PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Pharmacology of Pseudaconitine and Japaconitine Considered in Relation to That of Aconitine

J. Theodore Cash and Wyndham R. Dunstan

Phil. Trans. R. Soc. Lond. B 1903 **195**, 39-95
doi: 10.1098/rstb.1903.0002

II. *The Pharmacology of Pseudaconitine and Japaconitine considered in Relation to that of Aconitine.*

By J. Theodore Cash, *M.D., F.R.S., Regius Professor of Materia Medica in the University of Aberdeen, and* Wyndham R. Dunstan, *M.A., F.R.S., Director of the Scientific and Technical Department of the Imperial Institute.*

Received June 11,—Read June 20, 1901.

In a previous paper on the Pharmacology of Aconitine and some of its principal derivatives (' Phil. Trans.,' B, 1898, vol. 190, p. 239), we have given an account of the physiological action of this, the highly toxic alkaloid of Monkshood (*Aconitum Napellus*), and of its principal derivatives, and we have also discussed the ascertained physiological effects of these substances in relation to their chemical constitution. The results of this investigation have proved to be of much practical importance in connection with the pharmaceutical and medical employment of aconite, especially in demonstrating the partial antagonism of aconitine to benzaconine, and in a greater degree of aconine, both of which derivatives accompany the parent alkaloid in the plant, and in the pharmaceutical preparations made from it. Although it seems likely that these separate alkaloids, and especially aconine, may be useful as therapeutic agents, it is now clear that for the purpose for which aconite is employed, the pure alkaloid, aconitine, should be used in the place of the indefinite mixture of physiologically antagonistic alkaloids contained in pharmaceutical preparations made from the plant.

In a series of papers communicated to the Chemical Society and published in the 'Journal of the Chemical Society' (1891–1900), one of us, in conjunction with his pupils, has described the chemical properties of the toxic alkaloid contained in two other species of the plant, viz., *Aconitum ferox* or Indian or Nepal aconite, and *Aconitum Fischeri* or Japanese aconite. The medicinal employment of these potent drugs has been very restricted in the absence of any definite knowledge as to the nature of their constituents and the physiological action to which they give rise.

Aconitum ferox has long been known to botanists and travellers in India as a poisonous plant of great virulence. It is used in Indian medical practice under the vernacular name of "Bikh." There appear to be several other varieties of aconite passing under this vernacular name. This is a subject which we are at present investigating with the assistance of the Government of India.

In 1878, Alder Wright isolated a crystalline, highly toxic alkaloid from the root

of the plant and named it pseudaconitine. In 1897 ('Proc. Chem. Soc.,' 1895, p. 154; 'Trans. Chem. Soc.,' 1897, p. 350), one of us, in conjunction with F. H. Carr, gave an account of a complete investigation of the chemistry of this alkaloid, the results of which have obliged us to modify in certain important respects the conclusions arrived at by Wright and his co-workers. Our results have been confirmed by Freund and Niederhofheim ('Ber.,' vol. 29, p. 852).

For details of the chemistry of pseudaconitine and its derivatives, reference must be made to the paper already referred to (*loc. cit.*). We may here briefly record the chief properties of the alkaloid.

Pseudaconitine is a crystalline alkaloid whose composition differs from that of aconitine, being expressed by the formula $C_{36}H_{49}NO_{12}$. The crystals melt at 202°, and are sparingly soluble in water, but readily in alcohol. The salts are usually crystalline and soluble in water. Their solution and those of the base produce in excessively minute quantities, a persistent tingling of the tongue, lips, and other surfaces with which they are brought in contact, in this respect resembling aconitine and its salts, which produce the same effect.

When heated in the dry state at its melting point, pseudaconitine evolves a molecular proportion of acetic acid, leaving another alkaloid, pyropseudaconitine. This alkaloid, like the corresponding pyro-derivative of aconitine, does not give rise to the characteristic tingling effects of the parent base.

When a salt of pseudaconitine is heated in a closed tube with water, as in the case of aconitine, partial hydrolysis occurs with the loss of a molecule of acetic acid, an alkaloid, veratryl-pseudaconine, being left. This alkaloid, like the corresponding benzaconine, derived by similar means from aconitine, produces neither the tingling sensation nor the toxic effects of the parent base.

The complete hydrolysis of pseudaconitine, which is reached when the above-mentioned veratryl-pseudaconine is heated with alkalis, produces, instead of the benzoic acid furnished by aconitine, veratric or dimethylprotocatechuic acid, together with a base, pseudaconine, not susceptible of further hydrolysis. Whilst there is thus a strong general resemblance in chemical constitution between pseudaconitine and aconitine, the benzoic radical of aconitine is replaced in pseudaconitine by the veratric radical of veratric acid, whilst there are probably also constitutional differences in the central nucleus.

The composition and properties of the toxic alkaloid present in Japanese aconite "Kuza-uzu," regarded by botanists as *Aconitum japonicum* or *A. Fischeri*, have been very differently described by chemists who have examined it. Wright regarded it as chemically different from aconitine, differing in composition and structure, being an anhydro- or apo-derivative formed by the loss of water and conjugation of two molecules of an unknown alkaloid of the aconitine type. He assigned to it the formula $C_{66}H_{88}N_2O_{21}$. Lübbe afterwards studied the properties of japaconitine, and pronounced it to be identical with aconitine; and, more recently,

Freund and Beck ('Ber.,' 1894, 27, 723) have reached the same conclusion. Later, one of us, in conjunction with H. M. Read ('Journ. Chem. Soc.,' 1900, p. 45), subjected japaconitine to a very detailed investigation, in the course of which its properties and those of its principal derivatives were defined and compared closely with those of aconitine.* We believe that these results leave little room for doubting that japaconitine is a distinct alkaloid different from aconitine, although Wright was mistaken in the view he took of its composition and constitution. Superficially, japaconitine bears a very strong resemblance to aconitine; on closer examination, however, it appears that its physical properties and those of its derivatives differ markedly from those of aconitine. To this alkaloid we have provisionally assigned the formula $C_{34}H_{49}NO_{11}$, and have retained for it the name of japaconitine suggested by Wright.

In general the decomposition of japaconitine resembles that of aconitine, but the physical properties of the resulting derivatives are not the same. By the action of heat it furnishes acetic acid and pyrojapaconitine; on partial hydrolysis japbenzaconine is obtained, besides acetic acid, whilst on complete hydrolysis the products are acetic acid, benzoic acid, and japaconine. Whilst therefore the constitution of the central nucleus appears to be different, both aconitine and japaconitine contain the acetyl and benzoyl groups, whilst in pseudaconitine the acetyl and veratryl groups are present.

In the present paper the physiological action of pseudaconitine and japaconitine are recorded and compared with that of aconitine.

The differences found are nearly always differences of degree and not differences of kind, a result which bears out the close constitutional relationship which is to be inferred from their chemical reactions. Although there are probably constitutional differences in the central nuclei of the three alkaloids, the same constitutional type is to be seen in each, and the substitution of a veratryl group (in pseudaconitine) for an benzoyl group (in aconitine) counts for little in influencing the characteristic physiological action.

Section I.—Pseudaconitine and Japaconitine.

The experiments described in this paper have been made with specimens of specially purified alkaloidal hydrobromides in aqueous solution. These experiments refer to toxic and lethal dose, to the action upon circulatory and motor organs of warm and cold blooded animals respectively, as well as to temperature variation and tactile and thermic sensation in man.

Some observations are also included upon the possibility of the acquirement of tolerance towards these alkaloids after repeated administration.

In considering the results that have been obtained by previous observers in

* In the abstract of this paper ('Roy. Soc. Proc.,' vol. 68, p. 380, line 21 from bottom of page), "richer in carbon" should have been "slightly different in composition."

investigating the pharmacology of pseudaconitine and japaconitine, contrasted with that of aconitine, it is to be observed that all the evidence goes to show that many have used substances of varying degree of purity.

The majority of observers, whilst denying a qualitative difference, find that quantitatively aconitine, japaconitine, and pseudaconitine are not identical. Rossbach and Nothnagel attribute to pseudaconitine an action seventeen times more energetic than that of aconitine, and to this proportionate toxicity Böhm and Ewers also adhere. The chemical experiments of Ewers also supported the idea that pseudaconitine was much the more active. Cloetta states that pseudaconitine is more energetic than aconitine, and Adelheim estimates the toxicity of the former as twice that of the latter. Harnack and Meunicke conclude that whilst the "under margin of active dosage for all three is nearly equal" (*i.e.*, ·5 milligramme per kilogramme), yet japaconitine is rather stronger than the others. Langaard (who found ·1 milligramme lethal to rabbits in 29 minutes) concludes that this alkaloid surpasses European aconitine in toxicity. He also regarded it as more energetic in occasioning muscular fibrillation.

That no quantitative difference exists between pseudaconitine and aconitine is stated by Liebreich, whilst Kobert is of the same opinion, adding further that he can detect no true distinction between japaconitine and aconitine.

The action of various aconitines towards the motor nerves (in their intramuscular course) of *R. esculenta* and *R. temporaria* has been described as different in character by Böhm and Ewers, who further recognise a point of contrast in the early action of aconitine and pseudaconitine upon the mammalian vagus. The undoubted impurity of the alkaloids which have been employed by many observers may account for some of the differences in action which have been noticed, and for some of the divergences in statement.

Two of the main results established in the present research are :—

1. That quantitatively the three alkaloids differ from one another, but that the relative toxicity they exhibit towards mammals is not identical with that which is recognisable when they are administered to frogs or applied to the separated tissues of these animals.

2. That qualitatively, and so far as these alkaloids have been contrasted in this research, there is no effect or action producible by any one of the three alkaloids under consideration which is not capable ot production in degree and at some stage of their operation by the other two.

Pseudaconitine.

Action of Pseudaconitine on Pulse and Blood Pressure of Mammals.

The information obtained was derived from cats anæsthetised by means of ether or urethane. In some experiments natural respiration was preserved, in others exposure of the heart for observation and registration rendered artificial respiration essential.

In all experiments in which artificial respiration was used, doses much in excess of those which prove lethal to animals breathing spontaneously were requisite, in order to cause a similar action. Registration of blood pressure, pulse, and heart movement was effected by means of the apparatus already described by us ('Phil. Trans.,' B, vol. 190, 1898, p. 239), whilst stimulation on section of vagi, splanchnics, or sciatic nerves were made when necessary. All administrations of the alkaloid were made hypodermically where not otherwise stated.

The early as well as the developed effect of pseudaconitine is, in the main, closely parallel with that of aconitine, the slowing succeeded by acceleration and irregularity of the pulse, asequence of ventricular upon auricular action, incomplete systole and diastole and inco-ordination of the myocardium, all appearing as in poisoning by the latter alkaloid. There is the same rapid and extensive fluctuation in blood pressure with the same phenomenon of failing pressure in presence of very rapid and irregular heart's action towards the end of the poisoning. Although Böhm and Ewers have maintained the contrary, no distinction is recognisable between the effects exerted by the two alkaloids upon the central vagus apparatus. Both stimulate this mechanism, thereby slowing the heart, and if the question be regarded as a purely quantitative one, when they are given in doses proportionate to their lethal activity, aconitine proves itself the more active substance of the two. The time elapsing after injection before occurrence of serious arhythmia with great fluctuation of pressure, was found to be 65 minutes after ·000112 gramme pseudaconitine per kilogramme, and 57 minutes after ·000149 gramme respectively. In both experiments artificial respiration was employed. As in the toxic action of aconitine, it is usually the ventricles which show the earliest, and eventually, the widest departure from the original rhythm as contrasted with the auricles. A primary auricular intermission was seen in only one experiment out of seven, and even in this case the disorder of ventricular action soon predominated.

Peripheral Vagus (*Cardiac*).

No increase in excitability of the peripheral cardiac vagus is detectable during the early period of pseudaconitine action, when slowing of the pulse due to a central stimulation is frequently present. Inhibitory action is preserved throughout the stage of occasional intermission of the pulse, but when the auricles and ventricles tend to develop a more or less independent rhythm the results of vagus stimulation are very various. Thus the usual slowing of the pulse with fall of pressure may be substituted by a slowing accompanied by a rise, or unattended by a fall, due to an approximation in rhythm between auricles and ventricles favouring a more effective filling and emptying of the organ. As the rhythm of the different parts of the heart varies rapidly so vagus effect is very inconstant. Stimulation may appear to be inoperative at one time, and then operative at another. Before the stage of great

acceleration of rhythm and inco-ordinate ventricular action, accompanied by failure in vagus reaction, the heart may even accelerate on vagus stimulation, probably owing to a rapidly induced exhaustion of the inhibitory mechanism. Whilst stimulation is effective it is frequently followed by a period of more complete contraction of the heart-walls, whilst the incomplete diastole, induced by all the aconitines, is temporarily counteracted.

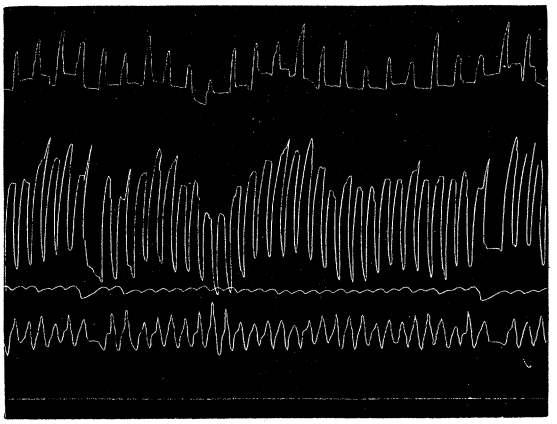
Atropine is antagonistic to the action of pseudaconitine in the same degree and manner as it is to that of aconitine.

Vaso-motor Apparatus.

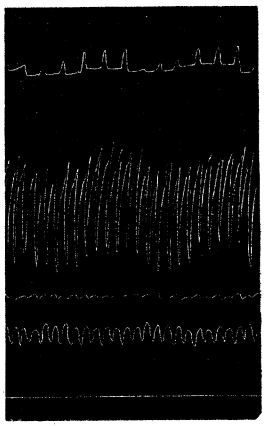
There is evidence of early central vaso-motor stimulation after pseudaconitine, and until poisoning is far advanced, some elevation of blood pressure is produced by

Pseudaconitine on the Heart. Mammalian.
The levers write upwards in systole.

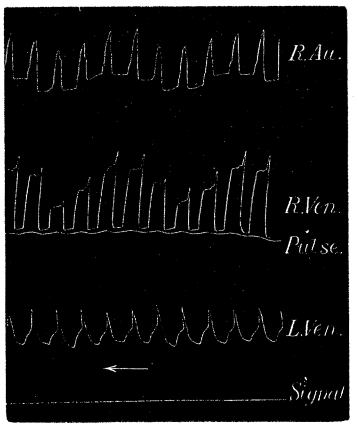
A4 taken 78 mins. after injection and 1 min. before ex. leth.



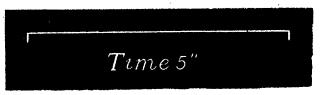
A2 taken 67 mins. after injection.



A3 taken 73 mins. after injection and 10 mins. after irregularity appeared.



A1 before injection.



suspending artificial respiration or stimulating the central end of a sensory nerve, but the function of the centre becomes eventually greatly impaired. Splanchnic stimulation determines an increase of pressure throughout poisoning. The condition of the heart during tolerable sequence of ventricular upon auricular action is naturally much more favourable to a rise in pressure when the vessels of the splanchnic area are narrowed than is the case when asequence is predominant. So the effect of stimulation is by no means uniform.

Experiment.—Cat of 2505 grammes fully etherised, in warm box. Cannulas in trachea, and left carotid artery; artificial respiration by warmed air. Sternum in part resected with three ribs. Pericardium opened and secured to thoracic wall anteriorly. Threads connected with right auricle (top line), right ventricle (second), and left ventricle (fourth). The third line is a pulse record from a straight spring FICK's kymograph. Lowest line, electrical signal. Electrodes on peripheral vagus (left), splanchnic (left), and central sciatic (left), all three nerves being divided.

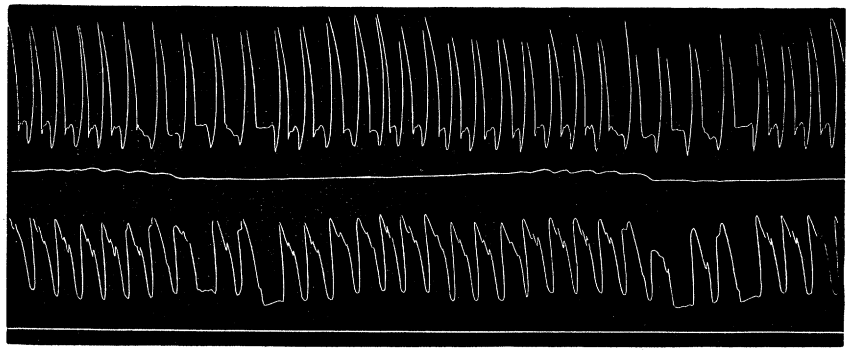
The results of the experiments are given in abridged and tabulated form.

