An experimental inquiry into the relationship of action to dose : especially with reference to repeated administration of indaconitine / by J. Theodore Cash.

An Experimental Inquiry

INTO

THE RELATIONSHIP OF ACTION TO DOSE ESPECIALLY WITH REFERENCE TO REPEATED ADMINISTRATION OF INDACONITINE.

BY

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THE RELATIONSHIP OF ACTION TO DOSE ESPECIALLY WITH REFERENCE TO REPEATED ADMINISTRATION OF INDACONITINE.

ALTHOUGH the consideration of amount of dose, as well as of the principles of readministration in the treatment of cases calling for more or less prolonged medication, are of prime importance therapeutically, it must be admitted that they have scarcely received the amount of attention to which they are obviously entitled. Stokvis asserts that there is perhaps no part of our science which does not defy our aspirations to precision more than the posology, whilst Harnack laments the inattention of medical practitioners towards such an important question, remarking that there exists a surprising carelessness and caprice in the matter. Sir Benjamin Brodie recognized the error of the assertion, which is even to-day occasionally propounded, that by doubling a dose a double effect is to be anticipated; and exact experimental observations have shown that such a theory if applied to many active remedies must certainly lead to disaster.

Harnack regards Juckuff's attempt to experimentally establish some mathematical correspondence between dosage and effect as one of the few serious efforts in the direction of a more exact therapeusis. Juckuff's work was based upon a historical case of poisoning in which a fatal issue was barely averted. The case was that of Koppe, who, experimentally, took 0.5 mg. of digitoxin. This dose proving ineffective, the experimenter, after an interval of twenty-four hours, swallowed double the quantity. The ensuing results were so trifling that Koppe at that time regarded them as chance occurrences, and so, after the lapse of four days, undeterred by any anxious anticipations, the dose of 2.0 mg. was taken, with the result that violent, almost fatal, poisoning was produced. This clearly indicated that the effect of 2.0 mg. of digitoxin was not merely of twice, but of many times the activity of 1.0 mg. This fact, established with regard to the action of a single glucosidal body, was expanded by Juckuff, who tested the

activity of chloroform (of which Paul Bert had already said that double the active dose constituted a surely lethal proportion), ether, amylenhydrate, and other bodies with reference to their action upon the respiration of certain animals and to their potency as haemolytics. His results led to the promulgation of certain laws which are of considerable practical utility, into the discussion of which it is not my present purpose to enter. Let it suffice that the nearest fulfilment of a mathematical relationship seemed to be achieved by working upwards from that amount of any drug which produced the minimum of appreciable action. For such a proportion Harnack has used the term "Grenzdose," or limit dose, and this minimal effective dose stands above that amount which is unproductive of action. Of this Buchheim speaks as the "Compensazions grösse," on the supposition that to that proportion the tissues offer a compensatory resistance, any appreciable action being thereby obviated. Perhaps the term "maximal ineffective" dose may be accepted as expressing the fact more clearly, without the introduction of theoretical consideration. To apply these terms to the case already detailed. In Koppe's experience of digitoxin 1 mg. was the minimal effective or "limit" dose, and presumably 0.9 mg. might be regarded as the maximal ineffective (though of this there was no experimental confirmation); his bare escape with life after a dose of 2 mg. led to the supposition that 2.1 mg. (or × 2.1 of the minimal effective) would have proved a lethal proportion. There was recognizable, therefore, a very narrow variation in dosage between that amount which caused a slight but by no means toxic effect (for its importance was ignored by Koppe in the first instance) and the lethal, for if this is correctly represented by 2.1 mg., the lethal is but × 2.1 of the "limit." Juckuff inferred that the addition of 0.1 mg. to the (active) 1 mg. practically doubled the effect of the larger dose; that by + 0.2 the result would be trebled; by + 0.3 quadrupled, and so on, though it is open to criticism whether the equal increments would not become progressively more active as the total dose underwent increase.

It is improbable that any universal law of uniform applicability associating dosage with its certain effect could be formulated which would prove adaptable to all classes of remedies, or even to such remedies as presumably belong to the same class. The individuality of the remedy must be closely studied before an appropriate position can be given it, and even when we have learnt its value towards the healthy organism, the reaction of disordered organisms which vary within such wide limits in the degree of departure which they manifest from the normal, even though they pass under one and the same name, must necessarily be of a less easily predicted character.

We may safely follow Harnack in his recognition of two main classes into which potent remedies may be divided:

(*a*) Those in which the lethal dose lies far above the active; and

(*b*) Those in which the lethal dose lies near the active.

Whilst atropine would be placed in the first group, its active dose being, relatively to the lethal, but very small, digitoxin would obviously be included in the second. Strychnine would also be grouped under (*b*), as whilst 1.5 mg. causes but a partial effect upon dogs (Harnack), twice this amount proves lethal. It is obvious that the contrast of activity of this poison with digitoxin is not quite satisfactory, as in the experimental testing of the latter the human subject could record symptoms indicative of a feeble toxicity; whereas in the strychnine observations only objective symptoms could be recognized. Whilst these two bodies are evidently members of the same class, it is not yet proved that they would submit themselves to an identical law or mathematically-expressed formula connecting activity with dosage. Though indaconitine possesses a medicinal action in doses remote from the lethal, it will be shown that this is by no means the case when objective phenomena are regarded as the standard of activity.

