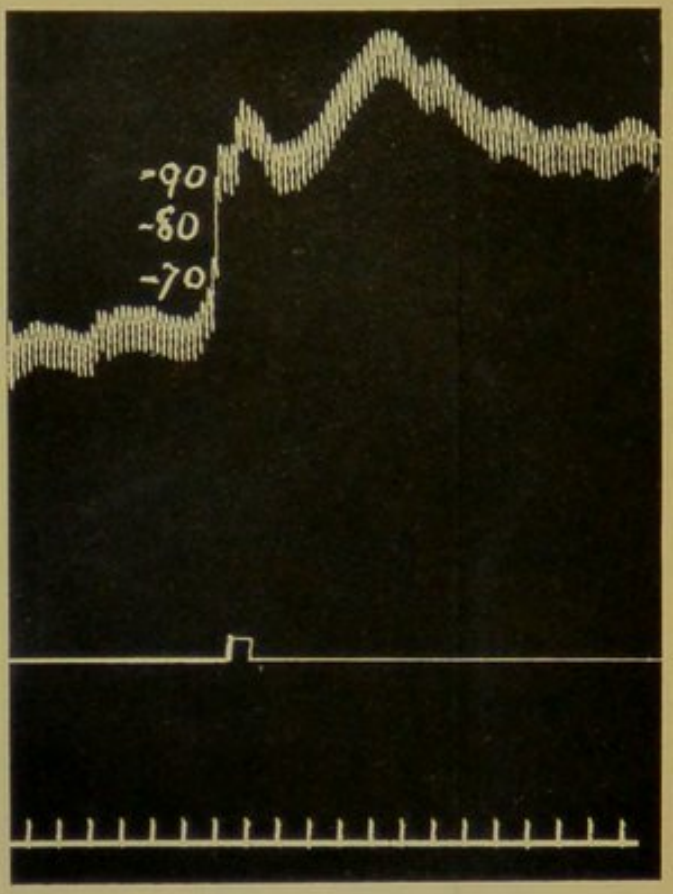


FIG. 17. Cat decerebrate. Blood pressure, base line and signal time in 10″. Effect of 20 mgrs. 6 : 7 dimethoxy, 2 methyl, 3 : 4 dihydro, *iso*-quinolinium chloride recovered from the urine of a dog.

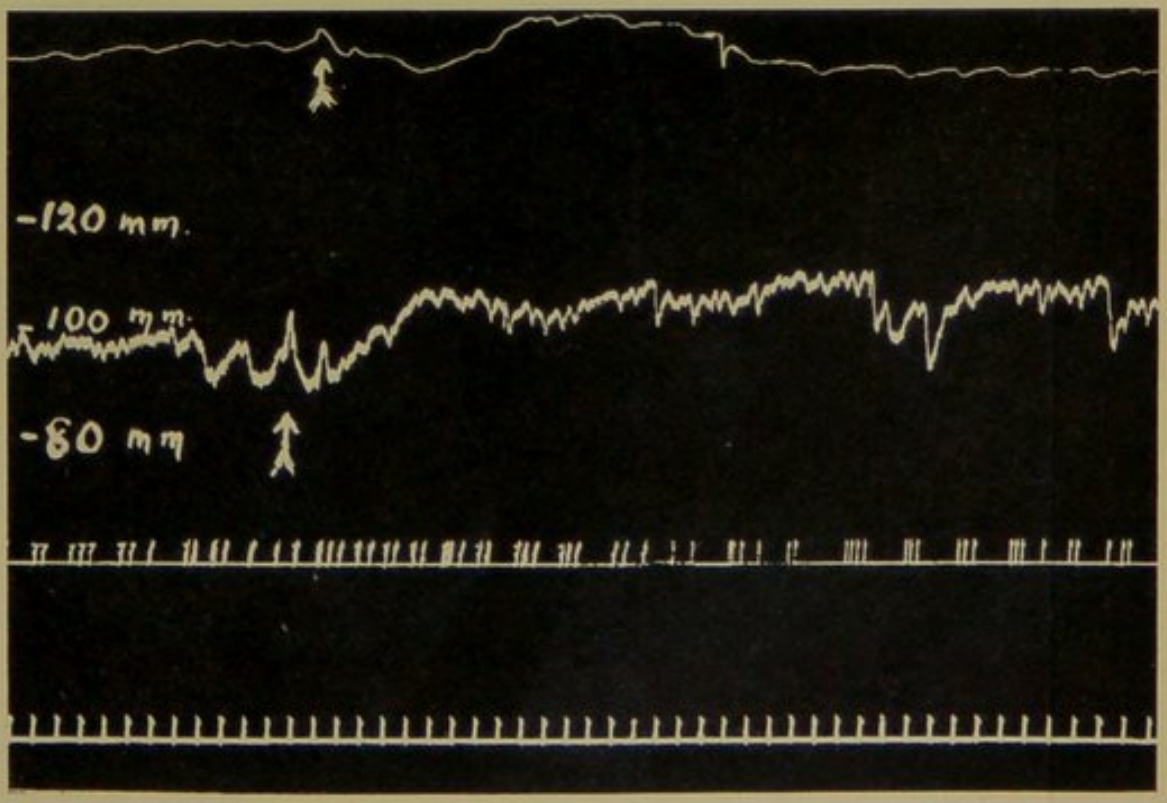


FIG. 16. Rabbit urethane, kidney volume blood pressure urine flow, time in 10″, effect of 20 mgrs. 6 : 7 dimethoxy, 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.