Time.	Blood pressure.	Pulse, auricle, and ventricles.	Notes.
0	76–79	116–130	(A1).
0 (20 min. later)	—	127–66–138	Stim. L. vagus (coil 10), fall of 20 millims.
0	—	—	Inject pseudaconitine, ·00015 gramme per kilogramme, hypodermically.
5 min.	76	114	
10 ,,	74	111	
20 ,,	82	144	Heart steady, filling well.
45 ,,	76	149	
50 ,,	76	156	Stim. L. vagus (coil 10), fall of 13 millims., stopping artificial respiration causes prompt rise in blood pressure.
57 ,,	57	—	Irregularity first seen in ventricle.
67 ,,	46–54	Auricle, 144 Ventricles, 216	(A2). Vagus stimulation does not affect blood pressure.
68 ,,	—	Auricle, 124 Ventricles, 190	
70 ,,	—	Auricle, 126 Ventricles, 216	Sections of right vagus (both now divided).
73 ,,	44	Auricle, 162 Ventricles, 296	(A3). The auricular movement is now very feeble.
78 ,,	31 and falling	Auricle, 165 Ventricles, 330	(A4). There are now two feeble ventricular to one auricular contraction.
79 ,,	—	—	Death.

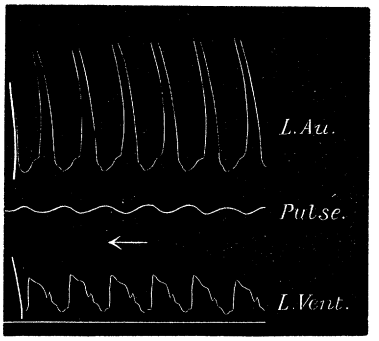
In this experiment irregularity of sequence is at a minimum, and though the auricle slows distinctly at the time it parts company from the ventricle and only at last accelerates, its rhythm is from first to last regular. Whilst this condition of affairs maintains in the great majority of cases, it very occasionally happens that

the auricle from the first departs from its regular rhythm. In such cases the fluctuations of blood pressure are very large and vagus stimulation is apt to produce an elevation in blood pressure. This arises from the steadying effect of such stimulations on the auricular rhythm, and resulting tendency of the ventricle to follow the auricle in its action. Auricular acceleration occasionally follows stimulation.

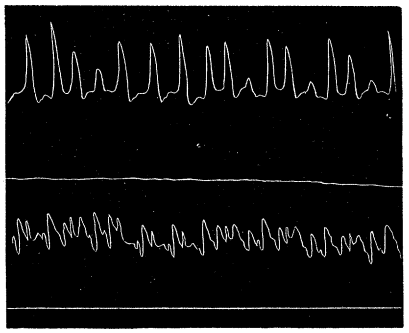
Pseudaconitine on the Mammalian Heart.
The levers write upwards in systole.



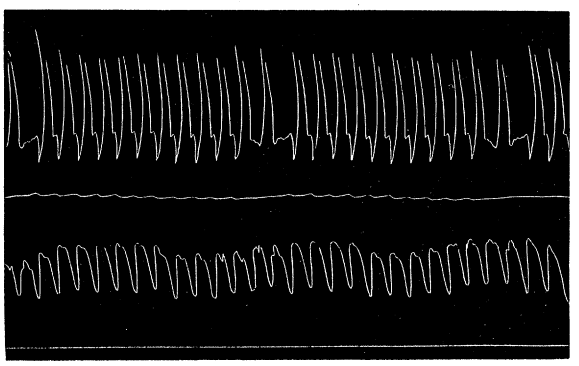
B2 taken 98 mins. after the injection of PsA. and 7 mins. after irregularity began.



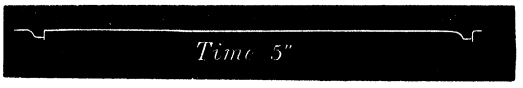
B1 before injection.



B4 127 mins. after injection. Ex. leth. 5 mins. later.



B3 115 mins. after injection. (Auric. record imperfect at summit.)



Experiment.—The preparation was essentially that described in the last experiment. The movements of the left auricle (top line), pulse (second line), and left ventricle (third line) are recorded. The original pulse-rate was 151 B1.

91 minutes. After a first injection of pseudaconitine, ·00005 per kilogramme, and 40 minutes after a second similar injection, irregularity of rhythm developed.

98 minutes. Periodic slowing with great ventricular dilatation giving place to acceleration with fall of pressure (B2).

115 ,, The heart though much accelerated (264) is regular, were it not for a periodically dropped auricular contraction (B3).

127 ,, The pressure had never fallen to a lower level than 76 when the auricle and ventricle struck an entirely different rhythm, the latter greatly accelerating (over 300) and becoming extremely irregular, whilst the auricular systoles (210), though more regular, greatly varied in force (B4). Death followed soon (5 minutes) after, but, as has already been recorded in the case of aconitine poisoning, there was a short antecedent period of steady sequence of ventricle on auricle at 240 per minute.

Action of Pseudaconitine on Respiration.

The respiration of etherised animals receiving a lethal dose of pseudaconitine shows little, if any, acceleration, before it begins to decline in frequency. The slowing is peculiarly marked at the time when cardiac arhythmia becomes established, but even at this phase section of the vagi may increase the decline by 3 to 6 movements per minute. The failure of respiratory function hereafter becomes more rapid, and only a transitory improvement follows a 2 minutes' insufflation of air, or of air mixed with a large proportion ($\frac{1}{4}$ to $\frac{1}{2}$ vol.) of oxygen.

Experiment.—Etherised Cat. Respiration natural, 24 per 1 minute.

0 minutes.	Administer pseudaconitine hypodermically, ·000107 per kilogramme.			
10 ,,	Respiration, 24 per 1 minute.			
35 ,,	,,		,,	
48 ,,	,,	26	,,	
50 ,,	,,	22	,,	No retching or spasmodic movement.
55 ,,	,,	20	,,	Intermission and irregularity of heart began.
69 ,,	,,	14	,,	
70				Divided both vagi.
71 ,,	,,	11	,,	
78 ,,	,,	11	,,	
83 ,,	,,	10	,,	
87 ,,	,,	8·5	,,	
96 ,,	,,	7		
97 ,,	Death.			

The central effort, which has become so reduced as barely to maintain life, further

degenerates into spasmodic inspiratory movements, which precede death. Although these facts are sufficiently apparent, it may be further demonstrated by the use of a diaphragm needle that the contractions of this muscle become much reduced as poisoning progresses, so that towards the end they assume the character of unsustained twitches.

Lethal Dose.

·0000465 gramme of pseudaconitine per kilogramme body weight is a proportion which is almost uniformly lethal to rabbits, but downwards to ·000038 gramme death may occasionally take place, especially if the viscera are loaded with food or the animal is not carefully sheltered from cold.

Whilst there is no invariable symptom which enables an observer to separate the action of one alkaloid from the other with certainty, it has nevertheless become clear from contrasting many experimental results that acceleration of respiration as an initial feature of pseudaconitine action is not so usual, nor when occurring so pronounced, as it is after aconitine. The advent of dyspnœa is also more sudden after the former, the animal which just before had shown little more than retching and salivation rapidly passing into a state of respiratory need. In this state the muscular movements upon which respiration depends are ataxic and apparently to some extent inco-ordinate. (Chewing movements, salivation, and retching appear to be similar in character, intensity, and time of occurrence after proportionate doses of the two alkaloids have been given, salivation is however longer continued after pseudaconitine.) The paralytic stage after large but sublethal doses is well marked and more enduring after pseudaconitine, the fall of temperature being somewhat greater. This preponderating effect of pseudaconitine upon the body temperature is not recognisable after smaller doses of the alkaloids.

Spasm of a rearing, running, or springing character, occasionally followed by unconsciousness, with insensitive cornea and dilated pupil, is witnessed in the course of serious and fatal poisoning. Though death is primarily due to impairment of function in the respiratory centres, it is not to be assumed that the heart will be found in a state of functional activity, if the thorax is opened immediately after death. On the contrary it is arhythmic and the ventricles inco-ordinate in movement, the effective systole rapidly failing when respiration ceases. At earlier stages of poisoning artificial respiration will often prolong or even save life, but in the presence of a dose largely in excess of the lethal, the heart fails notwithstanding.

If the dose though large is sublethal the respiration after remaining slow and dyspnœal for 70 to 150 minutes begins to accelerate, this change being anterior to the rise of temperature towards the normal. A second slowing of respiratory rhythm—not however dyspnœal or extreme in character—is usually observed after the temperature has almost reached its normal level. Of the three alkaloids examined pseudaconitine is distinctly the most depressant towards respiration.

It may be noted here that repeated daily administrations of sublethal doses of pseudaconitine are followed by a somewhat lessened effect upon the respiration. It is probably this fact which explains the degree of tolerance (trifling though it is) which is established towards the alkaloid.

Temperature.—The preliminary rise of internal temperature (frequently, though by no means invariably, seen in rabbits after aconitine) is even less usual after pseudaconitine, and this fact seems to stand in relationship with the slighter tendency to respiratory acceleration occasioned by the latter. The ensuing fall of temperature bears a direct proportion to the dose of the alkaloid, when the conditions under which the animal is maintained are similar. Thus a relatively smaller dose will cause a greater and more enduring reduction when the animal is in a cool atmosphere, and when the loss of heat from the surface is not guarded against, than under the reverse conditions. The maximal fall after large sublethal doses occurs within $2\frac{1}{2}$ hours of administration and amounts to 2 to 3°·5 C. Return towards the normal succeeds acceleration of respiration in point of time, the original level being reached and often exceeded in between 4 and $5\frac{1}{2}$ hours after administration.

Daily administration of such doses are followed by a slightly lessened depression of temperature and usually, though not invariably, by a shortening of the subnormal period.

TABULATED Results of Experiments upon Rabbits, mainly with reference to action of Pseudaconitine upon Respiration and Body Temperature.

Dose of pseud-aconitine per kilo-gramme animal body weight.	Respiration.			Temperature.			Notes.
	Initial accelera-tion.	Reduction.	Time of reduc-tion.	Initial rise.	Fall.	Recovery.	
					°C.		
·000038	None	By 13 per 1 min.	33 mins.	None	1·10	Normal in 250 mins. and then hyper-normal	Symptoms moderate in degree.
·00004	Slight	By 20 per 1 min.	—	·5	2·3	About 4 hrs. 30 mins.	
·000044	None	—	—	none	2·7	About 6 hrs.	Symptoms severe; re-covery.
·0000475	,,	To 24 in	140 mins.	,,	2·8	—	Recurrent spasm; death in 145 mins.
·0000546	,,	To 6 in	60 mins.	,,	2·7	—	Death in 88 mins.
·00008 to ·0001	—	—	—	—	—	—	Death in 40 to 50 mins.

Contrasted with aconitine and japaconitine, the fall of temperature produced by pseudaconitine (given in large doses bearing the same proportion to the lethal) is somewhat more extensive and enduring.

Observations on the Blood of Rabbits.

The blood was derived from puncture of an ear vessel. The percentage change in the hæmoglobin and the variation in number of coloured corpuscles per cubic millimetre are recorded.

First Experiment.—A rabbit (of 1700 grammes) in which the variations of hæmoglobin and corpuscles in three preliminary estimations were unimportant, received three successive doses of pseudaconitine each of ·000035 gramme per kilogramme (this is equal to $\frac{3}{4}$ of the lethal) at intervals of 24 hours. The effect of the alkaloid was moderately developed on each occasion.

The hæmoglobin was reduced by 8 per cent., the coloured corpuscles by 1,520,000 per cub. millim., and the body weight by 180 grammes.

In a *second experiment* the large dose of ·00003 gramme per kilogramme was administered every second day, with the result that after nine administrations the hæmoglobin had fallen by 7·5 per cent., and the corpuscles by 1,300,000 per cub. centim., the body weight having also declined by 120 grammes.

A single lethal dose of pseudaconitine caused a reduction of 1·5 per cent. of hæmoglobin and 130,000 corpuscles, but such variations are too slight, considering the possibility of errors in method, to deserve much attention.

It is clear that (probably as a result of interference with nutrition) the blood is distinctly and prejudicially modified by large doses of pseudaconitine.

As the effect of aconitine administration on the hæmoglobin and corpuscular contents of mammalian blood was not discussed in our former paper, it is necessary for the purpose of contrast to quote two experiments made with this alkaloid.

Daily Administration of ·00006 *gramme Aconitine.*

Experiment No. 1.—After the preliminary estimations, a full-grown rabbit was subjected to the action of aconitine as above. After six administrations the hæmoglobin had fallen by 9 per cent., the corpuscles by 1,300,000 per cub. millim., and the body weight by 125 grammes.

Administration every Second Day of ·00007 *gramme.*

Experiment No. 2.—After administration every second day of aconitine ·00007 gramme per kilogramme, seven such doses in all being given, a reduction of the hæmoglobin through 6 per cent., of the corpuscles through 1,100,000, and of the body weight by 145 grammes, was recorded.

In both experiments there was a slight increase in hæmoglobin and corpuscles after the first and second injections.

In the experiment No. 2, a dose of about three-quarters of the lethal was given, but the injurious effect was relatively less than in the companion experiment with pseudaconitine, which appears to be the more powerful body in this respect of the two.

Daily doses of ·00002 to ·00003 gramme of aconitine per kilogramme rabbit's body weight, are practically without effect on the blood or body weight of the animal.

As the aconitines do not appear to affect the blood injuriously in doses which fail to produce interference with nutrition, and as the indifference to food following their administration in larger doses is accompanied by loss of weight and *pari passu* by deterioration of the blood, it seems reasonable to infer that the aconitines act primarily and mainly not upon the blood itself, but upon the nutrition. The extensive reduction in hæmoglobin is probably attributable to the insufficient ingestion of iron-containing vegetable matter.

Action of Pseudaconitine on Guinea-pigs.

The lethal dose of pseudaconitine for guinea-pigs is about ·000045 per kilo. (*i.e.*, rather less than for rabbits). Between this proportion and ·000038 a lethal effect occasionally results. The following experiment had a fatal termination, though the proportion is somewhat below the certainly lethal.

Guinea-pig of 487 grammes. Respiration 64. Rectal temperature 38° F.

0 minute.	Injected ·000043 gramme pseudaconitine per kilogramme.
15 minutes.	Some local irritation. Chewing.
18 ,,	Respiration 48; slight dyspnœa. Temperature 38°·3. Wrapped in cotton wool.
48 ,,	Distinct salivation. Starting of body. Retching. Pupil dilated.
54 ,,	Respiration 47. Gasping. Temperature 37°·6.
60 ,,	Rises with difficulty. Slight dyspnœal spasm on attempting movement.
65 ,,	Respiration less dyspnœal. Temperature 37°·3. Gets on to legs with difficulty.
75 ,,	Respiration 40. Temperature 37°.
80 ,,	Convulsive movements at intervals of a few seconds. Pupils dilated.
95 ,,	Respiration 45, less vigorous. Temperature 36°·1.
108 ,,	No power to move into ventral position. Occasional faint spasm.
109 ,,	Respiration 40. Temperature 35°·5.

115 minutes. Much mucous discharge from nostril. Body limp. Respiration 36, failing rapidly.

126 ,, Respiration 9. Temperature 39°·4. Slight spasm. Pupil dilated. Insensitive.

127 ,, Breathing stopped.

Action of Pseudaconitine on Pigeons.

The lethal dose corresponds very closely with that for rabbits, whilst ·000035 gramme per kilogramme is sublethal, ·00004 is frequently and ·000045 invariably fatal.

After a lethal dose the bird develops mandibular and swallowing movements, sometimes with regurgitation from the crop, shaking of the head, and ataxic walking. The position is low. Serious dyspnœa, with a sort of barking respiration, develops, as a rule only a few minutes before death, which occurs in 30 to 40 minutes after injection. Thus, after ·00004 gramme per kilogramme, except for mandibular movements, swallowing and ruffling of the feathers, there were no serious symptoms until in 51 minutes dyspnœa suddenly developed, death supervening in 5 minutes. In another bird receiving ·00005 gramme per kilogramme, dyspnœa ensued in 20 minutes, death 6 minutes later.

A dose double the lethal amount causes spasm, which may be recovered from once or twice, and death in 12 to 14 minutes. When the dose is sublethal no great degree of paralysis is produced; the bird remains standing, though insecurely; it is apathetic, and movement, if attempted, is ataxic.

Note on the Reaction of Pigeons to Aconitine.

As this point was not discussed in the previous paper, a brief statement is added here for contrast.

The lethal proportion is ·000115 gramme of aconitine per kilogramme body weight, recoveries occurring after ·0001 and ·00011.

The lethal effect ensues in 40 to 50 minutes after the barely lethal dose, but after ·00013 it occurred in 8 to 10 minutes.