Experimental.

The following experiments, which are of a very simple character, were undertaken in the hope that they might yield definite information regarding the effect of a dosage bearing varying but known proportion to some uniform standard. They have necessarily been of an extensive character numerically, whilst in many long-continued observation has been essential. My assistant, Dr. Croll, has kindly taken my place occasionally in order that the record of such prolonged experiments might be completed.

As it seemed advisable in the first instance to work downwards from the lethal proportion, which may be regarded as the most definite standard available, one of the aconitine series of salts—namely, indaconitine hydrobromide, of which the lethal proportion for rabbits had been previously established—was selected. This salt, which had been prepared in the laboratories of the Imperial Institute, was kindly supplied to me by Professor Dunstan. It is, as already shown, active in reducing body temperature.

Method.

For the following observations either fully or two-thirds grown rabbits were employed. The animals were abundantly fed up to the evening of the day preceding the morning on which the observation was to take place. The body weight was ascertained immediately before the experiment, and from it the dose was calculated.

A sensitive thermometer, certified at Kew, with a long stem permitting of very close reading, was used. All temperatures were taken deeply in the rectum. The observation room was kept at a uniform medium tempera-

ture, the animals being contained in a large run enclosed by netting which was abundantly littered with straw.

As it had been found that such weak solutions of the alkaloidal salt as were suitable for accurate measurement (0.000005 per 1 c.cm.) soon underwent partial decomposition, only perfectly fresh solutions either in 0.75 per cent. NaCl or Ringer's solution were employed. Medication was invariably by hypodermic injection in the region of the flank. The solution of the alkaloidal salt was drawn into the barrel of the syringe (which had already been charged with a few minims of salt solution) from a narrow and accurately calibrated pipette, graduated to one-hundredths of 1 c.cm After the injection had been made the syringe was detached from the needle, partially recharged with salt solution, and discharged through the needle, so that the administration of the entire dose was assured The needle and solution were sterilized; in no case was any subsequent irritation or inflammation produced in the animals employed.

All doses are quoted in relation to the kilogram weight of the animal (a large rabbit weighs fully 2 kg.). The toxic action produced by doses bearing a stated relationship to the lethal unit will be briefly described in the first place, and afterwards the action of subtoxic doses upon the temperature. Though individual results are frequently given, most of the figures represent averages derived from several similar experiments in which different animals were employed.

Abnormal susceptibility and the reverse are met with in animals as well as in man, and the examination of many rabbits with regard to their receptivity towards indaconitine has yielded four or five well-marked examples of it. The deviation from the normal (average) effect more usually takes the form of insusceptibility than of the reverse condition.

The Lethal Dose of Indaconitine Hydrobromide and the Toxic Action of Smaller Proportions.

Lethal Dose.—The lethal dose, as previously established, is 0.12 mg. per kilo body weight. Such a proportion occasions brief acceleration of respiration followed by slowing with dyspnoea, pupillary dilatation, free salivation, paresis with characteristic movements of the head and lower jaw. Diversified circulatory changes, leading up to inco-ordinate action of the various parts of the heart, coexist, but are not appreciable, unless in blood-pressure experiments. Final dyspnoeal spasms followed by unconsciousness (these symptoms usually being intermittent in character) precede death. There is naturally a fall of rectal temperature, but, as death supervenes in fifty to sixty minutes, the reduction is only a fraction of that produced by a large but non-lethal dose.

Eleven-twelfths of the Lethal.—A dose of 0.00011 g. per kilo is occasionally, though exceptionally, lethal. When the unusual results follow, there is associated with the symptoms above described (in character if not entirely in degree) a fall of rectal temperature, averaging from 2.6° to 2.7° C.; this reduction usually passes its maximum two hours after administration, but the normal temperature is not regained

until two or three hours after the lowest point had been recorded.

Two-thirds of the Lethal.—Such a dose (0.00008 g. per kilo) causes all the symptoms enumerated above to some extent, but dyspnoea is distinctly less evident, though the respiratory movements are usually reduced to two-fifths of the original number. Salivation lasts for about twenty-five minutes. The fall of temperature is from 1.5° to 1.7°—average 1.6° C.

Half of the Lethal.—All the symptoms previously mentioned are present, but dyspnoea less marked, and brief paresis not so conspicuous. The reduction of rectal temperature averages 1.2° C., the maximum fall being from sixty to seventy minutes after administration. Usually a commencing rise is noted in seventy-five to ninety minutes, normality being reached in 170 to 220 minutes. (Renewal of muscular activity favours an earlier return towards the normal.)