When the dose is sublethal recovery ensues rapidly.

Thus one bird receiving ·00008 gramme per kilogramme was practically normal in 80 minutes, whilst another receiving ·0001 recovered in 100 minutes.

Action of Pseudaconitine on Frogs.

The lethal dose of pseudaconitine for *Rana temporaria* in May and June (warm weather) begins at ·0008 per kilogramme, but recoveries may occur until a dose of

·0012 is reached, which proves uniformly fatal, though frequently only after the lapse of 3 or 4 days. In another series of observations fresh specimens of *R. temporaria* in August (relatively cool weather) showed greater resistance, the lethal dose being set down at ·0013 per kilogramme. Barely lethal doses were frequently followed by a fatal result on the 4th to the 5th day.

After large sublethal quantities, recovery takes place more gradually than after corresponding doses of aconitine or japaconitine, 8 to 12 days often elapsing before the animal can move freely.

In contrast with aconitine (experiments being made at the same time upon animals under similar conditions) the toxicity of pseudaconitine is rather less, being roughly only as 10 to 11, or 21 to 22, and this relationship of activity is consequently different from that which obtains towards mammals.

Contrasting the action of the alkaloids, it may be said that no symptom is produced by pseudaconitine which is not occasioned by aconitine. After barely lethal and large sublethal doses, however, there is greater prevalence and duration of paretic symptoms produced by the former and more interference with cord reflexes and respiration. In *R. esculenta* the primary excitement after injection of pseudaconitine is more marked than that following the other two alkaloids, but it is far from equalling that occurring in *R. temporaria*, in which increased cutaneous secretion and the frothing resulting on movement are also more prominently present than in the edible frog.

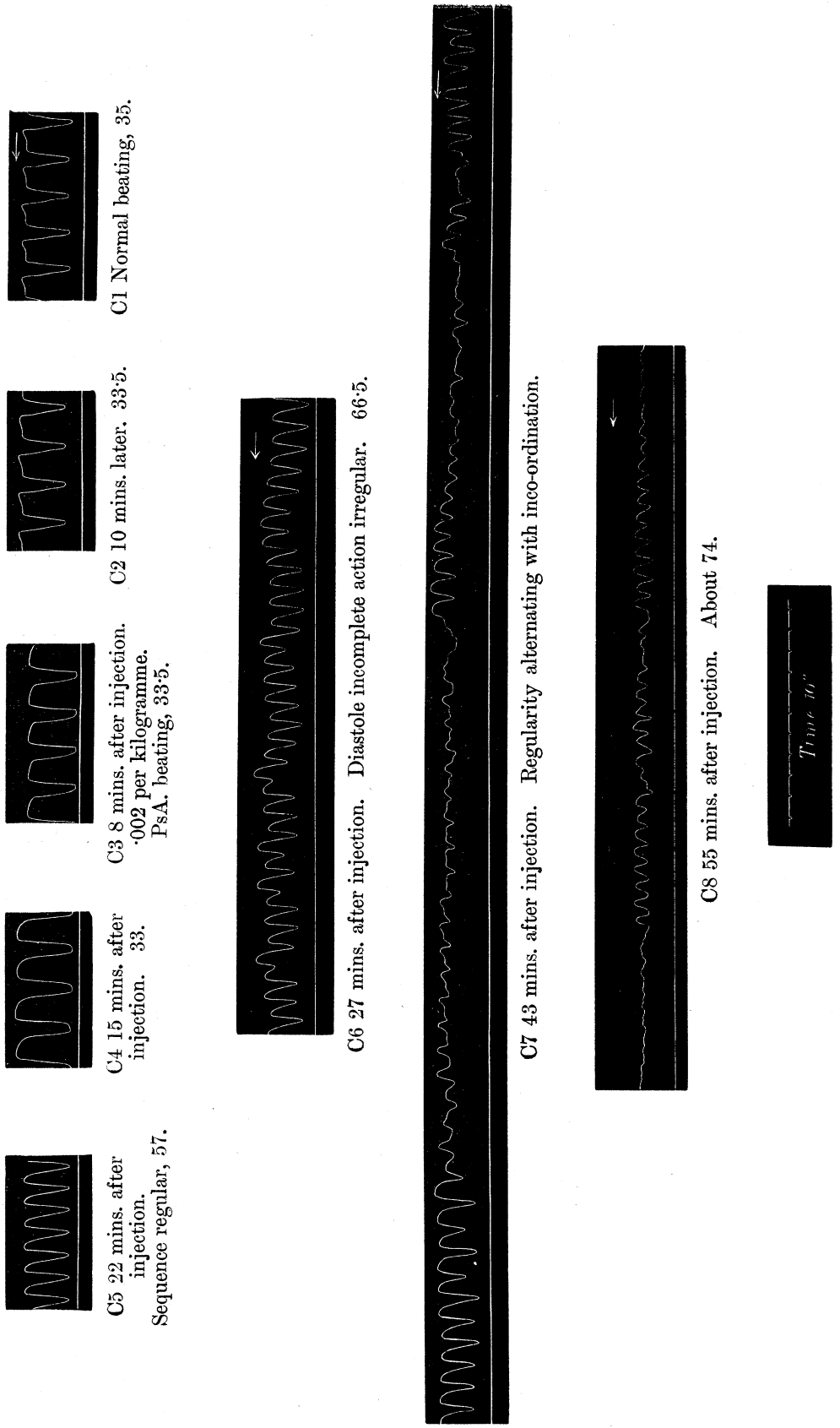
Many deaths occur on the 3rd to 7th day after barely lethal doses of pseudaconitine. Especially is this the case if the animal is kept with the body partially immersed in fluid, when considerable œdema develops. One point, which at first gave rise to misconception, has become very clear on repeated observation ; it is, that highly diluted pseudaconitine solutions in water suffer hydrolysis more rapidly than do solutions of the other two alkaloids. So much is this the case that very dilute solutions made only for 10 or 21 days have been found distinctly impaired in their activity.

Action of Pseudaconitine on Frog's Heart (in situ).

In the brainless frog, doses of ·0014 to ·0028 gramme per kilogramme produce acceleration of the heart of from 8 to 16 beats per minute. The same asequence of ventricular upon auricular action is seen as after aconitine, and a similar churning inco-ordinated movement of the ventricular wall, which yields temporarily to co-ordinate and effective systoles.

The ventricle after becoming feeble in its efforts at last fails, the auricles outlasting it in action.

Frog's Heart (circulating) on Float Cardiograph.



C1 Normal beating, 35.

C2 10 mins. later. 33·5.

C3 8 mins. after injection. ·002 per kilogramme. PsA. beating, 33·5.

C4 15 mins. after injection. 33.

C5 22 mins. after injection. Sequence regular, 57.

C6 27 mins. after injection. Diastole incomplete action irregular. 66·5.

C7 43 mins. after injection. Regularity alternating with inco-ordination.

C8 55 mins. after injection. About 74.

Time 10"

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Pegged frog of 18 grammes. Heart exposed, beating steadily 44 per 1 minute.

0 minute.	Injected a dose of pseudaconitine equivalent to ·0018 per kilogramme for the uninjured animal.
5 minutes.	48. Heart filling well, but rhythm obviously accelerating.
30 ,,	46. Inco-ordination at intervals. Temporary and shifting pouching or sacking of ventricle.
45 ,,	Great inco-ordination, with churning and sacking.
70 ,,	55. Rhythm regular. Both systole and diastole imperfect. Action feeble.
105 ,,	Permanent sacking at base. Again inco-ordinate action.
120 ,,	52. Steady sequence but very little movement in web.
150 ,,	Ventricle slow, follows every second or third auricular beat only.
180 ,,	Ventricle has ceased, auricle beats fairly about 10 to 1 minute. Atropine started the ventricle again. (Applied during inco-ordination of the ventricle it promotes return to a natural sequence without, however, appearing to strengthen the beat to any marked extent.)

Placed upon the float cardiograph the changes which the heart undergoes under the action of pseudaconitine may be recorded, the circulation continuing through the vessels in ordinary course. An examination of the web made simultaneously shows that at the time of rapid and inco-ordinate action during which the ventricle fails to dilate, the circulation is of the most meagre character possible, but during temporary return to co-ordinate action the movement in the vessels is at once augmented.

Heart beating 35, circulation in web excellent. C1.
(15 minutes later.) Heart beating 33·5, circulation in web excellent. C2.

0 minute.	Inject under skin of side pseudaconitine ·002 gramme per kilogramme.
8 minutes.	Heart as before 33·5 greater dilatation. C3.
15 ,,	Heart 33. Cycle shortened. C4.
22 ,,	Heart 57. Up stroke is purely systolic. No irregularity. C5.
27 ,,	Heart 66·5. Diastole very imperfect. Beats often bigeminal. C6.
43 ,,	Very irregular. Inco-ordination (churning movement) yielding at end of tracing to steady beat with fuller diastole. C7. Circulation in web just moving at first, improving at end.
55 ,,	Churning movements without intermission or relaxation. About 74 of these per minute representing systoles in different parts of the ventricle. C8.
85 ,,	Ventricle beating slowly and feebly about 14, *i.e.*, once to every second auricular beat. Stopped soon after.

Perfusion of the whole ventricle with traces of pseudaconitine does not interfere with the rhythm, and may even increase the force of contraction if the organ is beating feebly in the first instance. There is also observed a heightened tendency to spontaneous activity and a more sensitive condition towards weak stimulation, whilst group beating is frequently witnessed. The diastolic phase is slightly retarded. Larger doses, whilst at first producing these effects, afterwards cause a condition in which neither systole nor diastole are fully developed, the excitability declines, and finally a feeble vermicular movement of the ventricular wall takes the place of a co-ordinate systole. Neither calcium nor potassium are found to materially improve the latter condition of the organ, but atropine partially relieves it.

Dose for dose, pseudaconitine is no more energetic in its action upon the frog's heart than aconitine, in fact it appears to be somewhat feebler, but exact contrast is impossible as different organs must be used.

Vagus and Venous Sinus Stimulation.

Numerous experiments, confirmed by others made by Dr. Esslemont, have shown that the heart poisoned by pseudaconitine is, in its reaction to vagus and sinus stimulation, altogether similar to that under the influence of aconitine ('Phil. Trans.,' B, 1898, 160). One effect frequently observed in pseudaconitine poisoning (and also in that of aconitine, though it has not been specially mentioned in the paper just referred to) is that after partial poisoning, when vagus stimulation has corrected the disordered sequence of the walls of the heart chambers, and so for the moment re-established more effective circulation, the auricle in presence of the exhausted state of the inhibitory apparatus (brought about by prolonged stimulation) becomes very rapid, whilst the ventricle lapses into movements more vermicular and incoordinate than it showed before. That pseudaconitine renders the inhibitory apparatus more excitable in the first instance appears probable from the fact that a stimulation, ineffective before injection, becomes, to a greater or lesser extent, effective subsequently. That is, however, only a transitory phenomenon. When, as is frequently the case, the auricles are beating more rapidly than the sinus venosus, vagus stimulation at first establishes sequence between them, but ultimately the auricles break away into a more rapid rhythm of their own.

Ultimately the inhibitory action of the vagus upon the ventricle is lost. The last result of its stimulation is motor in character, as in a perfectly quiescent heart (deeply poisoned by pseudaconitine) the auricles and the venous sinus may often be started again (rarely the ventricle) as a result of strong excitation. (Though but little attention has been directed in this research to antagonism, it is evident from the few observations made that atropine and digitalin are both to some extent antidotal in their action towards pseudaconitine, as they are towards aconitine.)

Action of Pseudaconitine on Respiration.

Dose for dose, pseudaconitine appears to be distinctly more energetic in interfering with, and finally in abolishing, respiration than aconitine.

There is no essential difference in the phenomena.

The hyoid movements after transitory acceleration approximate in number to those of the flank; then large movements of inflation, followed by partial emptying of the lung, succeed each other at increasing intervals; these become fainter and finally disappear. In profound poisoning the flanks are collapsed and the hyoid retracted.

Action of Pseudaconitine on Reflex Function of Spinal Cord and Sensory Nerves of Frogs.

Following upon the injection of pseudaconitine there is occasionally a temporary increase of excitability, which is evidenced by the shortening of the reflex period. This is observed mainly upon the open side, and is therefore chiefly attributable to excitement of cutaneous sensory nerves. As it, however, may occur to a lesser extent upon the side of vascular ligature, some degree of excitation in the reflex centres of the cord, accompanying the peripheral effect, may be inferred. But this acceleration of reflex soon gives place to a delay in the withdrawal of the limb from the acid solution. This decline of reaction is primarily due to sensory impairment at the periphery, as the motor nerve is at this time unimpaired in function, and the foot upon the side of vascular ligature is withdrawn more rapidly than its fellow, which is open to the circulation, thus demonstrating the activity of reflex centres of the cord. (The reflex time of the excluded limb is subsequently increased, owing to depression of function in the cord centres, and in part to the stasis existing in the vessels of the limb.)

Experiment.—Frog of 29 grammes. Brain destroyed 24 hours previously. Vessels of the Left Leg ligatured. Reflex tested with 1–800 sulphuric-acid solution.

	Left.	Right.	
	secs.	secs.	
0 minute	2·5–3	3–3·5	
0 „	—	—	Injected into dorsal lymph sac, pseudaconitine ·002 gramme per kilogramme.
15 minutes	3	2·5	
35 „	3	3	
50 „	3	3·5	
70 „	3	4–4·5	
85 „	3–3·5	5–6	
95 „	3–3·5	6·5	Circulation in R. web very sluggish.
105 „	4–5	6–11	Variable and uncertain.
125 „	5·5	(24-sec. twitch, not out in 50 secs.).	
	Acid 1–500		
130 „	2·5–3	(20-sec. „ „ „).	

At the end of this observation the ventricle was found to be in a condition of intermittent delirium, the auricles being much accelerated. Minimum excitability was greater on the ligatured side, but stimulation of the sciatic nerves elicited an equally good series of muscular contractions on the two sides.

Whilst the sensory nerves are early affected, there is an evident reduction in the reflex excitability of the spinal cord, which makes its appearance later, but before longitudinal conduction is distinctly interfered with. Pseudaconitine is apparently more active in depressing the cord reflexes than are the other two aconitines.

An even more striking demonstration of the immediate action of the aconitines upon cutaneous sensibility may be obtained by keeping one of the feet of a brainless frog exposed to a solution having the alkaloidal strength of ·5 to 1 per cent. for 2—5 minutes, and then contrasting the reflexes obtained from the two feet dipped alternately in acid solution. Many such experiments terminate fatally, owing to alkaloidal absorption having occurred, probably from small wounds or abrasions, but if this is not the case it may be demonstrated that the reflexes are only restored to their usual condition on the affected side after a lapse of 60 to 90 hours.

After winter frogs have received large sublethal doses of pseudaconitine, or of its allies, there is a period of 8 or 9 days of great reduction of voluntary and reflex movement. Circulation is feeble, and respiration may be suspended entirely for the earlier part of the time, whilst the phenomenon described in the next paragraph will probably be witnessed.

Action of Pseudaconitine in favouring Occurrence of Œdema.

The fact was incidentally observed that frogs (*R. temp.* and *R. esc.*) kept wet (*i.e.*, sitting in water ·5 to 1 centim. in depth) increased greatly or enormously in weight during the days following administration of a large sublethal dose of pseudaconitine, and to a slighter extent after the other aconitines. This increase in weight depends upon the increase of fluid, either

(*a*) In the urinary bladder; or
(*b*) There is a general œdema of the tissues and within the lymph spaces; or
(*c*) These conditions co-exist.

The following daily weighings are taken from a series of frogs kept in different conditions. All the frogs, except No. 1, received pseudaconitine after it had been ascertained that their normal weight (with empty bladder) was practically constant. The total weight of Frog 3 includes the urine.

Before injection.	No. 1.* Control kept in 10 millims. water.	No. 2. Kept covered on plate under funnel lined with moist filter paper.	No. 3. Kept in 10 millims. of water.
	37	35·25	49·1 (3·5 cub. centims. urine).
	Injected into 2 and 3, pseudaconitine ·0007 gramme per kilogramme.		
1st day . . .	—	34·9	51·9 (4 cub. centims. urine).
2nd ,, . . .	—	34·6	62·2 (6 ,, ,,).
3rd ,, . . .	—	34·1	65·5 (8 ,, ,,).
4th ,, . . .	—	35·2	75·3 (15 ,, ,,).
5th ,, . . .	—	33	
6th ,, . . .	—	32·1	66·5 (22 ,, ,,).
7th ,, . . .			
8th ,, . . .	—	—	63·6 (16 ,, ,,).
9th ,, . . .	—	31·3	
10th ,, . . .	—	—	69 (21 ,, ,,).
12th ,, . . .	—	29	
14th ,, . . .	—	—	69·5 (17 ,, ,,).
16th ,, . . .			
18th ,, . . .	—	—	71 Dead.
20th ,, . . .			
22nd ,, . . .			