One-third of the Lethal.—This proportion causes little evidence of dyspnoea or paresis. The animal is capable of hopping, though for a short time its movements are somewhat uncertain, and it rests on the abdomen more than is customary. The pupil is not widely dilated; salivation, though distinct, lasts for not more than eighteen minutes. The average fall of rectal temperature is 0.95°C., its maximum being attained in about sixty minutes; normality is regained in 130 to 160 minutes.

One-quarter of the Lethal.—A rabbit having received this dose appears for a time somewhat apathetic—indifferent to the proximity of other animals or to the approach of food. Excepting that there may be an occasional chewing movement at the height of action, no motor symptoms are present. There is no external salivation (exceptions to this statement are quite rare); if the secretion is increased it is swallowed. The average fall of rectal temperature is 0.8° C., the duration of effect being slightly shorter than that recorded after a one-third dose.

We may, then, conclude that the objective toxic action of indaconitine is comprised within limits, the lowest of which is 0.25 of the lethal, or in exact figures 0.00003 g. per kilo body weight (rabbit). In the case of digitoxin poisoning, already cited so often, subjective symptoms were recorded after a dose bearing the proportion of 1 to 2.1, the latter figures in milligram representing the lethal. In these observations dealing with indaconitine only objective symptoms are available, but assuming that the subjective symptoms would disappear at one-fifth of the lethal (the objective symptoms are ill-defined and not certainly characteristic of the poison at one fourth), then the proportion of the minimal effective towards the lethal would be as 1 to 5. Although such a variation is evidently greater than that stated for digitoxin (Juckuff) or strychnine (Harnack), indaconitine would be classified with these rather than with the atropine group, as being productive of an obvious action only when given in doses which are not far removed from the lethal.

When the dose of indaconitine is reduced below the proportion at which simple observation fails to detect any action, it nevertheless remains effective in modifying the body temperature. In order to shorten description a diagram is given below (Fig. 1), which

shows the average reduction of internal (rectal) temperature caused by Indaconitine given in fractional doses of the lethal. The greatest effect is that produced by $\frac{1}{1}\frac{1}{2}$ths of the lethal (the nearest attainable to the unit), the second is by two-thirds, the subsequent proportions being in series of geometrical progression from the unit and two-thirds respectively, so that we have $\frac{1}{1}\frac{1}{2}$ths (for one), $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{2}{3}$, $\frac{1}{3}$, $\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{24}$ as the fractions examined.

Average Reduction of Temperature by Single Doses of Indaconitine Hydrobromide.

Proportion of Dose to Lethal.	Fall of Rectal Temperature.
$\frac{1}{1}\frac{1}{2}$	2.2° C.
$\frac{2}{3}$	1.6°
$\frac{1}{2}$	1.2°
$\frac{1}{3}$	0 95°
$\frac{1}{4}$	0.8°
$\frac{1}{6}$	0.7°
$\frac{1}{8}$	0.6°
$\frac{1}{12}$	0.5°
$\frac{1}{16}$	0.4°
$\frac{1}{24}$	0 3°
$\frac{1}{32}$	—

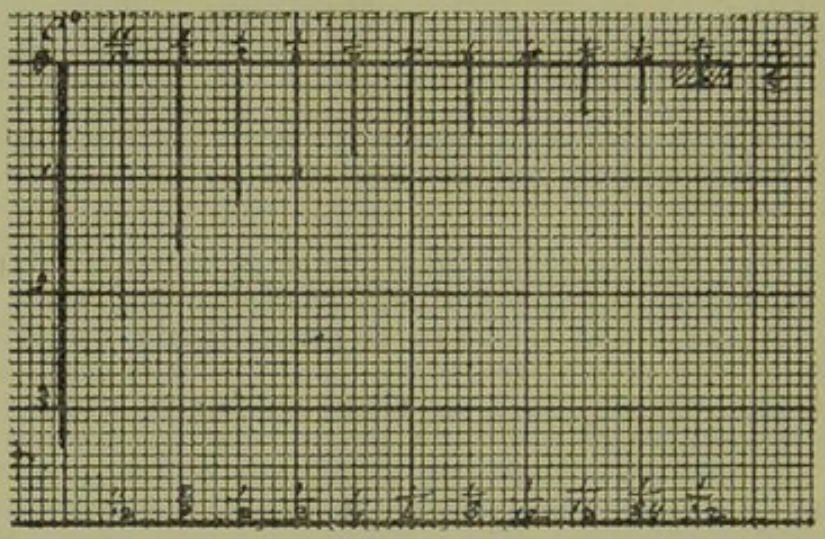

Fig. 1.—Reduction of temperature by single doses given in fractions of the lethal unit. The shaded block indicates area of physiological variation.

It is, therefore, obvious that some effect upon temperature is producible by doses of the Indaconitine salt which bear such a relatively small proportion as the $\frac{1}{16}$th and even the $\frac{1}{24}$th to the lethal, but the latter may be regarded as being upon the border line of activity and inactivity; in other words, it is the "limit" or "minimal effective" dose so far as temperature is concerned.

It is undeniable that slight fluctuation of temperature may sometimes be recorded where still smaller single doses are administered, but these I have not felt justified in regarding, inasmuch as the temperature of a normal animal to which no administration has been made has been frequently found to vary from 0.15° to 0.2° C. during

the time occupied in making an average observation. Such variation may be without detectable cause. On the other hand, we anticipate a change of temperature under certain conditions. Unfed rabbits when offered food show at first a transitory fall in rectal temperature of 0.1° to 0.2° C, but this is rapidly succeeded by a rise of 0.3° to 0.35° C., so that the original level is exceeded. There may, therefore, be an oscillation about the normal of 0.35° C., occasionally more, attributable to local changes in vascular areas adjacent to the bulb of the thermometer. Pembrey and Nicol have indicated the occurrence of increase in rectal temperature of man by 0.17° to 0.39° C. when a meal is taken in the earlier part of the day. Movement is also a factor bearing upon the general body temperature; Woodhead mentions that in horses subjected to exercise by trotting for thirty minutes an average rise of internal temperature by 0.16° to 0.2° C. was produced.

Such variations as these may be anticipated and their occurrence obviated, but when everything is done to avoid adventitious causes, a small variation of temperature, amounting to about 0.2° C., may yet be recorded. As this appears to be a usual physiological variation, it would not be justifiable to attach importance to reduction of temperature not exceeding this figure. Now when a dose of indaconitine bearing a lower relationship than the $\frac{1}{24}$th to the lethal unit is administered, the oscillation of temperature which may ensue will fall within this area of physiological variation, and therefore it may be concluded that at or about the $\frac{1}{25}$th lethal the "maximal ineffective" dose for temperature must be supposed to lie. This statement applies to the healthy normal animal, in which we may regard the temperature around the normal as being in a state of instability in so far as it varies, though of course within narrow limits, under the influence of impressions which are natural to the daily life of the animal. The slight effect caused by the very small proportions of the lethal dose of indaconitine, such as the $\frac{1}{20}$th or $\frac{1}{24}$th, are yet considerable when contrasted with the action of $\frac{1}{8}$th or $\frac{1}{12}$th, which produce their effect in part upon an area without the limit of imperfect stability, and consequently meet with an increased resistance to their operation. So it is probable that a febrile temperature, unstable as it is and dependent upon causes against which the organism is, in one way or other, exerting itself, is still sensitive to doses of so small a proportion as to be altogether outside the limit of effective action where the normal fever-free organism is concerned.

Fig. 2 shows an approximate toxicity curve for indaconitine hydrobromide, associated with the temperature-reducing power of fractional doses down to the $\frac{1}{16}$th lethal. The intermediate line indicates the geometrical progression in dosage.

Repetition of Dosage.

The main object of this inquiry has been to ascertain what relative values may be attached to repeated doses

bearing a known proportion to the lethal when administered at varying time intervals. The method of procedure was that already detailed for single dose administrations, except that the dose was repeated on two or more occasions. The shortest time elapsing before readministration was 45 minutes; the longest four hours. The doses employed were from one-half to one-fortieth of the lethal proportion.

Proportion of Dose to Lethal.

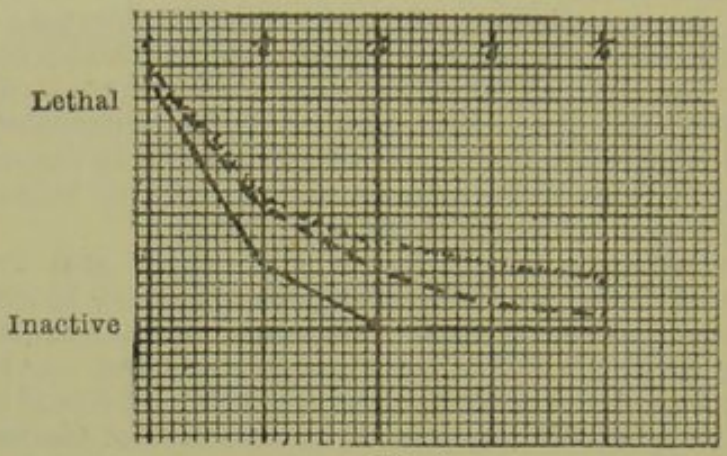

Fig. 2.

Geometrical regression of dosage — — —

Temperature reduction ---------

Approximate toxicity curve ———

Now to turn to the reduction of temperature brought about by large (one-half lethal) doses of indaconitine hydrobromide administered at stated intervals.