Whilst frogs kept on a dry glass plate, after receiving pseudaconitine, lose weight from the free skin secretion, but mainly by diuresis induced by the alkaloid, loss of weight is also witnessed, as in the experiment just quoted, when the animal is kept in a moist atmosphere but without immersion of any part of the body in water or contact with a moist surface, such as a saturated sponge.

On using brainless frogs for similar observations, it was found that œdema occurred also in the control animal placed in water, though to a much slighter extent than in the animal receiving pseudaconitine. In order to obviate the possibility of fluid gaining access to the alimentary canal through the mouth, the frogs were hung on threads, so that only the lower limbs and half the trunk were immersed. These experiments were entirely confirmatory of the previous observation, that after poisoning by pseudaconitine free absorption of fluid takes place through the skin, resulting in great accumulations in the tissues, especially in the lymph sacs and in the urinary bladder. As in the former series of experiments, no appearance of hæmoglobin nor of albumen was detected in the urine. Series of parallel experiments made with aconitine and japaconitine showed in the main similar results, but the œdema of the tissues appeared to be less readily induced by them than by pseudaconitine.

The reduced functional activity of the cord succeeding pseudaconitine effect is favourable to a retention of the augmented renal secretion in the bladder.

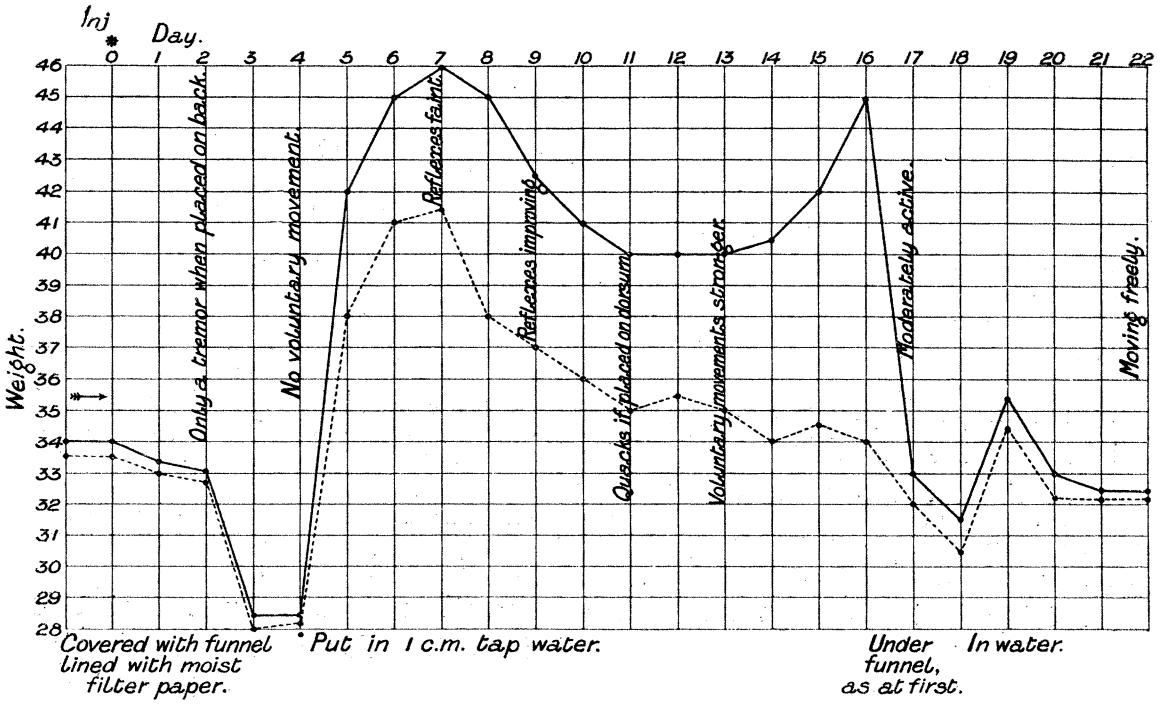
The tendency to hyperabsorption is present for many days after pseudaconitine has

* This frog varied by only 1 gramme during observation.

been given, so that if the animal is kept in a merely moist atmosphere for 3 or 4 days after injection, for so long it loses weight (Diagram A), but at once becomes œdematous when placed in water, the urinary secretion also increasing greatly. The œdema is naturally outlasted by the diuresis.

Diagram A.—PsA. on Body Weight of *R. esc.* kept Dry or in Water.

(The top line represents weight (total) in grammes; the lower is the weight after emptying the bladder. The difference between these lines therefore represents urine—that between the abscissæ and the lower line, œdema.)



Withdrawal from water of a frog which, whilst partially immersed, has received pseudaconitine and become dropsical, is followed by a prompt decline in its weight (Diagram A), so that the falling weight curve soon cuts the curve obtained from an animal less dropsical originally, which remains partially immersed in water. Two pegged frogs, esculentas (Experiment X.), were placed in water and received ·0006 gramme per kilogramme pseudaconitine respectively; both became markedly œdematous, that more so having increased by 42 per cent. of its original weight. This frog was now removed from water, whilst the other was retained in it, with the result that œdema declined so fast in the former that on the eighth day after injection and third after removal from water it was relatively free from œdema when compared with the other.

In both normal and brainless frogs the occurrence of œdema favours suspension of cord reflex function and gradual heart failure, so that death is more readily induced after poisoning by the aconitines when the frog is kept wet rather than in a merely moist

atmosphere. The hydræmic state of the blood has been demonstrated repeatedly in such cases. The distension of the bladder bears evidence to the continuance of renal function. No colouring matter of the blood or albumen has so far been found in the urine, but it would be unwise to conclude that they never appear, in view of the altered condition of the blood in the vessels.

1. There is an increased absorption of fluid from the surface of the body during prevalence of pseudaconitine action; probably also from the alimentary canal, this being favoured by the flat position often assumed, which facilitates passage of fluid into the mouth.

2. This is, to a corresponding extent, productive of hydræmia and of impaired function, especially of cord and heart. Fluid accumulates in the tissues, but there is no evidence that the kidneys fail in elimination, as a large amount of urine is found in the bladder, and if the animal is removed to a dry or damp chamber, œdema is drained off by this channel.

3. The fact that some œdema occurs in animals merely pegged and suspended in fluid seems to indicate a change, probably vaso-motor, which causes increased absorption, the same effect being produced, but to a greater extent, by the aconitines. This may perhaps be best described as a reduction of a restraint upon absorption which is in operation in the uninjured and unpoisoned animals.

4. Salt solution (·7 per cent.) as the medium of immersion seems to be more productive of œdema than is distilled water.

5. Pseudaconitine is more favourable to the production of œdema than are the other two aconitines.

Action of Pseudaconitine on the Blood of Frogs.

When frogs are the subjects of experiment it is at once evident that obtaining blood, even in the small quantities required for examination, is a proceeding of some difficulty. A single estimation is easily made with blood drawn directly from the heart, but this can only be done as a terminal experiment, and lacks the necessary contrast. Or in brainless frogs a toe may be amputated, or an artery divided, or a vain punctured. Dr. Esslemont has carefully contrasted these various methods in the Aberdeen Pharmacological Laboratory. He finds that the first proceeding yields unreliable results, as there is free exudation of lymph from the divided toe which serves to dilute the blood—the second method is apt to be attended with hæmorrhage, which interferes with further observation, while the last plan is free from the first, and is less open to the second objection. Puncture of the vein was therefore practised as a means of procuring blood, though, as some loss of blood is apt to occur, it is only claimed that the method is sufficiently reliable for the recognition of considerable changes in the hæmoglobin and coloured corpuscles. A single injection of pseudaconitine was given after the necessary estimations had been made. The

results, which are appended, are summarised with the exception of the first, which, for the sake of example, is given in detail from Dr. ESSLEMONT'S notes :—

Experiment—Rana esculenta.—Blood obtained from punctured femoral vein. (Animal in 1 centim. distilled water.)

Hæmoglobin, 40 per cent. Corpuscles, 3800 for field.

0 h.	Inject ·0007 pseudaconitine per kilogramme.	
22 h.	Hb. 23 per cent. corpuscles 1920.	Frog œdematous. Circulation still moderate.
70 h.	Hb. 18 per cent. corpuscles 1940.	Moderate œdema.
118 h.	Hb. 22 ,, ,, 2100.	Considerable œdema.
166 h.	Hb. 15 ,, ,, 1460.	Great œdema.
238 h.	Hb. 15 ,,	,,
358 h.	Hb. 15 ,, ,, 1350·0	,,

Experiment terminated.

Experiment—Rana esculenta.—(Animal in 1 centim. distilled water.) A dose of ·00085 in the course of 48 hours reduced the hæmoglobin by 32·5 per cent., and the corpuscles by 57 per cent. Distinct hydræmia of the blood was produced, and general œdema of the tissues.

But these results stand in contrast with others, in which no œdema was produced, the frog being kept dry upon a porcelain plate. In one experiment in which the large dose of ·001 gramme of pseudaconitine per kilogramme was survived, five estimations were made in the course of the 6 days following injection, with the result that there was no more variation than could be accounted for by the errors of observation. It therefore seems probable that no active destruction of blood elements follows pseudaconitine, but that the hydræmia, which is mainly due to absorption of an excess of extraneous fluid into the tissues, is accountable for the altered constitution of the blood. It may be a point for further enquiry whether the powerful effect which the aconitines exert on the skin may prepare a channel for an abnormal degree of absorption.

Frogs' Nerve and Muscle poisoned in situ.

It is to be noted that, as was the case with aconitine, the effect of pseudaconitine as an agent reducing the activity of intramuscular nervous structures, has been hitherto overstated on the one hand, whilst its depressant effect, when poisoning is thorough, upon muscular tissue, has not received adequate recognition on the other. As contrasted with aconitine and japaconitine, pseudaconitine does not prove itself a more powerful poison towards nerve and muscle, but rather the reverse. This

statement is founded on the examination of a series of nerve-muscle preparations derived from frogs poisoned by doses varying from sublethal to "overwhelming" in action, as well as on the results of "immersion" experiments. Either of these methods makes it clear that pseudaconitine—the most toxic alkaloid of the series towards mammals—is, with regard to the peripheral motor system of frogs, the least active member of our series. Whilst all the aconitines may cause in certain dose a transitory increase of excitability in intramuscular motor nerve structures, pseudaconitine is in this respect more active than the other two.

Method.—After destruction of the brain and the application of vascular ligature to one hind limb, the frog was poisoned by doses of pseudaconitine of very various proportions to the body weight. These were administered at once, or, in order to avoid early stoppage of the heart, and thereby hindrance to the free circulation of the poison, in divided doses, repeated at intervals of 1 or 2 hours. Nerve-muscle preparations were made and examined after poisoning had reached a certain stage, as that of failure of reflex, cessation of circulation in the web vessels, &c.

The primary effect of pseudaconitine was to increase, the ensuing effect to reduce, excitability of intramuscular nervous structures and (though to a lesser extent) of muscular tissue. After a single dose, just sufficient to prove lethal, the muscle yields a series of good responses to indirect stimulation (examination immediately on cessation of reflex), though there is on repeated stimulation an earlier occurrence of fatigue on the control side.

A more distinct action followed repeated administration of smaller doses, which arrest the heart only after several hours. The nerve is then found to be somewhat less excitable, and on continued stimulation its work capacity is reduced when contrasted with the control. When the preparation is postponed until the next day, the tissues remaining soaked in the poison, the nerves may be found much less sensitive or altogether insensitive (this exceptionally) to an electrical stimulation, and the muscle greatly reduced in its excitability and capacity for work. It is necessary, however, to add that the excitability of the nerves on the control side is somewhat impaired under these circumstances.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Preparation made before Reflexes arrested.

R. esculenta pegged, vessels to left leg ligatured. Injected ·0015 pseudaconitine per kilogramme.

In 4 hours reflexes failing, made two nerve-muscle preparations. Lever × 10.

	Ligatured.		Poisoned.		
	N.	M.	N.	M.	
Min. excit	18 centims.	8 centims.	22 centims.	9 centims.	
Height of contraction—					
Induction shock (0) .	48 millims.	48 millims.	47 millims.	47·5 millims.	Both give excellent series of 30 contractions.
Tetanus	84 „	84 „	82·5 „	84 „	Fatigue slightly sooner on poisoned side.

Preparation made Two Hours after Reflex Arrested. Circulation ceased in Web.

R. temporaria pegged, left vessel ligatured. Injected ·002, and in the course of 90 minutes ·001 gramme pseudaconitine per kilogramme.

The reflexes had disappeared for 2 hours when the preparations were made (5 hours after the first injection), ventricle motionless, feeble movement of the auricles, but all circulation has ceased for some time.

Lever × 4. Weight 10 grammes.

Ligatured preparation min. excit. . . .	N 30·5	M 12
Poisoned „ „ . . .	N 26	M 14

A series of stimulations (0 induction shocks) give a good series of contractions, control side 23—21 millims., poisoned side 20—18 millims.

The muscles were subjected to a long series of stimulations at ·5 second interval, the result being fatigue in both—control side from 22 to 20 millims., the poisoned 19·5 to 15, the muscle more fatigued than the control, and tending to relax.

After stimulating for 3 minutes and resting for 1 minute, two short series of contractions were taken, the nerve being stimulated. The control gave contractions 16—8 millims., the poisoned 8·5—3·5. The nerve is slightly more involved than the muscle in this experiment, but the latter does not entirely escape.

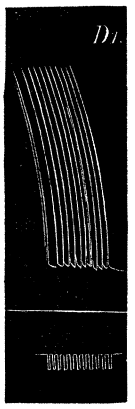
Large and "Overwhelming" Doses.

When doses which may vary from five times the lethal up to forty or fifty times that amount ("overwhelming" proportion) are given, the heart is arrested speedily, and

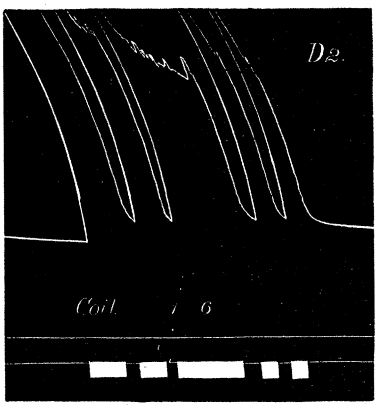
there may be a question as to the distribution of the absorbed poison. Undoubtedly, however, such enormous doses as those last named are found greatly to impair the reaction of the motor nerves, and even occasionally to abolish it altogether; the muscle is also damaged to a lesser extent in its ability to perform laborious work.

When observers speak of the curare-like action of pseudaconitine, they usually refer to the effect of large doses. In the experiment (Fig. D), a proportion of ·013 gramme

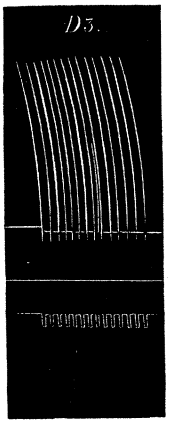
PsA. ·013 gramme per kilogramme on Muscle-nerve (*R. temporaria*).



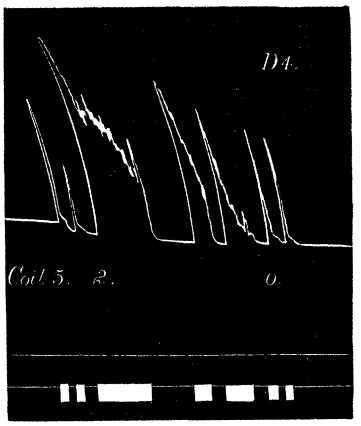
N. unpoisoned. Opening ind. shock, 5 secs.



N. unpoisoned. Tetanus. (The summit of contraction is not shown.)



N. poisoned. Opening ind. shock, 5 secs.



N. poisoned. Tetanus.

per kilogramme was given to a decerebrated *R. temp.* of 42 grammes, a total dose of ·000446 gramme, which would be a serious dose for a man. The following figures show the reaction to induction shock and tetanising currents respectively. Preparations made and examined 3 hours after poisoning.

	Single induction shock.	Faradisation.	
Ligatured. .	N. 34 millims. (D_1)	52	After tetanising 6 times tracing D_2 taken (coil 16 and 14 centims.).
	M. 37·5 millims.	55	
Poisoned . .	N. 29 millims. (D_3)	41	
	M. 35 ,,	50	After tetanising 6 times tracing D_4 taken (coil 5–2–0).

The statement of Böhm and Ewers ('Arch. Exp. Path. u. Pharm.,' 1873, vol. 1, p. 385) to the effect that intramuscular nerves are differently affected in specimens of *R. temp.* and *R. esc.*, is not confirmed by the present research for pseudaconitine or its allies. The degree of activity does, however, show a variation, the aconitines being slightly more active towards the *R. esc.*

Whether given to frogs in lethal, hyperlethal or overwhelming doses, pseudaconitine shows itself less energetic than aconitine and japaconitine in depressing or suspending the function of motor nerves at the periphery.