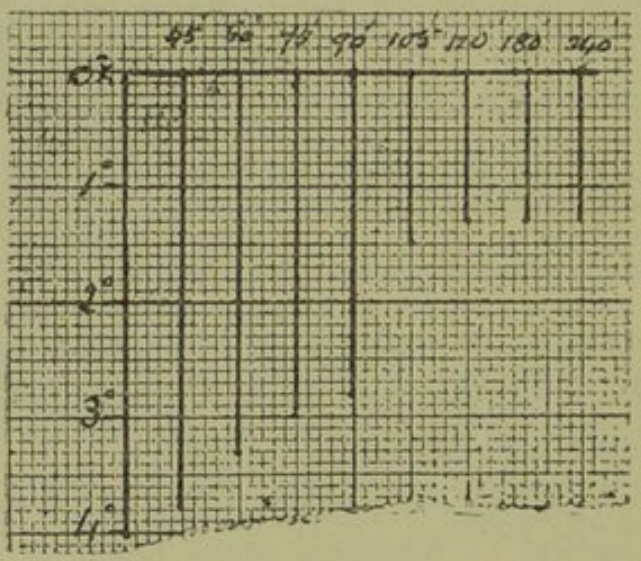

Fig. 3.—Three one-half lethal doses at varying time intervals. Ordinates indicate extent of greatest fall of rectal temperature.

In every instance two repetitions were made, so that in all one and a half times the lethal proportion was administered.

Table Showing Effect on Temperature of Three Doses of Indaconitine Hydrobromide, each of One-half Lethal Proportion. Administration at Various Time Intervals.

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Rectal Temperature.	Time of Greatest Reduction.	Notes.
½		45′	3.8° C.	After third dose	Maximal reduction after first dose, 0.9°; second dose, 2.3°; third dose, 3.8°.
½	3	60′	3.3	After third dose	—
½	3	75′	3.0	—	—
½	3	90′	2.8	After second dose	First dose, 1.5°; second dose, 2.8°.
½	3	105′	1.5	After second dose	—
½	3	120′	1.2	After first dose	First dose, 1.2°; second dose, 1.0°; third dose, 0.9°.
½	3	180′	1.3	After first dose	—
½	3	240′	1.2	After first dose	First dose, 1.2°; second dose, 0.7°; third dose, 0.5°.

The results above are graphically represented in the figure below (Fig. 3), in which the ordinates represent the greatest fall of rectal temperature (centigrade scale). Thus, repetition of the one-half lethal dose at intervals of 45′ and 60′ respectively is followed in each case by summation of effect of the second upon the first, and subsequently of the third upon the second. When the interval is increased to 75′, the greatest fall may be either after the second or third dose, but when it is 90′ the second only summates, and the temperature is never so far subnormal after the third. The 105′ interval is interesting, inasmuch as it is at about this time that the summation of the second dose action upon the first tends to fail; for, though the greatest reduction of temperature may still be recorded after the second, it ensues occasionally upon the first administration of the alkaloidal salt. In either case the fall of temperature after a third dose is less than after those preceding. When time intervals amount to 120′, 180′, and 240′ respectively, the greatest reduction of temperature occurs after the first and before the second dose, whilst the effect of the third is less than that of the former. If reference is made to the previous statement

regarding the time of commencing rise after the initial fall occasioned by one-half lethal proportions, we note that this takes place in about 75', so that a second dose timed at 90' after the first—allowing a few minutes for absorption—would begin to be effective when the rise is established, and when manifestly the disorder occasioned by the first dose is waning. The temperature-reducing effect of the first dose is not reproduced by the second, nor is that of the second reproduced by the third when the time interval is of 120' or longer, whereas if the time interval is short (45') a progressive increase is recorded. A glance at the subjoined table will make this clear:

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Rectal Temperature.	Lowest Temperature recorded below Normal after each one of three Doses.		
				1.	2.	3.
½	3	45'	3.8° C.	0.9°	2.3°	3.8°
½	3	90'	2.8	1.5	2.8	1.2
½	3	120'	1.2	1.2	1.0	0.9
½	3	240'	1.2	1.2	0.7	0.6

Readministration of One third of the Lethal Dose.

The average reduction of temperature for a single administration of this proportion is 0.95°. Recovery to the normal is complete in 130' to 160'.

Table taken from a Series of Observations in which such a Dose was twice readministered at varying Time Intervals (see also Fig. 4).

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Rectal Temperature.	Time of greatest Reduction.	Notes.
⅓	3	45'	2° C.	After third dose	—
⅓	3	60'	1.7	After third dose	—
⅓	3	75'	1.4	After second dose	—
⅓	3	90'	1.15	—	—
⅓	3	120'	0.95	After first dose	After second – 0.7° C.
⅓	3	240'	0.95	After first dose	After second – 0.45° C.

Summation of effect of the second upon the first, and of the third upon the second, occurs when time intervals are of 60′ and less, but the third dose does not summate on the second when 75′ to 90′ elapses. The first dose is succeeded by the greatest fall when two hours or longer elapses before incidence of the second. The second dose is more effective when the interval is of two rather than of four hours.

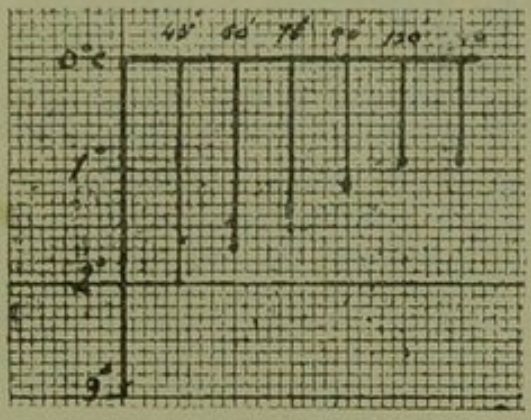

Fig. 4.—Three one-third lethal doses at varying time intervals. Ordinates indicate extent of greatest fall of rectal temperature.