Immersion of Muscles in Pseudaconitine Solution.

Two nerve-muscle preparations were made from the legs of a frog (*R. esc.* or *R. temp.*). The plan followed in making these preparations was to keep the sciatic extended by ligaturing it round the upper end of the femur, the bone being denuded of its muscles, and the foot left in its normal connection.

These preparations were placed in small covered dishes containing 10 cub. centims. of Ringer's solution. After the lapse of a certain time, the minimal excitability was ascertained toward direct and indirect stimulation, after which the pseudaconitine solution, accurately measured, was added to one of the glasses, with agitation. Thereafter the two preparations were tested from time to time.

The following results have been arrived at:—

1. Persistence of excitability of muscle is to a very slight extent limited by solutions of pseudaconitine as dilute as 1—800,000, or occasionally by 1—1,000,000. The effect increases with the employment of stronger solutions, whilst weaker ones are inoperative.

2. The intramuscular motor nerves are sensitive to even more dilute solutions, so that some impairment in function is evidenced when pseudaconitine is present in the proportion of 1—1,500,000, and very occasionally if the proportion is only 1—2,500,000.

3. Excitability is temporarily increased to a slight extent in muscle, and to a larger extent in nerve by such solutions as 1—200,000 to 1—800,000.

4. When such relatively strong solutions as 1—20,000 or stronger are used, the decline of activity of motor nervous and muscular tissue (*i.e.*, to indirect and direct stimulation) becomes more parallel than is the case with weaker solutions.

Strong Solution.

Experiment.—*Rana esculenta.*

Time.	Two nerve-muscle preparations from a 30 gramme frog, placed in RINGER'S solution.			
	N.	M.	N.	M.
0 min.	48	19	47	19·5
0 ,,	—	—	Add PsA to make 1–16,000	
15 mins.	46	19·5	42	18
30 ,,	44	20	43·5	19·5
45 ,,	43·5	20	39·5	18
65 ,,	44	19·5	32	17·5
120 ,,	44	20	23	16
150 ,,	43	18	21·5	16
180 ,,	41·5	21	16	17
210 ,,	41	21	6·5	10
240 ,,	41·5	20	0	8·5
270 ,,	—	—	0	4

Note.—The figures in the N.M. columns indicate the distance of the secondary coil from the primary in centims.

5. The results obtained with preparations derived from *R. esc.* and *R. temp.* respectively are in the main parallel, the excitability to direct stimulation disappears very nearly at the same time, but excitability of the muscle of esculenta is somewhat longer preserved than that of temporaria.

6. When equal quantities of the two aconitines are added to the two companion preparations respectively, it was found that the activity of pseudaconitine was uniformly less than that of japaconitine, and in the majority of observations (see p. 82) slightly less than that of aconitine. On the other hand, pseudaconitine favours a temporary increase in excitability more than do the other two alkaloids.

SECTION II.—JAPACONITINE.

The great toxicity of this alkaloid has been admitted by all experimenters, and as already indicated, it has been regarded as equally, or even more, energetic when contrasted with aconitine. No essential difference in its manner of action has been described by previous observers.

Results of Present Research.

In order to avoid repetition, the points in which japaconitine and aconitine have been found to closely correspond will be only very briefly touched upon.

Action of Japaconitine on Mammalian Blood Pressure.

The effects are in all respects parallel to those obtained from aconitine excepting that, dose for dose, japaconitine is qualitatively slightly more active, and this activity is manifested when artificial respiration is employed. The toxic dose is, however, distinctly larger under such circumstances than when the animal breathes spontaneously. The action of japaconitine on medullary centres, on the peripheral vagi and on the vascular systems, central and peripheral, shows no essential difference from that of aconitine. With reference to the last point it may be added that entire abolition of activity of the vaso-constrictor centre has never been reached. This point is illustrated by an experiment in which 73 minutes after injections of ·00013 gramme per kilogramme japaconitine, the ventricles beating about half as fast again as the auricles, strong sciatic stimulation induced a very distinct rise of pressure, and developed two respiratory efforts, the auricles subsequently beating at the rate of 192, the ventricles at 264 per minute. The ventricles passed into a state of wildly tumultuous action 5 minutes later, death rapidly supervening.

Lethal Dose of Japaconitine to Rabbits.

An occasional lethal result follows hypodermic administration of ·000065 gramme per kilogramme body weight and upwards. In one instance a dose of ·000075 gramme actually occasioned death in 30 minutes. A proportion of ·00008 gramme causes considerable vascular depression, with motor paralysis, and the lethal results from this proportion to ·000105 are frequent. As a first dose the latter proportion is regarded as the absolutely lethal, death ensuing in 1–2 hours.

On Respiration of Narcotised Animals.

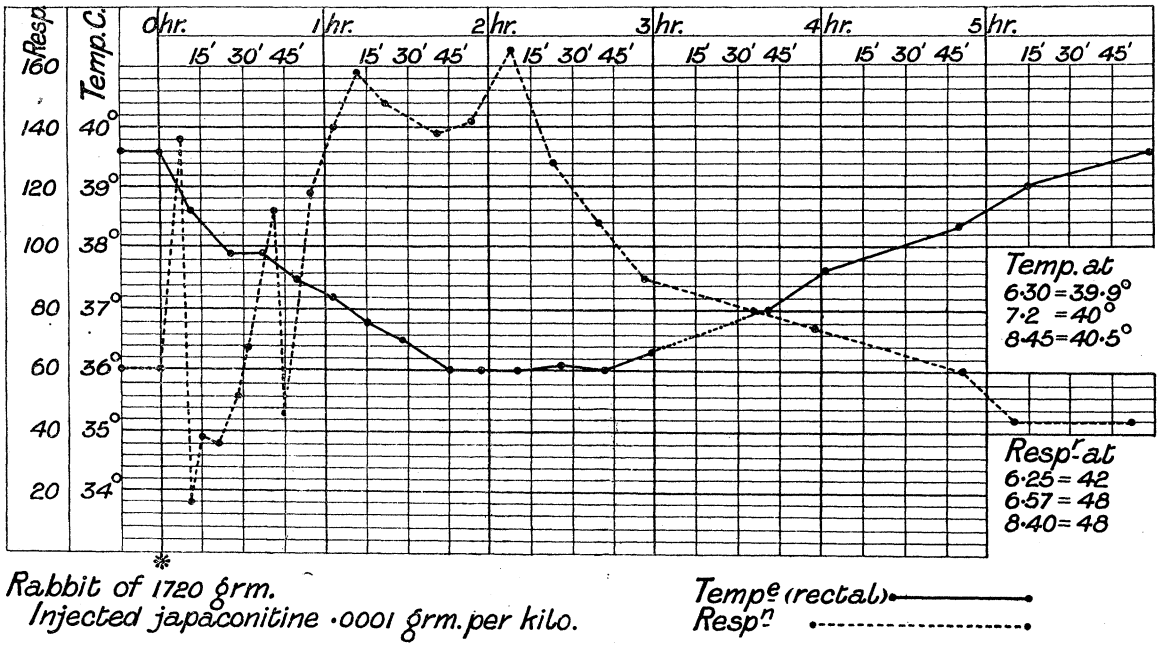
The respiration of etherised animals either slows from the first after japaconitine administration or a very temporary and inconspicuous acceleration precedes the slowing. The death of the animal is respiratory in character, but it is preceded by the same disorder of the circulation as in the case of aconitine and pseudaconitine, and at an advanced stage of poisoning neither insufflation with air, nor with air mixed with a large proportion of oxygen, will permanently restore the heart to normal action.

Action of Japaconitine on Respiration and Temperature of Rabbits.

Considerable acceleration of respiration is the usual result of large sublethal doses of japaconitine, quickly yielding to slowing, the condition being dyspnœal and the respiratory movements spasmodic and largely abdominal in character, the inspiratory phase unduly prolonged.

The preliminary acceleration is more often witnessed than when pseudaconitine is the poisonous agency, and the ensuing slowing is more transitory in character, indeed the rapid, though dyspnœal, stage of respiration is more rapidly established than after aconitine itself (*vide* Diagram B).

Diagram B.—Japaconitine on Internal Body Temperature and Respiration of Rabbit.



Action of Japaconitine on Internal Body Temperature.

The temperature following japaconitine undergoes an elevation of a fraction of a degree after sublethal doses, followed by a very steady reduction, and on an average the fall produced by this alkaloid given in large sublethal and lethal doses averages ·25 to ·5 centigrade more than that produced by similar doses of aconitine.

The average duration of sub-normal temperature in these observations is from $4\frac{1}{2}$ to 5 hours, the normal being regained on an average 20 minutes later than would be the case after a parallel dose of aconitine. Diagram B illustrates the action of a large sublethal dose on temperature (rectal) and respiration.

The following synopsis of the results of seven observations shows the main points of interest with regard to respiration and temperature :—

Tabulated Results of Seven Experiments. Action of Japaconitine on Respiration and Body Temperature of Rabbits.

Dose of japaconitine per kilogramme animal weight.	Respiration.			Internal temperature.			Notes.
	Initial acceleration.	Slowing to	Time of greatest slowing.	Initial rise.	Fall.	Time of recovery to normal.	
				° C.	° C.		
·00004	20 per 1 min.	40	40 mins.	·4	·5	In 4 hrs.	
·00006	None	32	40 „	·2	·8		
·000075	32	47	—	·5	2·2	In 258 mins. and then +	Moderate toxic effect.
·000085	50	44	—	·2	3·5	In 297 mins. and then +	„ „
·000095	Great	30	45 mins.	·3	4·5	In 6 hrs.	Deep toxic effect.
·0001	„	34	50 „	None	4·7	In 7 „	Nearly lethal.
·000105	„	20 in 26 mins., stopped in 53 mins.	—	„	1·3	—	Lethal in 106 mins.
·00013	„	—	—	„	1·6	—	Lethal in 64 mins.

Lethal Dose of Japaconitine for Guinea-pigs.

The certainly lethal dose for guinea-pigs lies between ·000095 and ·0001 gramme per kilogramme body weight, although an occasional lethal effect ensues after the proportion of ·00006 gramme and upwards. The main symptoms under various doses are noted in the synopsis.

Japaconitine for kilogramme Body Weight.	Main Symptoms.
·000035	Retching, salivation. Respiration reduced by 20 per 1 minute.
·00007	As above. Sharp dyspnœa, paresis.
·00009	As above. Dyspnœal attacks accompanied by spasm. Respiration reduced to 16 per 1 minute. (This proportion often lethal.)
·0001	Fatal effect in 90 minutes.
·00012	Dyspnœal attacks with spasm in 15 minutes. Much paralysis. Fatal in 30 minutes.
	In fatal cases the right heart and coronary vessels much engorged. Auricles beating, ventricles twitching. Minimal excitability of sciatic 24 and 19 centims. respectively.

On Pigeons.

The action of japaconitine is similar to that of aconitine upon pigeons, only that it is slightly more toxic, whilst as contrasted with psuedaconitine the dyspnœa develops more gradually and the paresis is neither so extensive nor so long continued as after the latter.

Whilst recovery has occurred after ·000085 gramme per kilogramme bird weight, the proportion of ·00009 is to be regarded as a lethal dose.

Death ensued very rapidly in all cases of fatal issue after injection. Thus, after ·0001, there was a spasm at 9 minutes and again at 10 minutes, death occurring in 11 minutes. Conversely, if the dose was sublethal recovery took place rapidly, a bird receiving ·0000605 gramme per kilogramme recovered in 65 minutes, and another which showed mandibular movements, shaking of head, dyspnœa, gaping, and regurgitation from the crop after ·00008, was practically normal 94 minutes after injection.

On the Blood.

Experiment I.—The hæmoglobin and corpuscular contents of the blood of a full-grown rabbit having been carefully determined, japaconitine was administered in doses of ·00006 gramme per kilogramme every second day. After six injections had been made the hæmoglobin was reduced by 16 per cent., the corpuscles by 1,120,000 per cub. millim., and the body weight by 55 grammes, whilst after three more injections the hæmoglobin was still further reduced by 2 per cent., the corpuscles by 320,000 and the body weight by 105 grammes.

There was clearly an impairment in nutrition produced, and it was noticeable that after the alternating days when no japaconitine had been given there was an evident, though very partial, recovery in all respects, but that this reaction became less marked as the experiment proceeded.

In order to ascertain whether such deterioration would arise if there was no impairment in nutrition resulting from japaconitine, it seemed advisable to give a dose sufficiently small to guard against this result. A daily dose of ·00002 gramme per kilogramme (about one-fifth of the lethal) was therefore decided upon. The weight of the full-grown rabbit varied from 1995 to 2040 grammes (the initial being 2020, the terminal 2005); in other words, the weight was very constant and the condition of the animal excellent throughout, its appetite was in no way affected. The hæmoglobin, as estimated before administration of japaconitine commenced, is reckoned at 100 per cent.

Experiment 2.		Body weight.	Hb.	Corpuscles.
1st day	—	2020	—	6,840,000
2nd ,, ...	Injection 1			
3rd ,, ...	,, 2			
4th ,, ...	,, 3	2012	– 6 per cent.	5,384,000
5th ,, ...	,, 4	2020	– 5 ,,	5,710,000
6th ,, ...	,, 5	2000	– 6 ,,	5,653,000
7th ,, ...	,, 6			
8th ,, ...	,, 7	1995	– 5 ,,	5,813,000
9th ,, ...	,, 8	2010	– 6 ,,	5,360,000
10th ,, ...	,, 9	2010	– 1 ,,	6,048,000
11th ,, ...	,, 10	2015		
12th ,, ...	,, 11	2005	– 4 ,,	5,733,000
13th ,, ...	,, 12	2025	—	5,946,000
14th ,, ...	,, 13	2040	normal	6,800,000
15th ,, ...	,, 14	2035	—	6,640,000
16th ,, ...	,, 15	2005	– 3 per cent.	5,733,000

This series of observations, carefully made by Dr. ESSLEMONT, whilst allowing for the slight errors which thee mployment of FLEISCHL'S hæmoglobinometer and the ZEISS-THOMAS' hæmacytometer may admit of, are sufficient to show that there is no marked alteration, either in hæmoglobin or in the corpuscular contents of the blood as a result of the administration of a daily dose of ·00002 gramme japaconitine per kilogramme.

Action of Japaconitine on Peristalsis.

The statement stands largely on the assertion of POHL ('Arch. f. Exp. Path. u. Pharm.,' vol. 34, p. 94), that ·5 per cent. solutions of aconitine brought into direct contact with the muscular coat of the excised intestine caused powerful and repeated peristaltic movements. The degree of action approached that of muscarine. By other methods, however, the result is not identical. It has been frequently noted in the course of this research that evidence of increased peristalsis following the hypodermic administrations of the aconitines is by no means an invariable or prominent symptom of their action, although in advanced poisoning the low blood pressure prevailing is in itself favourable to this condition.

What it is specially desired to direct attention to here, however, is that the contact of the various aconitine solutions with the intestinal mucous membrane tends to hinder the occurrence of peristalsis, and to arrest such movements or diminish them for a considerable time. Thus the occurrence of increased peristalsis in the VELLA'S fistula of a dog, after ingestion of food or from the action of drastics, has been found to be greatly under the control of very dilute solutions of the aconitines. To avoid repetition, only one member of the group—japaconitine—will be considered here.

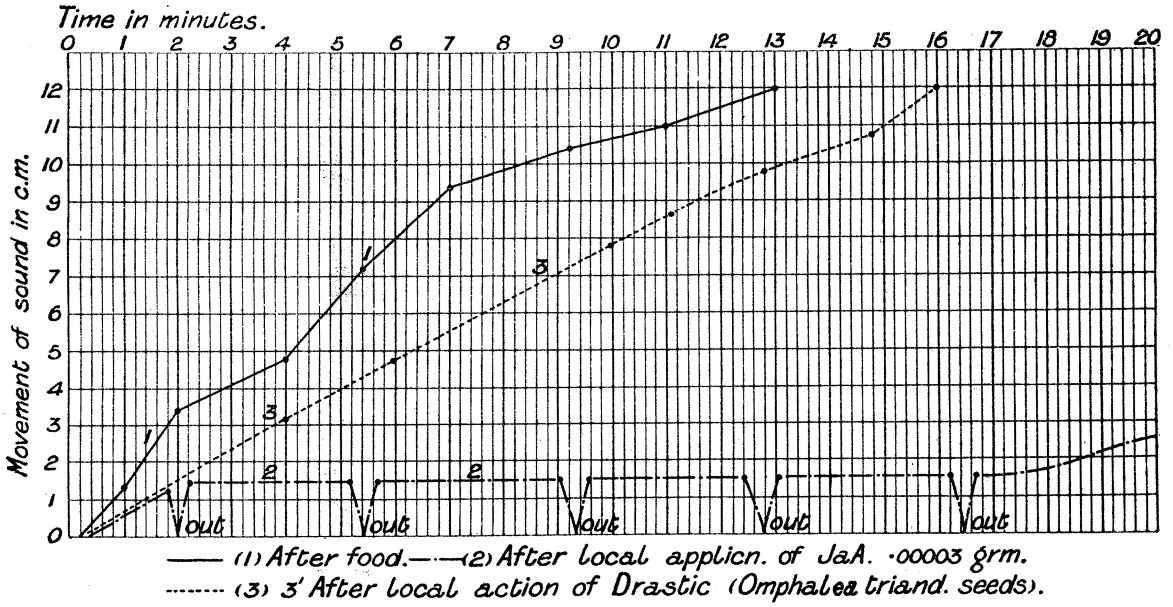
The dog, in which a VELLA'S fistula had been established some weeks previously, was at the time of the observation in excellent health, and its nutrition in the most satisfactory condition.