Readministration of One fourth of the Lethal Dose.

The average reduction of temperature following a single dose of one-fourth of the lethal is 0.8° C. (It will be recalled that this is the smallest proportion which produces an obvious toxic action; external salivation is quite rare; there is some degree of apathy, but the symptoms have ceased to be characteristic.) Recovery of original temperature takes place in 120 to 140 minutes.

Table giving the Result of the Administration of Four Doses each of One-quarter of the Lethal Proportion (see also Fig. 5).

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Rectal Temperature.	Time of Greatest Reduction.
¼	4	45′	1.6°C.	After third dose.
¼	4	60′	1.4	After third dose.
¼	4	75′	1.1	After second dose.
¼	4	90′	1.0	After second dose.
¼	4	105′	0.8	After first dose.
¼	4	120′	0 8	After first dose.

There is, therefore, a summation of effect up to the third dose, when the intervals between administrations are of 45′ and 60′. At and after 105′ summation disappears, the greatest temperature reduction being after

the first dose, the subsequent reductions becoming progressively less extensive.

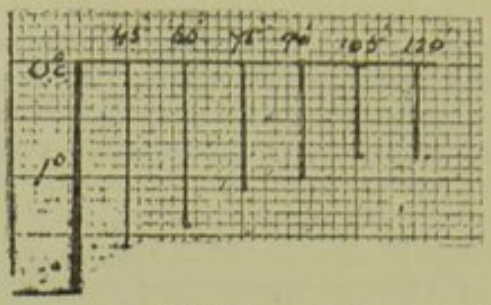

Fig. 5.—Four one-quarter lethal doses at varying time intervals. Ordinates indicate extent of greatest fall of rectal temperature.

The unit dose of one-quarter of the lethal having such slight toxicity it is possible to repeat it many times, even at short intervals, without the least danger to life.

Thus eight administrations, at intervals of forty-five minutes (that is a total dose of double the lethal distributed over six hours), caused a temperature reduction of 2 3° C., the lowest point being attained after the fifth administration; thereafter each successive dose showed a diminishing effect.

Six administrations of one-quarter proportion, at intervals of ninety minutes (that is one and a half times the lethal dose in nine hours), caused a total fall of 1.2° C, only, the greatest reduction being after the second dose.

Readministration of One-sixth of the Lethal Dose.

The average temperature reduction following a single dose of one-sixth lethal proportion is 0.7° C. The temperature attains the original level again in about 120 minutes. No obvious toxic effect follows this dose.

Table giving the Result of Six Administrations each of One-sixth of the Lethal Proportion.

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Rectal Temperature.	Time of Greatest Reduction.	Notes.
1/6	6	45'	1.25°C.	After second dose	Fell 0 65° after first and 0 6° further after second.
1/6	6	60'	1.1	After second dose	—
1/6	6	90'	0.9	After first or second dose	Rose after third to −0 5°. Normal after fourth.
1/6	6	105'	0.7	After first dose	—
1/6	6	120'	0.7	After first dose	Normal 30' after fourth.

This table indicates that when the dose is reduced to one-sixth of the lethal proportion the maximal effect falls

after the second administration when this occurs in 45', 60', or 90' (occasionally), but after the first when the interval is longer, and occasionally when it is of ninety minutes.

Readministration of One eighth of the Lethal Dose.

A single dose of this proportion occasions a fall of 0.55° to 0.6° in rectal temperature, the normal being regained in the course of two hours. The occurrence of hypernormality (to be referred to later) modifies the time of maximal effect produced by repeated doses. There is less regularity in response to this dosage than to the larger proportions already considered.

Readministration of One-eighth of the Lethal at Varying Time Intervals. (Fig. 6)

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Rectal Temperature.	Time of Greatest Reduction.	Notes.
1/8	6	45'	0.95°C.	After second dose	—
1/8	6	60'	0.85	After second dose	Rising after third, hypernormal after fourth.
1/8	6	90'	0.7	After first or second dose	—
1/8	6	105'	0 6	After first dose	—
1/8	6	120'	0.55	After first dose	Rise well established before second and continued to hypernormality without check by subsequent doses.

Fig. 6.—Six one-eighth lethal doses at varying time intervals. Ordinates indicate extent of greatest fall of rectal temperature.

Exceptionally the result of the first injection is to develop a phase of hypernormality, which may last until a second injection, falling in 45' occasions a fall which, though passing below the normal, does not amount to such an extensive reduction as would have resulted if hypernormality had not preceded. (Larger proportions of indaconitone frequently cause initial hypernormality, but under such conditions this effect is

transitory, being replaced by a fall before 45′ have elapsed. Thus the duration of a hypernormal temperature disappears within 30′ after proportions of $\frac{3}{4}$ and $\frac{1}{2}$ lethal proportion.)