Registration was made in several experiments by the method and apparatus already described by one of us ('Roy. Soc. Proc.,' No. 247, 1885, p. 212), in others the rate of passage of the travelling sound was observed without registration. After the condition as to peristalsis for the time being had been established, doses of from $\frac{1}{50}$ to $\frac{1}{20}$ milligrammes of japaconitine were injected (in 2 cub. centims. of water) into the fistulous intestine and soon thereafter the observation was repeated. The following experiments in brief will serve to illustrate the results.

Experiment A.—The dog had received a light meal 2 hours and 15 minutes previously, when the sound in connection with the enterograph was introduced. The transit was at first very steadily accomplished, so that 12 centims. were traversed in 13 minutes (Diagram C1). (There was slight hunger and the results were

Diagram C.—Japaconitine on Peristaltic Movement.

(Travelling Sound in Isolated Loop of Intestine (VELLA'S Fistula).



fairly constant.) Local injection of ·00003 gramme (C2) of japaconitine in 2 cub. centims. of water was now made into the upper opening of the loop and in 15 minutes the sound was again introduced. There was some pendulum movement of the loop and the sound was drawn in to the extent of 1·5 centim., but thereafter the hold of the intestine upon it relaxed, and it was ejected from the upper opening; this took place five times in the course of the next 25 minutes—no propulsion taking place, though after the last introduction it passed 2·5 centims. into the loop and was held there without progressing.

Two hours later the same result. In order to ascertain whether this inertia would yield to a well-marked drastic, ·05 gramme of emulsionised seed of *Omphalea triandra* (a drug at this time undergoing investigation in the laboratory) was

injected, and 5 minutes thereafter the sound again introduced (C3). A moderate degree of peristalsis at once developed, and in 18 minutes the sound had advanced 12 centims. (It may be stated that if no such substance as japaconitine is previously used a very rapid transit follows the local action of Omphalea.)

Experiment B.—The first passage of the travelling sound was made 2 hours 25 minutes after a light meal, and occupied 12 minutes 45 seconds. Local injection of japaconitine ·00005 gramme in 1·5 cub. centim. water was now made into the loop, and in 30 minutes the sound was again introduced. Although feeble rolling movements persisted, there was but faint progression at first, and then entire absence of peristalsis, so that after 22 minutes the total advance was little more than 2 centims. Four hours after the japaconitine application the experiment was repeated, when it was found that feeble peristaltic movement had returned, but only a partial transit was made. In 5 minutes the sound had advanced 3·3 centims., in 10 minutes to 5·8; in 15 minutes to 6·2, and in 20 minutes to 6·4 (as hunger was beginning to develop conditions were favourable to rapid peristalsis). The next morning 3 hours 15 minutes after food, peristalsis was found to be moderately active, the sound passing through the fistulous loop in 17 minutes.

Experiment C.—The travelling sound was introduced 90 minutes after the morning meal. Peristalsis was active, and the transit was completed in 11 minutes 30 seconds. Japaconitine, ·000075 gramme in 1 cub. centim. water, was injected at once into the loop and in 50 minutes the sound was re-introduced, but in the succeeding 12 minutes there was absolutely no movement forwards. Six hours 32 minutes after injection there was evidence of the presence of very feeble peristalsis, the sound advancing almost 1 centim. in the course of 12 minutes. On the succeeding day (the animal having had free access to food all the time) and being in a condition of satiety, the transit occupied 22 minutes.

These experiments all point to a powerful action of japaconitine when applied to the mucous lining of the intestine in checking peristalsis, whether this be due to the presence of a mechanical or irritant body in the intestinal loop. In so far its effect is sedative upon the sensory visceral nerves, to the origins of which it gains easy access, but beyond this it tends to restrain peristalsis, which is such a prominent symptom at certain phases of hunger and ingestion. Japaconitine, whilst diminishing or abolishing peristalsis by its local action, does not entirely suspend the occurrence of unpropagated constrictions in the intestinal loop, as has been proved by introducing an india-rubber bag filled with air and connected with recording tambours.

Action of Japaconitine on Frogs.

For *R. temp.* (May and June) the lethal proportion of japaconitine was determined at ·001 per kilogramme, though deaths were frequent down to ·0006. In August fresh and vigorous specimens occasionally recovered after less than ·000115 per

kilogramme. This proportion may be accepted as the absolutely lethal for that season. In May and June ·0009 to ·001 per kilogramme is the ascertained proportion for *R. esc.* Excitement of movement with frothing on and around the body is much more marked in *R. temp.* than in *R. esc.* Both show spasmodic extension of the limbs at intervals, succeeded by a stage of greater quiescence during which reflex is uncertain and voluntary movement is still possible. The asymmetrical and often grotesque disposal of the limbs is met with after japaconitine as after the other aconitines. When large but sublethal doses have been given, recovery takes place more rapidly than after pseudaconitine, which is also more active in depressing respiration and causing paralysis and œdema, though less active in disordering the heart's action than is japaconitine.

Action of Japaconitine on Frog's Heart.

The action of a small dose is to slow the rhythm by a few beats per minute, and this is the initial effect of larger doses, which subsequently cause a considerable acceleration. Large sublethal and lethal doses interfere with full and co-ordinate ventricular systole at a time when the auricles are beating regularly, producing thereby a fault in sequence, which may be constant or intermittent in character. The ventricle often evidences a churning incessant movement, one part contracting whilst another dilates. This movement imparts, at most, only a very feeble and oscillating impulse to the blood in the peripheral vessels.

Stimulation of the vagus and venous sinus is attended by the same results as after aconitine ('Phil. Trans.,' B, 1898), and the same partial degree of recovery follows the application of atropine solution.

Whilst the action of japaconitine is qualitatively like that of aconitine, it appears to be slightly superior to it quantitatively.

Perfusion of the excised heart capable of spontaneous contraction, by RINGER'S fluid containing traces of japaconitine increases the excitability of the intrinsic motor mechanism, and strengthens the systole, especially if this is originally of a feeble character. The beat accelerates, and after the perfusion of large amounts of the alkaloid (·0001) great rapidity with imperfect systole and diastole, and a tendency to group beating, develops. The extreme irregularity in ventricular action, observed when the heart is poisoned, *in situ*, is absent if the ventricle of the excised organ is tied upon the cannula at the auriculo-ventricular groove, or is at most represented by a slight vermicular movement of the myocardium, indicative of inco-ordinate action.

As an early result of japaconitine perfusion the apical two-thirds of the ventricle yields a vigorous systole with some retardation of its maximum. This gives place to a feeble and less sustained systole, the excitability of the organ being increased so that spontaneous beating, often of a grouped character, is witnessed. Great

acceleration of rhythm appears in alternation with or succeeding this condition. Ultimately excitability declines, the systole becomes feeble and ineffective. It is at this time that vermicular action is occasionally present.

Action of Japaconitine on Frog's Blood (R. temp.).

In these experiments only two estimations (initial before injection of japaconitine and terminal 2–3 days after injection) were made. The estimation of hæmoglobin is only approximately correct, as the tint of FLEISCHL's slide matches the tint of the blood of the frog very imperfectly. The frogs were kept in a moist atmosphere throughout.

	Initial estimation.		Dose of japaconitine per kilogramme.	Day of final estimation.		Final estimation.
	(Hb.)	Corps. in 100 squares.			(Hb.)	Corps. in 100 squares.
No. 1 ...	70	182	·0005	3rd	72	183
No. 2 ...	80	170	·0008	3rd	80	191

The result here as affecting the action of japaconitine in large doses on the frog's blood is practically negative.

In case of japaconitine administration to uninjured frogs kept in shallow water, or to brainless animals partially suspended in water (*vide* Pseudaconitine section) some œdema developed, but this condition and the resulting increase of weight were not so great as they were after pseudaconitine. The blood, however, became hydræmic and the corpuscles as well as hæmoglobin contained were relatively diminished. The diuretic action of japaconitine towards frogs is well marked.

On Respiration.

Excepting that japaconitine occasions rather more initial excitement and its subsequent depressive action is not quite so long continued, its effect is closely similar to that of aconitine.

On Cord Reflex.

The action of japaconitine in abolishing reflex is rather stronger than that of aconitine, but it is only a minor difference in degree at most and not in kind.

Action of Japaconitine upon Frog's Muscle and Nerve, in situ, *and exposed to the local action of Alkaloidal Solutions.*

In situ poisoning, brain previously destroyed, and circulation to one leg arrested by vascular ligature.

After medium sublethal doses there is increased excitablity to stimulation as contrasted with the control side, and the work capacity is but little if at all diminished for indirect, whilst it is unimpaired for direct stimulution.

Doses of lethal proportion and somewhat in excess are followed by a greater tendency to failure of the preparation to indirect stimulation, but this tendency is frequently only developed after repeated stimulation. The result of direct stimulation indicates either no effect or a slight reduction in ability to resist fatigue.

If after poisoning is completed the making of the preparation is postponed for some hours, the japaconitine effect is enhanced and the reaction to muscular stimulation may show considerable impairment.

Out of a series of nine experiments in which doses of ·001 to ·002 gramme of japaconitine per kilogramme had been administered to brainless frogs with vascular ligature applied to one leg, the heart being arrested in from 210 to 500 minutes according to dose, excitability of nerve was increased on the poisoned side in 6, equal in 2, and less in 1. (In the last 3 the poisoning was rapid, so that the reduction of excitability from blood stasis on the ligatured side had not time to develop itself.)

In six of these experiments there was some evidence of a reduced power of resistance to fatiguing influences occasioned by repeatedly stimulating the nerve with faradic currents at short intervals, and in two of them the muscle contracted less perfectly than did that of the unpoisoned preparation. It is easy to produce a more striking effect by an injection of such overwhelming proportion as ·008 to ·015 gramme per kilogramme body weight. (Such and larger doses of various aconitines were used by Plugge ('Virchow's Archiv,' 1882, vol. 87, p. 410) and other German observers, who claimed that, inasmuch as the result was to abolish the excitability of intramuscular motor nerves, therefore the aconitines are to be considered as essentially curare-like in their effect.) Our contention is that in order to produce anything approaching such an effect doses must be employed so greatly in excess of the lethal proportion as to be without the proper limits of pharmacological contrasts. Such doses would be large even for an adult human subject.

A few experiments will be recorded here made with large and very large doses of japaconitine and aconitine respectively.

The minimal excitability of the muscle-nerve preparation will be given where the nerve still reacted to stimulation.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

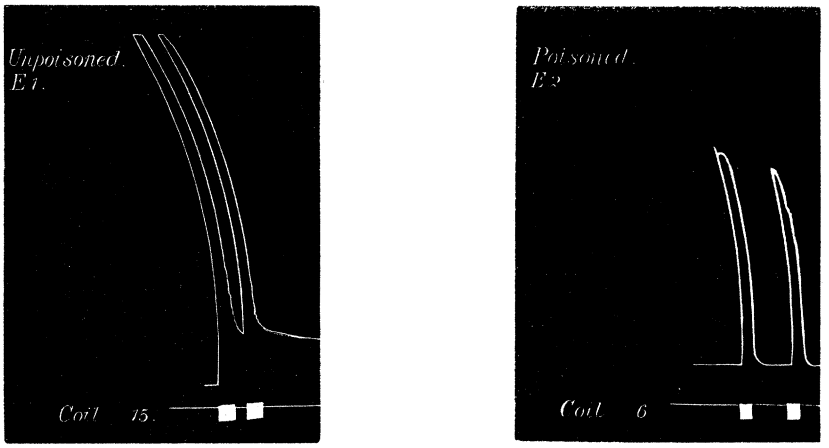
Frog.	Alkaloid and dose per kilogramme.	Arrest of heart.	Time of examination after poison.	Min. excitability in centims.				Work.			
				Ligatured.		Poisoned.		Ligatured.		Poisoned.	
				N.	M.	N.	M.	N.	M.	N.	M.
		hrs. mins.	hrs. mins.								
R. esc. . .	JA ·001	5 0	24 0	28	16	24	16	Moderate		Tetanus fair at first then breaking down.	
,, . .	,, ·005	1 30	3 0	26	16	12	13	Sustained		Breaks down on tetanising repeatedly	Impaired.
R. temp. . . (Fig. G)	,, ,,	2 5	3 0	30	15	22	11	,,		Tetanus firm twice and then breaks down. (Fig. E.)	,,
R. esc. . .	,, ·013	1 0	4 30	22	18	0	12	Fair	—	0	,,
,, . .	PsA ·0012	?	24 0	16	10	14	9·5	Fair		Fair	Fair.
,, . .	,, ·0015	?	6 0	13	9	18	9	,,		Tetanus fair at first but less sustained.	
R. temp. . .	,, ·005	3 0	3 30	32	4	32	14	Sustained		Sustained.	Sustained.
,, . . (Fig. E)	,, ·013	2 0	3 0	27	15	13	14	,,		Impaired	Good.
R. temp. . .	,, ·04	1 20	2 0	24	18	6	17	Fair		Fails rapidly on repeated tetanus . .	Impaired.
R. esc. . .	,, ·05	1 18	6 0	10	9·5	0	8	Poor		0	Much impaired.

From an examination of a large number of preparations obtained from poisoning *in situ*, japaconitine is to be placed in the foremost position with regard to its action upon intramuscular motor nerves; it is followed by aconitine, and, more distantly, by pseudaconitine.

Experiment.—Pegged *R. temp.* received japaconitine ·005 gramme per kilogramme after ligature of vessels of one leg (see notes on p. 78), fig. E, 1–2 nerve. Tetanising stimulations of 3 seconds; ligatured, 66 millims.; poisoned, 36 millims.

The reactions failed in the poisoned muscle stimulated indirectly after the two contractions registered, the control continuing to react freely. The muscle on the open side is impaired in reaction (direct stimulation).

Motor Nerve Stim. (*R. temp.*) after Japaconitine ·005 gramme per kilogramme.



1. Nerve-muscle preparation from ligatured leg. Indirect faradisation.
2. „ „ „ poisoned „ „

Exposure of Preparation to Japaconitine Solution.

Muscle-nerve preparations were made from companion limbs and placed in RINGER'S solution of 10 cub. centims. After this immersion had lasted 30 minutes a measured proportion of japaconitine solution was added to the contents of one of the glasses and the minimal excitability and character of contraction were then observed from time to time in the poisoned and control nerve-muscle preparations respectively. In these experiments solutions of 1–5000 to 1–5,000,000 were employed.

Immersion of One Muscle-nerve Preparation in Japaconitine Solution, the other serving as Control.

Synopsis of Results.

1–5000. Occasional fibrillations, especially after stimulation. Excitability to direct stimulation ceases only a few minutes before that of muscles directly stimulated. Reaction gone in 20–30 minutes.

1–50,000. In 30 to 60 minutes reaction of muscle to indirect stimulation feebler, and gone in 60 to 100 minutes. Muscle directly stimulated fails 10 to 12 hours later.

1–500,000. The reaction to indirect stimulation fails in about 12–14 hours. To muscle (direct) stimulation in 36–40 hours.

1–1,000,000. Nerve stimulation fails in 18 hours, muscle in 48–50.

1–2,000,000. Nerve stimulation fails in 21 hours (control in 30). Muscle unaffected.

1–3,000,000. In three experiments, two negative, nerve excitability slightly reduced in the third.

Fibrillation is a frequent phenomenon when immersion is in solutions stronger than 1–200,000, and is chiefly developed after indirect or direct stimulation.

Whilst the nerve and muscle are both affected by solutions having a strength of 1–1,000,000 and occasionally 1–1,500,000, the former is in the end affected by more dilute solution, such as 1–2,500,000 and occasionally 1–3,000,000.

Action on muscular tissue is distinct when this is exposed to solutions of a greater strength than 1–500,000.

Some increase of excitability to stimulation (especially indirect) follows early upon immersion of muscle-nerve preparations in medium-strength solutions of japaconitine, but it is a very transitory phenomenon if stronger solutions are employed.

Relative Activity of Japaconitine towards Muscle-nerve Preparations of R. esculenta *and* R. temporaria.