Readministration of One-twelfth of the Lethal Dose.

The average fall of temperature following a dose of one-twelfth of the lethal is 0.45° to 0.5° C. Normality is regained in 110 minutes; thereafter the temperature exceeds the normal. There is still evidence of summation on repeating the administration, but it is only to a slight extent, as the following table shows. (See also Fig. 7.)

Readministration of One-twelfth of the Lethal Dose at Varying Time Intervals.

Proportion to Lethal.	Number of Doses.	Time Interval.	Greatest Reduction of Temperature.	Time of Greatest Reduction.	Notes.
$\frac{1}{12}$	4	45′	0.65° C.	After second dose	—
,,	5	60′	0.6	After second dose	—
,,	3	90′	0.55	After first or second dose	—
,,	3	105′	0.5	After first dose	—
,,	3	120′	0.5	After first dose	—

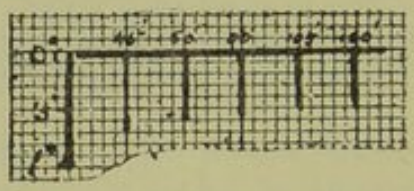

Fig. 7.—One-twelfth lethal dose, repeated at varying time intervals. Ordinates indicate extent of greatest fall of rectal temperature.

A point has now been arrived at in the reduction of dosage which only admits of slight summation; the increase occasioned by a second administration after such intervals as 45′ and 60′, being in excess of the single dose action by 0.15° and 0.10° respectively.

Even up to a 90′ interval some summation may be observed, although it does not average more than $\frac{1}{10}$ to $\frac{1}{20}$° C.

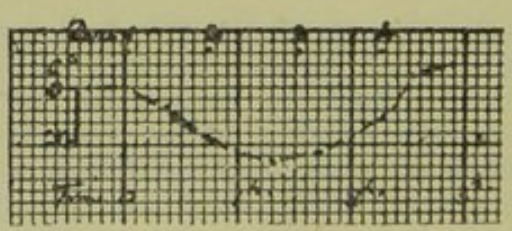

Fig. 8.—Four doses, each of one-twelfth lethal.

The summation does not, however, extend beyond the second dose; even when the interval is only of 45′ a third dose does not check the tendency to rise, and hypernormality is reached within 20′ to 30′ after the fourth administration (Fig. 8). It is worthy of note that, feeble though the temperature reduction is by such a proportion, almost as long a period is required for reinstatement as after a much larger proportion.

One-sixteenth of the Lethal Proportion.

A single dose of this proportion is still productive of a positive effect, either an immediate fall or an initial rise succeeded by a fall. In the former case the reduction is of 0.35′ to 0.4° C., but in the latter it is somewhat less. Summation of effect of a second dose upon the first is present after intervals of 45, 60, and occasionally 90 minutes. If the second dose is separated from the first by intervals of 105′ or more there is no increase or summation of effect attributable to it.

Fig 9 shows the course of temperature under four doses each of $\frac{1}{16}$ lethal proportion.

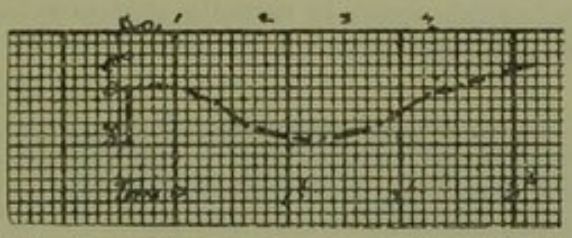

Fig. 9.—Four doses, each of one-sixteenth lethal.

One-twentieth of the Lethal Proportion.

The effect of such a single dose is an immediate fall, or, as often, a rise succeeded by a fall, after which return to normality and thereafter hypernormality ensues. The average fall is 0.3° C. If readministration occurs in 45′ after the first dose the greatest effect will be observed after the latter if the fall is initial (Fig. 10, A), but if a rise above normal is initial the greatest reduction will be recorded after the second (Fig. 10, B), in this case reduction will be less and the advent of hypernormality relatively earlier.

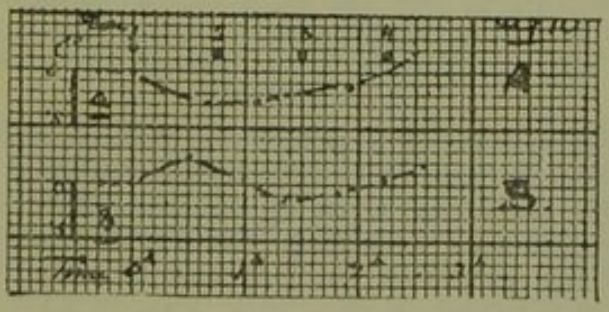

Fig. 10.—Four doses of one-twentieth lethal, interval 45′.

The result of repetition of such a dose may best be described as an oscillation of temperature around the normal, the extent of the variation being (for intervals of 45') about 0.4° C. For longer intervals there is no evidence of summation.