On the same day, and under precisely similar conditions, two muscle-nerve preparations were obtained from specimens of esculenta and temporaria of similar weights. The minimal excitability having been noted, after the preparations had rested for some time in RINGER'S solution, an equal quantity of aconitine was added to each trough, and the examination was continued. The figures indicate the position of the secondary coil at the time when contraction appeared :—

	R. esculenta.		*R. temporaria.*		
	M.	N.	M.	N.	
Immersion in RINGER'S solution. Add to each japact. to make 1–50,000	28	46	32	53	
hrs. mins.					
0 10	26	49	31	54	
0 15	27	47	29	54	
0 20	26	44	28	52	Active fibrillation in both.
0 25	26	41	24	49	
0 35	20	37	22	43	
0 50	19	18	19	22	
1 10	19	0	19·5	0	
2 10	13	0	19	—	
10 35	11	0	8	0	
14 0	10	0	0	0	

When exposed to solutions of 1–500,000 and 1–1,000,000 of the aconitines, the muscle of the esculenta in a majority of experiments, *indirectly stimulated*, ceases to contract at (practically) the same time as that of *R. temporaria*. The muscle of the former, *directly stimulated*, continues to react feebly for 1 to 3 hours after the latter has ceased. This may be due, in part but not entirely, to the denser character of the fascia investing the gastrocnemii of esculenta, as the poison clearly penetrates to the intramuscular nervous structures. Bearing in mind the length of duration of many of these experiments the results obtained have been surprisingly parallel.

Relative Activity of the Aconitines towards Nerve Muscle. Immersion Experiments in Solutions of the various Aconitines.

Companion nerve-muscle preparations were exposed to the action of two of the aconitines under similar conditions.

As the results of these experiments, performed with solutions of from 1–3,000,000 to 1–100,000, japaconitine has been found to be the most active in ultimately reducing excitability of nervous tissue; it is succeeded at an interval by aconitine; last in the series is pseudaconitine, which, whilst least energetic in this respect, has a somewhat greater activity than the other two in at first increasing excitability of the nervous tissue.

These differences are not so well marked when stronger solutions, 1–20,000 to 1–50,000, are employed, for the reason that such act powerfully upon muscular tissue, which is apparently equally affected by all three aconitines in equal strengths of solution.

Fibrillation has been observed most frequently under the action of japaconitine, nearly as often under aconitine, and least frequently under pseudaconitine.

Japaconitine v. *Aconitine.*

In eight experiments the solutions varied from—

1–600,000 to 1–2,500,000.

The muscle was unaffected by the weaker solution.

In four, japaconitine showed itself more active in reducing and finally in abolishing nervous excitability.

In the other four the results were practically equal.

Japaconitine v. *Pseudaconitine.*

In eight experiments the solutions varied from—

1–500,000 to 1–2,500,000.

In seven, japaconitine was at first more active in reducing and in all eight in abolishing nervous excitability.

Muscular excitability is also more rapidly impaired by 1–500,000 japaconitine than by pseudaconitine.

Stronger solutions, up to 1–50,000, also show a predominating effect of japaconitine. The increase of excitability rapidly ensuing on immersion in pseudaconitine solution is greater than when japaconitine is used.

Pseudaconitine v. *Aconitine.*

In eight experiments the solutions varied in strength as in the experiments epitomised in last section.

In four, aconitine was the stronger in its effect upon nervous excitability; in two it proved weaker, and in two practically equal, to pseudaconitine.

Comparative Action of the Aconitines on Tactile and Thermic Sensibility.

Unguents were prepared having a basis of lanoline and oleic acid, containing similar proportions of the three alkaloids. An æsthesiometer with bristle points, and a calorimeter through which water could be circulated (a thermometer being sunk in the metal apex), were used for the discrimination of tactile and of thermic perception respectively.

The temporal cutaneous areas were carefully tested in the first instance, and on the right or left side the application of the alkaloidal (2 per cent.) unguent was made, the area being carefully defined, whilst the other side was reserved for contrast observations, or two alkaloidal preparations were applied one to either temple simultaneously. Sensations were then tested from time to time. From observations

of a somewhat similar character made by EWERS, pseudaconitine was found to be more active in reducing discrimination of distance of two points and of variation of temperature than was aconitine, but the contrast experiments may have been made with an impure preparation of the latter alkaloid.

Our observations made with the three alkaloids may for the present purpose be summarised in a very few words :—

1. More evidence of local irritation was witnessed after application of pseudaconitine than after the other two substances (irritation being evidenced by local redness and slight swelling, the latter not invariably present).

2. All three alkaloids rapidly impair the delicacy of tactile perception at the seat of their application, *i.e.*, the distance at which the two points are recognised is frequently increased by from one-third upwards of the original distance. This effect is active for 10 or 12 hours, and some remnant of it is usually observable 24 hours after application.

3. Thermic appreciation is rapidly and distinctly impaired by the aconitines mainly in this manner, that temperatures are underestimated and discrimination for the middle ranges, especially 22–27° C., is considerably reduced, so that a variation of, say, 3° to 4° C. may pass unnoticed.

The change produced by the aconitines in this respect is more marked and enduring (it has been recognised in degree 36 hours after application) than that produced on the tactile organs.

4. Regarding the sensations recorded by the two observers who prosecuted this enquiry, it may be stated in general terms that the prickling succeeded by numbness with a sensation of fulness or tension is occasioned by all the aconitines. Further, the local sensation of enduring heat which they promote in the first instance gives place to occasional sensations of heat waves and the latter to a feeling of coolness or positive coldness in the part.

5. From a number of observations, which it would be superfluous to recapitulate here, it was deduced that pseudaconitine is slightly less depressant towards thermic and sensory perception than the other two alkaloids, which seem to be equally active in this respect. The difference is, however, very slight.

Therapeutical employment in several cases of facial neuralgia showed that the three aconitines were all active, and apparently equally so, in counteracting this painful condition.

SECTION III.—REPEATED ADMINISTRATION OF ACONITINE, JAPACONITINE, AND PSEUDACONITINE AFTER LONG AND SHORT INTERVALS OF TIME.

Several series of observations were made with the object of determining—

1. Whether any degree of tolerance is established as a result of repeated administration of the three alkaloids ;

2. What interval of time must elapse in order to present summation in action of doses which are individually sublethal;

3. What differences are detectable in the effects of the alkaloids examined in these particulars.

These experiments, many of which were carried out by Drs. Esslemont and Fraser, involved injection of the carefully measured dose of the alkaloid at determined intervals, notes being kept of the weight and general condition of the animal, and the reaction it showed towards the poison. In cases in which a loss of body weight was detected the dose was calculated upon the ascertained weight of the day. The experiments which are given here in abstract are too voluminous for publication in extended form, but it is intended to utilise them more largely in connection with an enquiry which has for its main consideration the problem of an acquirement of tolerance towards remedies.

Note.—As some of these experiments necessitated the sacrifice of many animals, they might not have been attempted on the present scale had it not been that the opportunity arose of utilising several animals which were required for purposes of dissection and had therefore to be destroyed.

Aconitine. Daily Administration.

In rabbits a limited degree of tolerance is established towards aconitine when it is administered daily. This is demonstrated in two ways:—

1. The symptoms produced by a given dose become somewhat less marked on daily repetition of that dose. (This remark applies chiefly to the effects produced on respiration and temperature. Aconitine was found on three occasions to be less effective in producing disorder of the rhythm of the heart when the alkaloid had been administered in large dose on the previous day. Salivation and paresis do not appear to be materially modified.)

2. By gradually increasing the daily dose by small increments (·0000025 to ·000005 gramme per kilogramme) the lethal dose (calculated on the body weight of the animal) may at last be exceeded. The limit so far reached of this excess is not more than 28 per cent. of the calculated lethal amount.

The slight tolerance established towards aconitine when it is daily administered does not last more than a few days (probably two or three) after it ceases to be given, and thereafter the symptoms appear to resume their original character, so that death may occur from a dose equal to, or even less than, that last administered in the series.

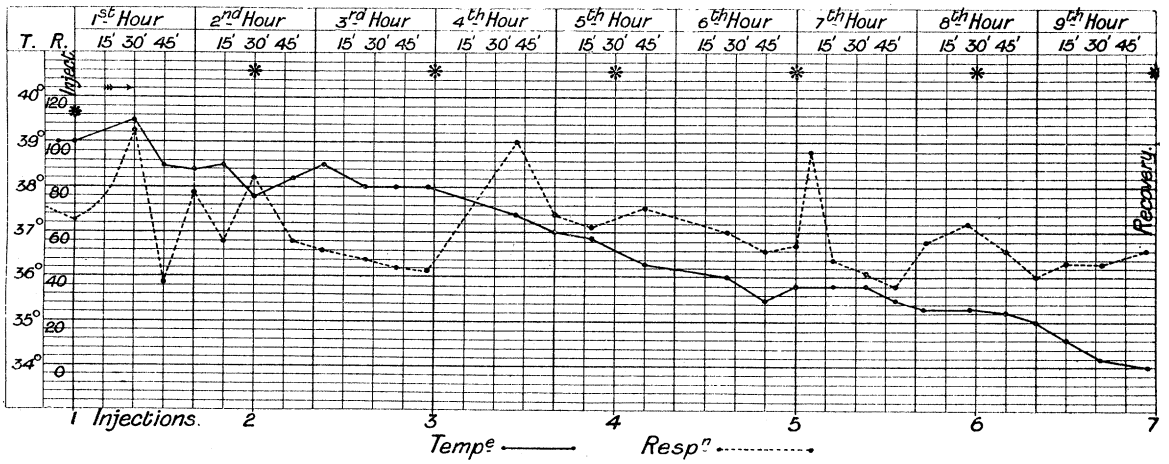
Experiment.—A medium-sized rabbit (1662 grammes) received daily progressive doses of aconitine as hydrobromide, commencing with ·00005 gramme per kilogramme body weight. This dose was increased by one-tenth daily until the thirteenth day (the rabbit weighing 1675 grammes); a dose of ·00014 gramme per kilogramme (an

amount distinctly in excess of the toxic proportion for an unprepared animal) was administered without a lethal issue. The use of aconitine was then intermitted for ten days, when a single dose of ·00011 gramme was just sufficient to prove fatal. It may be added that this is the most marked example of tolerance towards aconitine which has been observed, but it is not to be inferred that it represents the limit attainable under all conditions.

When such daily administrations interfered with nutrition, as evidenced by the marked decline in weight, there was even smaller evidence of tolerance.

Administrations every second day had likewise a feeble effect, whilst those given every third day had apparently not any.

Diagram D.—Internal Temperature and Respiration of Rabbit receiving ·000035 gramme Aconitine per kilogramme every 90 mins.



Aconitine. Re-administered at Short Intervals (Summary).

·000025 gramme per kilogramme administered every 15 minutes.

Urgent symptoms began after the fourth injection, and death occurred 12 minutes after the fifth.

(Five doses amounting to ·000125 per kilogramme given in 75 minutes.)

·000025 gramme per kilogramme administered every 90 minutes.

The temperature (internal) fell 2°·1 C., and the respiration to 40 per minute, but there was no urgent symptom.

(Six doses amounting to ·00015 per kilogramme given in 540 minutes.)

·00003 gramme per kilogramme administered every 90 minutes.

No urgent symptoms.

(Six doses amounting to ·00018 per kilogramme given in 540 minutes.)

·000035 gramme per kilogramme administered every 90 minutes. (Diagram D above.)

The temperature fell to 5° C., but the respiration only to 40.

Another injection would probably have proved lethal.

(Six doses amounting to ·000210 per kilogramme given in 540 minutes.)

·00004 gramme per kilogramme administered every 90 minutes.

Symptoms urgent after third injection; a fourth would certainly have proved lethal.

(Three doses amounting to ·00012 given in 270 minutes.)

·00005 gramme per kilogramme administered every 40 minutes.

Death occurred 18 minutes after the third dose.

(Three doses amounting to ·00015 given in 120 minutes.)

·00005 gramme per kilogramme administered every 120 minutes.

Symptoms at no time urgent.

(Three doses amounting to ·00015 gramme given in 360 minutes.)

Japaconitine. Daily Administration.

There is evidence of some degree of tolerance being established towards this alkaloid. The most prolonged experiment in the whole series was made with japaconitine, and is quoted below. It occupied between seven and eight weeks. Instead of a progressive dose being given from the first, small equal doses were repeated for several days before augmentation; thus the animal received ·00005 gramme per kilogramme daily for seven successive days, then ·000055 for fifteen successive days, ·00006 for six days; after ·000075 had been reached, a daily increase of ·000005 gramme was instituted. The forty-fourth injection was a dose of ·00014 gramme (largely hyperlethal), but death ensued the day after, when ·000145 gramme per kilogramme was given, the limit under this method of treatment having apparently been reached. The weight of the animal varied only through 90 grammes (1880 to 1790) during the administration of, in all, ·00326 gramme per kilogramme, exclusive of the lethal dose.

In the second experiment, which will be quoted, very small doses (·000025 gramme per kilogramme) were given daily for between seven and eight weeks, and thereafter there was a rapid increase by ·01 milligramme daily.

In this case there was much less evidence of tolerance than in the preceding, the lethal dose only slightly exceeding that which would have proved lethal on a first administration.

In the third experiment, from which the schedule is compiled, a grown rabbit received every second day a dose of japaconitine of ·0000389 per kilogramme, which was rapidly increased at first, and then more slowly (by increments of ·000025) until a dose of ·00015 per kilogramme was received at the twentieth injection. This large dose was all but lethal, and may be viewed as probably representing the limit of tolerance.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Dose of japaconitine per kilogramme of weight ascertained each day.	No. of injection.	Rectal temperature.					Respiration.							Salivation.				Paralysis.		
		Initial rise.	Fall.	Time of min.	Time of recovery to min.	Subsequent hypernormal.	Initial rise to.	Min.	Time of min.	Spasm and other symptoms.	Max.	Time of max.	Progress during recovery.	Chewing begins.	Salivation begins.	Amount.	Cessation.	Begins.	Degree.	Recovery.
·0000389	1st	—	0°·6	h. 1′·17	h. 2′·50	—	128	90	16′	—	150	h. 1			none		h.		none	h.
·0000732	4th	·3°	2°·1	1′·23	4′·7	+·8	140	94	30′	—	216	58′	gradually fell to 60	17′	26′	slight —	1′·12	34′	standing insecure at 47′	1′·23
·000089	5th	·2°	3°·5	1′·27	4′·50	—	176	66	24′	head thrown back, dyspnœa —	200	1·6	gradually fell to 56	18′	24′	profuse —	1′·37	35′	distinct paresis —	2′·28
·0000947	7th	·3°	4°·5	2′·31	6′·20	+·1	176	36	45′	dyspnœal springing —	220	2·0	fell to 50	9′	13′	profuse —	2′·18	23′	great —	2′·31
·00012	12th	—	5°·9	2′·14	7	—	154	18	21′	spasm, with loss of consciousness —	184	3·7	fell to 64	14′	20′	very profuse —	1′·44	21′	extreme —	2′·45

The details in the schedule show the progressive effect of the alkaloid in increasing doses. Injection took place every second day. The results of five of these observations are given. The fact that the dose of ·00012 per kilogramme is not lethal indicates that a degree of tolerance has been acquired.

Japaconitine administered at Short Intervals (Summary).

·00002 gramme per kilogramme administered every 90 minutes.
Slight fall of temperature and respiration.
(Seven doses amounting to ·00014 given in 420 minutes.)

·000025 gramme per kilogramme administered every 90 minutes.
Distinct effect.
(Six doses amounting to ·000150 given in 540 minutes.)

·00003 gramme per kilogramme administered every 90 minutes.
Urgent symptoms after fourth dose.
(Four doses amounting to ·00012 given in 360 minutes.)

·00003 gramme per kilogramme administered every 90 minutes.
Death occurred after the fifth.
(Five doses amounting to ·00015 given in 450 minutes.)

·00004 gramme per kilogramme administered every 120 minutes.
Symptoms marked, not urgent.
(Four doses amounting to ·00016 given in 480 minutes.)

·00005 gramme per kilogramme administered every 120 minutes.
Third dose almost fatal.
(Three doses amounting to ·00015 given in 360 minutes.)

·00005 gramme per kilogramme administered every 120 minutes.
Lethal after the fourth dose.
(Four doses amounting to ·0002 given in 480 minutes.)