I convinced myself that doses as small as $\frac{1}{2^{1}2}$ and $\frac{1}{2^{1}3}$ of the lethal are yet capable of producing a fluctuation of temperature very slightly beyond the limit of physiological variation. As the result of repeated observation, it appears that the limit of action ("minimal effective dose") must be located at or about the $\frac{1}{2^{1}4}$ of the lethal.

One Twenty-fourth of the Lethal.

A single dose of this proportion may exceptionally have no effect whatever upon temperature, but usually it originates an oscillation totalling about 0.25° C. This may be of the nature of a rise, having its maximum in 30 to 40 minutes, after which a gradual return to, but seldom below, normality ensues, or else a slight reduction occurs without antecedent rise. On repetition of dosage there may be a slight additional effect (Fig. 11), followed by a reversion to normality and hypernormality. After such a proportion there is usually more oscillation above than below normality, whether the dose is repeated or otherwise.

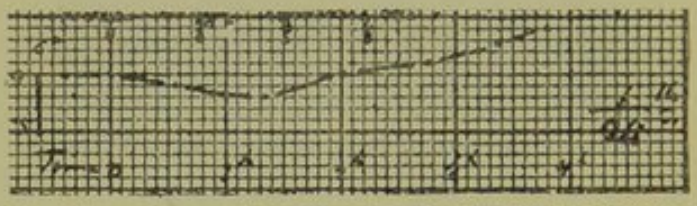

Fig. 11.—Four doses of one twenty-fourth lethal, interval 45'.

Whilst the results discussed in this paper appear to be generally applicable to the large group of aconitines in so far as these have been investigated, it cannot be assumed that they are necessarily strictly applicable to other bodies which would find their place in the same class as indaconitine.

Divergent potentiality for absorption and excretion might obviously cause variation in the advent of maximum intensity of effect, and consequently of the probability of summation by subsequent doses.

Conclusions.

1. Objective toxicity is produced by indaconitine hydrobromide when administered hypodermically in doses which are not less than $\frac{1}{4}$ of the lethal proportion. The characteristic symptom of obvious salivation is produced by $\frac{1}{3}$, rarely by $\frac{1}{4}$ of the lethal. Perceptible toxic effect is therefore restricted to a very limited range of dosage.

2. The temperature-reducing activity caused by single doses of the salt, which decrease in geometrical progression, does not show a parallel diminution, but is more gradual, excepting for doses of $\frac{1}{2}$, $\frac{1}{3}$, and $\frac{1}{4}$, which are approximately proportionate in effect.

3. Some modification of the internal (rectal) temperature of the normal animal is observed as the result of hypodermic medication after doses bearing a proportion down to the $\frac{1}{24}$ of the lethal, which may, therefore, be regarded as the "minimal effective dose" for temperature. Smaller proportions may occasion a slight effect within the area of physiological variation. At or near the $\frac{1}{25}$ of the lethal is to be located the "maximal ineffective dose."

4. Although the total reduction of temperature becomes very small as the reduction of dosage proceeds, there is by no means a commensurate abbreviation in duration of effect.

Repetition of Dose.

5. A second dose causes a summation of temperature reduction for practically all fractions of the lethal down to the $\frac{1}{25}$ (provided the interval before readministration is not more than forty-five minutes), but the extent of this summation declines very rapidly after the dose falls below the proportion of $\frac{1}{4}$ to the lethal, and is quite inconspicuous when below $\frac{1}{12}$.

6. When there is an interval of sixty minutes before readministration there is still summation of effect of the second dose, though to a lesser degree than after a forty-five minutes' interval. When ninety minutes elapse it is still recognizable for doses which bear a larger proportion than $\frac{1}{16}$ to the lethal unit.

7. When the interval is of 105 minutes, summation, though greatly reduced in extent, follows doses of $\frac{1}{2}$ and $\frac{1}{3}$ of the lethal proportion, but for $\frac{1}{4}$ and smaller proportions there is no summation, the greatest reduction of temperature falling after the primary administration. After intervals of 120 and 240 minutes there is no summation, successive doses causing a diminished effect, which is more or less in direct proportion to the increase in time interval.

8. It has already been shown that a slight degree of tolerance is established towards the action of the aconitines when administered daily or every second day, but this is inconspicuous compared with the decline in temperature-reducing activity of all proportions from $\frac{1}{2}$ of the lethal downwards when given at intervals of 120 and 240 minutes.

9. Hypernormality of temperature may occur as a transitory condition after administration of indaconitine in large proportions. The larger the dose and the sooner this phase is superseded by a fall to subnormality. When the proportion is reduced to $\frac{1}{8}$ or less, hypernormality may endure for 45', and under these circumstances there is a relative delay in the time of occurrence of sub-

normality by a second administration. As the limit of active dosage is approached in the descending scale, the result is often of the character of an oscillation of temperature around the normal, in which hypernormality is conspicuous.

It is observed that as the effect of practically all doses which are sublethal passes over, there is a tendency to a condition of hypernormality after the original temperature has been regained.

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