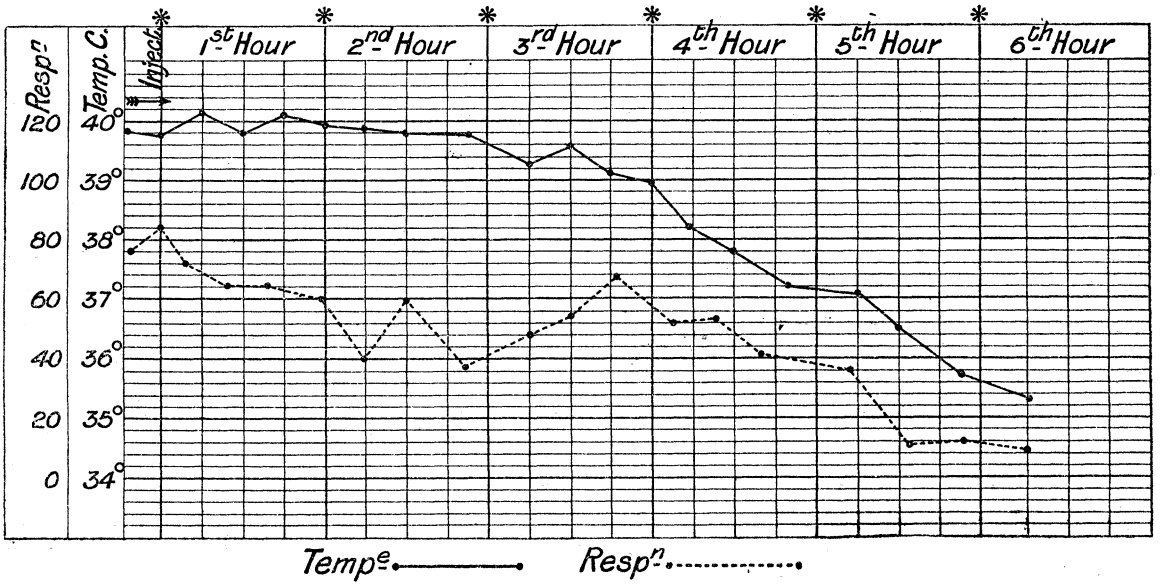
Pseudaconitine.

There is evidence of but little tolerance resulting from re-administration of this highly toxic body. The result of daily doses of ·00004 gramme (*i.e.*, more than ·8 of the lethal) on each of six successive days, was to cause a slight but progressive diminution in the fall of temperature and the slowing of respiration during this time. Nevertheless, tolerance did not seem to expand the non-lethal area with any certainty.

Experiment.—An animal received three doses on successive days of ·00003 gramme of pseudaconitine per kilogramme, on the fourth day of ·000045 (a dose which frequently proves lethal), on each of the two next days ·00005 (a lethal dose it

initial), but on repeating for the second time it proved lethal. The most that can be said of this result is, that though a very slight degree of tolerance appeared to be created, this disappeared on continuing the same dose.

Diagram E.—Internal Temperature and Respiration of Rabbit receiving ·00001 gramme Pseudaconitine per kilogramme every 60 mins. at *.



Pseudaconitine re-administered at Short Intervals (*Summary*).

The doses were reduced to one-half or less of those given of the other two alkaloids.

·00001 gramme per kilogramme every 60 minutes. (Diagram E.)
After third dose temperature began to fall; after fifth dose it was down 4°·3 C., and the respiration to 16 per minute. Death occurred after the last injection (six doses amounting to ·00006 in 360 minutes).

·0000114 gramme per kilogramme every 90 minutes.
Severe symptoms, but just recovered.
(Five doses amounting to ·0000570 in 450 minutes.)
The next dose would inevitably have proved lethal.

·00001543 gramme per kilogramme every 90 minutes.
Severe symptoms, but recovery.
(Four doses amounting to ·00006172 in 360 minutes.)

·00001543 gramme per kilogramme every 90 minutes.
Death occurred after the fifth injection.
(Five doses amounting to ·00007715 in 450 minutes.)

·00002 gramme per kilogramme every 120 minutes.
Caused fall of temperature by 3° C., and respiration to 25.
(Three doses amounting to ·00006 in 360 minutes.)
·00003 gramme per kilogramme every 120 minutes.
Temperature fell by 7°·5 C., and respiration to 20.
Death occurred after the second injection.
(Two doses amounting to ·00006 in 240 minutes.)

Contrasted Results of the Aconitines given in Repeated Doses Daily, or every Second Day.

1. That some tolerance is shown towards aconitine and japaconitine when these were administered daily or every second day, but the dose must constitute a considerable fraction of the lethal before such an effect is witnessed. Pseudaconitine may have a feeble effect in the same direction, but it comes distinctly after the other two.

2. That such tolerance towards aconitine and japaconitine is limited to a dosage excessive of the initial lethal dose by one-fifth to (the outside) one-third of the lethal. This higher degree of tolerance has only once been attained.

3. The tolerance soon (within 3 days) disappears when the regular administration of the alkaloid is interrupted.

Repeated Doses at Short Intervals.

1. That the result of repeated administration of these alkaloids at short intervals shows that the total lethal dose for all of them may be exceeded if a sufficient time interval is permitted between the administrations.

2. For aconitine the difference in toxicity between divided doses, together amounting to about the lethal in 60 minutes, and a single toxic dose, is not great (·000025 per kilogramme every 15 minutes five doses). Three doses, each about half the lethal, given every 40 minutes, were also speedily lethal.

On the other hand, frequent hourly repetition of an amount equal to nearly half the lethal is necessary to cause death (six doses of ·00005). If the interval is 2 hours such doses produce a relatively small effect.*

3. Re-administration of parallel doses of japaconitine cannot be carried so far as in the case of aconitine. Thus, whilst six doses of ·00003 of the latter were given at intervals of 90 minutes without serious effect, five equal doses of japaconitine so given proved lethal.

* Quarter lethal doses (·000025 gramme) may be administered every 90 minutes with but slight effect, and so with ·00003, but ·000035 gramme produces active poisoning, whilst a dose of ·00004 with the same interval is soon lethal on repetition.

The bi-hourly administration of japaconitine in about half lethal doses produces much more urgent symptoms after the third dose than when aconitine is given.

These facts may imply (1) greater toxicity of japaconitine, or (2) slower disappearance of the toxic effect due to individual doses, whether this is due to delayed elimination or otherwise is not decided; but as it has been already shown that in a single initial dose japaconitine is slightly more active, its stronger action on repetition is probably mainly due to the first cause.

4. There is distinctly less tolerance towards repetition of pseudaconitine given in the same relationship to the probable lethal, as in the case of the other two aconitines. This holds equally for quarter lethal doses given at 90 minutes as for third lethal at 90 minutes, and half lethal at 2 hours. In fact, the summation of effect after the longer interval is peculiarly striking.

Lethal Dose of the Aconitines per kilogramme weight of Animal.

(Time of Year for Frogs noted.)

	Rabbits.	Guinea-pigs.	Pigeons.	Frogs.*			
				R. temp.		*R. esc.*	
				Summer.	Winter.	Summer.	Winter.
Aconitine . . .	·00011† begins at ·000085	·00011†	·000115	·00115	·00075	·000105	
Japaconitine . .	·000105 begins at ·000065	·0001	·00009	·001 begins at ·0006	·0007	·0009 begins at ·00055	
Pseudaconitine .	·0000465 begins at ·000038	·000045	·000045	·0012 begins at ·0008	·0008	·00011	

* These figures are derived from three series of observations made with the aconitines simultaneously during the last three seasons. The lethal proportion varies with the season, with the condition of the animals employed, and with the prevailing temperature. The large proportion of ·0014 gramme of aconitine per kilogramme ('Phil. Trans.,' B, vol. 190, p. 392) has been found hyperlethal during these later observations, and indicates an exceptional tolerance towards this alkaloid.

† See note, 'Phil. Trans.,' B, vol. 190, p. 268.

Toxicity.

From the annexed table it is clear that towards mammals and birds pseudaconitine is distinctly the most toxic alkaloid of the group examined, whilst japaconitine is slightly more active than aconitine. Towards frogs (both *R. esc.* and *R. temp.*), however, pseudaconitine is rather less toxic, dose for dose, than japaconitine and aconitine. A more powerful involvement of the respiratory centres by pseudaconitine is the main cause of its much greater toxicity towards mammals.

General Toxic Symptoms.

Towards mammals pseudaconitine as a toxic agent causes variations in respiration and in the more sudden development of the dyspnœal state already described, which serves to bring it into contrast with the other aconitines. The paresis it induces is longer continued, the return to the normal is retarded.

Whilst there is no absolutely distinctive effect produced in frogs (*R. temp.*), a predominating action may be indicated as in the following table, in which the most active alkaloid is placed at the head of the list. Brackets indicate equality of action.

Respiration.	Circulation.	Paralysis.	Œdema.
Pseudaconitine.	Japaconitine.	Pseudaconitine.	Pseudaconitine.
Aconitine. }	Aconitine.	Aconitine.	Aconitine. }
Japaconitine. }	Pseudaconitine.	Japaconitine.	Japaconitine. }

As between *R. temporaria* and *esculenta*, it may be stated in general terms that all aconitines cause more excitement and frothing in the former than in the latter; but that, on the other hand, the degree of paralysis produced is greater in the latter. The lethal dose for all three is slightly larger (per gramme body weight) for *R. temporaria* than it is for *R. esculenta*.

In order to bring the action of aconitine, pseudaconitine, and japaconitine into contrast, which may be readily apprehended at a glance, the following summary will be useful.

Heart.—All three alkaloids have a similar effect upon the heart of such mammals as have been observed. Pseudaconitine is quantitatively more energetic than the other two towards cats, but is certainly not nearly twice as toxic when artificial respiration is practised. Towards the frog's heart pseudaconitine is slightly less powerful than the other two, of which japaconitine is rather the more active.

Vagus Nerve and Inhibitory Mechanism in Heart.—Heart slowing from increased central vagus activity is produced at first by all these alkaloids, and similar results follow section and stimulation of the nerve at this and later stages of poisoning, by one and all of them, both in mammals and frogs.

Respiration.—There is less tendency to acceleration of respiration in mammals poisoned by pseudaconitine than when the other two alkaloids are employed; further, the dyspnœal conditions develop more suddenly, and the central depression of respiration is greater. Japaconitine is at first slightly more depressant than aconitine, but thereafter the tendency to acceleration of respiration is sooner developed, otherwise the general features of their action are similar.

Blood.—All the aconitines produce a deleterious effect upon the hæmoglobin and coloured corpuscles of the blood of mammals when they are given in large doses. As far as has been ascertained, this is due to impairment in the nutrition of the animal rather than to a directly destructive action.

Frogs kept in a watery medium, or in contact with a moist surface, develop œdema after receiving any of the aconitines, but this condition is most marked, and the hydræmia of the blood is more pronounced and lasting after pseudaconitine.

Brain and Spinal Cord.—All aconitines appear to have a similar effect qualitatively on the brain and cord of rabbits, pigeons, and frogs. Quantitatively, pseudaconitine appears the most active in reducing reflex function of the cord.

Temperature.—The initial elevation of temperature often seen in rabbits which have received aconitine or japaconitine, is less frequently observed after pseudaconitine. A slightly greater and more enduring fall of internal temperature is witnessed after the latter, when the dose is large and bears a like relationship to the lethal amount.

Repeated Administration.—Some tolerance is established on the part of rabbits toward all the aconitines, and this is manifested with reference to temperature reduction, to the cardiac effect, and, to a lesser extent, to respiration; the general toxicity undergoing a reduction which is not, however, very extensive. Less tolerance is shown towards pseudaconitine than towards the other two; it has been found impossible hitherto to determine how far rapidity of elimination varies between the alkaloids.

Sensory Nerves.—Local application of the aconitine ointments of equal strengths are followed by a somewhat more powerfully depressant and enduring effect when these contain aconitine or japaconitine than pseudaconitine. This statement has reference to cutaneous sensory, and thermic impressions in the human subject. The difference is at most but slight.

Motor Nerve and Muscle.—The action of the individual alkaloids is much the same whether specimens of *R. esc.* or *R. temp.* are used. It is more difficult to reduce reaction or to produce insensitiveness of the intramuscular motor nerves by pseudaconitine than by the other alkaloids. The so-called curare-like action has been found for all the alkaloids to be much feebler than was at one time supposed.

Direct contact of the alkaloidal solutions with muscle-nerve preparations reduce excitability, the muscle being affected by solutions containing less than 1 in 1,000,000,

and the nerve by solutions still weaker. Pseudaconitine is recognised as producing a slightly weaker effect than the other two alkaloids, which are nearly equal to one another, japaconitine being slightly the more energetic.

The results of the experiments detailed in this paper do not in all respects agree with previous observations; especially is this the case with regard to the relative toxicities of the three aconitines. The general order of toxicity towards mammals is pseudaconitine, japaconitine, and aconitine, which is the least toxic. Pseudaconitine has been found (roughly speaking) twice as toxic as aconitine towards the small mammals and birds used in the research. This agrees closely with the results of ADELHEIM,* BÖHM, and EWERS, 'Arch. f. Exp. Path. und Pharm.,' 1873, vol. 1, p. 385. CLOETTA† states that pseudaconitine is the stronger body, but gives no proportion, whilst it differs from the conclusions of NOTHNAGEL and ROSSBACH‡, who state that pseudaconitine is seventeen times as active as aconitine, and of HARNACK and MEUNICKE,§ who find the under margin of active dosage equal. KOBERT finds pseudaconitine and aconitine to be pretty equal in activity.

The relative toxicity of japaconitine to aconitine is approximately as about 10 to 9 towards the small mammals and birds which were used. Previously japaconitine has been seldom contrasted with the other two aconitines, but has been recognised as stronger than aconitine by LANGAARD,‖ and in one series of observations by HARNACK and MEUNICKE. KOBERT,¶ on the other hand, does not separate japaconitine from aconitine and pseudaconitine in toxicity.

Dosage.—Based upon the observations made, the relative doses for therapeutical purposes would be approximately, regarding that for aconitine as the unit, for pseudaconitine ·4 to ·45 and for japaconitine ·85 to ·9.

Towards frogs, the toxicity of these alkaloids is by no means so great (per gramme body weight) as it is towards the same unit of the mammals and birds included in this research. Thus the lethal dose per kilogramme, mammalian weight, may only be lethal to from 140 to 170 grammes of frog weight, or even less, according to the time of year. A medium-sized rabbit may therefore be poisoned by a dose of aconitine or japaconitine which would suffice to destroy four or five frogs.

Japaconitine is slightly more toxic towards both mammals and frogs than is aconitine, but the higher toxicity of pseudaconitine towards birds and mammals is not associated with an equal activity towards frogs, for it exerts towards both *R. esc.* and *R. temp.* a slightly lower toxicity than do either of the other alkaloids.

There is no essential difference in the reaction of *R. esc.* and *R. temp.* respectively

* ADELHEIM, 'Forens. Chem. Unters. Dorpat,' 1869.
† CLOETTA, 'Lehrb. d. Arzneim. u. Arzneiverordnungsl.' Freib., 1885.
‡ NOTHNAGEL and ROSSBACH, 'Mat. Med. et Therap.' (Fr.), 1880, p. 685.
§ HARNACK and MEUNICKE, 'Berl. Kl. Wchsch.,' 1883, No. 43, p. 657.
‖ LANGAARD, 'Arch. f. Path. Anat.,' 1880, vol. 79, p. 229.
¶ KOBERT, 'Lehrb. d. Intox.,' p. 657.

to individual aconitines beyond a greater or less accentuation of one or other symptom, as for example more excited movement in the latter, more reduction of reflex in the former, but in all parallel series of observations the resistance of *R. temp.* has proved to be slightly greater to all the aconitines examined.

As concerns the local action of the aconitines upon sensory (cutaneous) structures in man, the differences are so trifling as to be negligible.

As regards the therapeutical employment of aconitine, japaconitine, and pseudaconitine the great similarity in their physiological actions, amounting almost to a qualitative identity, which is established by this investigation, justifies the employment of any one for internal administration provided that the dosage is properly regulated. If aconitine is taken as the standard and the usually accepted dosage taken as the unit, the dose of japaconitine is ·85 to ·9 and that of pseudaconitine ·4 to ·45. Given in such proportions the three alkaloids would exert the same action. We strongly recommend the use of a pure alkaloidal salt in preference to preparations made from the plants, since the latter would be difficult to standardise, and even if this were done, the action of the aconitines would be modified to a greater or less extent by the other alkaloids present in the vegetable preparations.

For local applications the three alkaloids may be introduced into ointments in identical proportions. The greater power of pseudaconitine need not prevent its use in this department of treatment, if it is remembered that all applications of the aconitines externally are to be considered dangerous if any abrasion or lesion of the skin is present.

The chemical part of this enquiry has been conducted in the laboratories of the Scientific and Technical Department of the Imperial Institute, with the assistance and co-operation of the Government of India. Our thanks are specially due to Dr. George Watt, C.I.E., Reporter on Economic Products to the Government of India, for the interest he has shown in the investigation, and for the care he has taken in the collection of the necessary material.

The physiological experiments have been conducted in the Department of Materia Medica and Pharmacology of the University of Aberdeen, and have been assisted by a grant made by the Royal Society from the Government Fund. The assistance of Drs. Esslemont and T. Fraser has been valuable in carrying out certain experiments in this portion of the research.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES