

## ON THE MECHANISM OF TONIC IMMOBILITY IN VERTEBRATES.

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### I.

Tonic immobility (Crozier, 1923), often more popularly known as "playing possum," "animal hypnosis," or even "feigning death," is exhibited by a great variety of animals. Rabaud (1919) discusses at length the condition as seen in arthropods. He points out that most arthropods have regions of the body which seem to be the loci of tactually excitable reflexes causing the animal to pass into a state of immobility characterized by heightened tonicity of the skeletal musculature. The durations of the periods of immobility vary considerably from species to species, and to a lesser degree for individuals within the species. The antennæ, thorax, and bases of the wings are in general regions which when stimulated tend to produce immobilization; while other parts of the body, particularly the feet, seem to be the seat of antagonistic reflexes causing recovery of activity. The state is conspicuous by the fact of its apparent all-or-none nature and because of the characteristic positions assumed by the animals.

Verworn in 1898 discussed tonic immobility as seen among lower vertebrates, and Mangold (1920) has described the condition in higher vertebrates. With birds and mammals the state is most readily produced by suddenly turning the animal dorsum downward. Guinea pigs are more readily immobilized by this method than most mammals, although the condition has been produced in young dogs, cats, apes, and sheep. Among birds the state is well demonstrated

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in hens and pigeons. As early as 1562 the Abbé Kircher records the phenomenon in a hen (*cf.* Piéron, 1913; Mangold, 1920).<sup>1</sup>

Foxes and opossums are said to "feign death" sometimes when pursued by enemies and to remain quite immobile even if mauled about. In such cases it has been urged that the phenomenon is a death feint of definite survival value, and as a result it has been spoken of as an "instinct of death-feigning."

In all cases from coelenterates to mammals the condition is marked by a high degree of plastic tonus of skeletal musculature and a pronounced lack of reactivity to environmental disturbances. Verworn (1898) speaks of it as animal hypnosis, because of the similarity of the plastic tonus to that shown in the cataleptic trance of the human hypnotic subject. The term, however, is not a very happy one in this connection because of its psychological implications.

Work by Rabaud (1919) and also by Holmes (1906) has tended to show that in arthropods the condition is not dependent on any one neurone center. On severally destroying various ganglia the state has been found to continue. *Rana* even when cut in two continues the "feint" for its customary period (Holmes, 1906).

Crozier (1923) gave a summary of work with the isopod, *Cylisticus convexus*. By correlation with the Arrhenius equation<sup>2</sup>

$$\frac{k_2}{k_1} = e^{\frac{\mu}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)},$$

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<sup>1</sup> Tonic immobility or a state akin to it has been described in children by Piéron (1913). I have recently been able to produce the condition in adult human beings. The technique was brought to my attention by a student in physiology, Mr. W. I. Gregg, who after hearing a lecture on tonic immobility suggested that a state produced by the following form of manhandling which he had seen exhibited as a sort of trick might be essentially the same thing. If one bends forward from the waist through an angle of 90°, places the hands on the abdomen, and after taking a deep breath is violently thrown backwards through 180° by a man on either side, the skeletal muscles contract vigorously and a state of pronounced immobility lasting for some seconds may result. The condition is striking and of especial interest since this type of manipulation (sudden turning into a dorsal position) is the most common one used for producing tonic immobility in vertebrates.

<sup>2</sup> *Cf.* Taylor, 1924; Crozier, 1924, *a, b*.

he examined the effect of temperature on successively induced periods of tonic immobility and concluded the effects might be accounted for by assuming the durations proportional to the amount of an inhibitory substance which was the mid-product in a catenary series. Two values of  $\mu$ , the temperature characteristic, were obtained (24,000 and 9,200) from lines of different slope meeting at a critical temperature of 16°.

A similar investigation was undertaken with vertebrates. The mechanism of tonic immobility in vertebrates is of interest since there is invoked a general hypertonicity of the skeletal muscles apparently associated with widespread inhibition of impulses from the so called "higher centers." Tonic immobility is useful as a possible means of studying tonus and selective inhibition, and is actually a special case of the problem of alternate periods of movement and quiescence of an organism.

## II.

Birds and mammals are out of the question for such experiments because of the impossibility of changing the controlled temperature. The lizard *Anolis carolinensis* is readily immobilized by turning it into the dorsal position and applying pressure lightly with the finger on the thorax. The onset of immobility is usually marked by a period of deep gasping respirations, followed by a gradual decrease in amplitude and frequency of respiratory movement until breathing is hardly perceptible. The immobile state is characterized by a heightened tone of the leg muscles and by a certain degree of non-reactivity to the environment although the eyes frequently remain open and alert. *Anolis* may remain immobile for periods ranging from a few seconds to several hours, depending upon conditions presently to be discussed.

The recovery of activity is well marked and even dramatic. It is generally heralded by the progression of a wave of activity starting at the tip of the tail and passing forward to the lumbar region, when the animal springs violently into activity; if lying immobile on the back it leaps to its feet and endeavors to escape.

Owing to a comparative responsiveness to environmental disturbances during the immobile state great difficulty was at first found in working with *Anolis*. It was desired to begin with the application of the temperature method of analysis.

Attempts were made in preliminary experiments to measure with a stop-watch the successive intervals between induction of immobility and spontaneous recovery. It soon became apparent, however, that this was a difficult task since the mere movement of objects in the environment was often sufficient to bring about a premature recovery and so to interfere with possible cyclical phenomena. Moreover, it was evident from the start that a large amount of data would be required for significance in statistical treatment. This necessitated watching the animal for long periods of time in order to ascertain the durations of the periods.

Because of the instability of the immobile state of *Anolis* another lizard was obtained, the horned "toad" *Phrynosoma cornutum*. This animal is readily put in a stable immobilized condition by a procedure similar to that used for *Anolis*. *Phrynosoma* seems to be quite indifferent to most environmental disturbances, so that once immobilized it continues the "feint" even though moderately handled.

Automatic immobilizing and recording had to be devised, since excessive durations of 5 to 10 hours were sometimes encountered. Ideally a method was desired whereby the animal could be automatically reimmobilized as soon as it recovered. This should be accomplished by a procedure in which light and temperature conditions could be kept constant and in which the excitement incident to handling and immobilizing the animal should be minimized.

Control of temperatures above that of the room was effected by the use of a Freas electrically regulated air incubator. For low temperatures a thermostat (Crozier and Stier, 1927) was used consisting, in the main, of a water bath with a stirrer in which is immersed a cooling coil connected to a motor-driven SO<sub>2</sub> condenser pump and external air-cooled coil. The motor of the pump is started and stopped by the action of a relay system which is in turn controlled by a mercury thermoregulator in the bath.

Preliminary experiments were made in which the animal was fastened in a holder, ventral side up, within the thermostat. One of its legs was connected by levers to a switch operating a signal magnet against a smoked drum. An automatic, electrically driven mechanism for stimulating the animal on recovery was also devised. This mechanism was so arranged as to be started by the recovery of the animal and stopped when the animal ceased to struggle and became immobile again.

Fortunately, a simple discovery rendered unnecessary this more or less elaborate set-up. The duration of tonic immobility in all cases has been found to be independent of the immobilizing stimulus. Any manipulation that produces the condition does so in an all-or-none fashion. While observing the properties of tonic immobility it was found that if, while lying on the back, one prevented the immobilized *Phrynosoma* from righting itself on recovery that it immediately became reimmobilized. This was demonstrated for all of the eleven horned "toads" in the laboratory at the time. If one but applied light pressure to the skin on the sides of the animals so as to prevent their turning over they relapsed after a brief struggle into immobility, *the same in all of its properties* to that produced by the usual sternal pressure.

It now only became necessary to fasten the animal on its back, in the thermostat, connect it with the writing lever of the kymograph, and have it make its own record of spontaneous recovery and immobilization. A holder was made for the lizards,

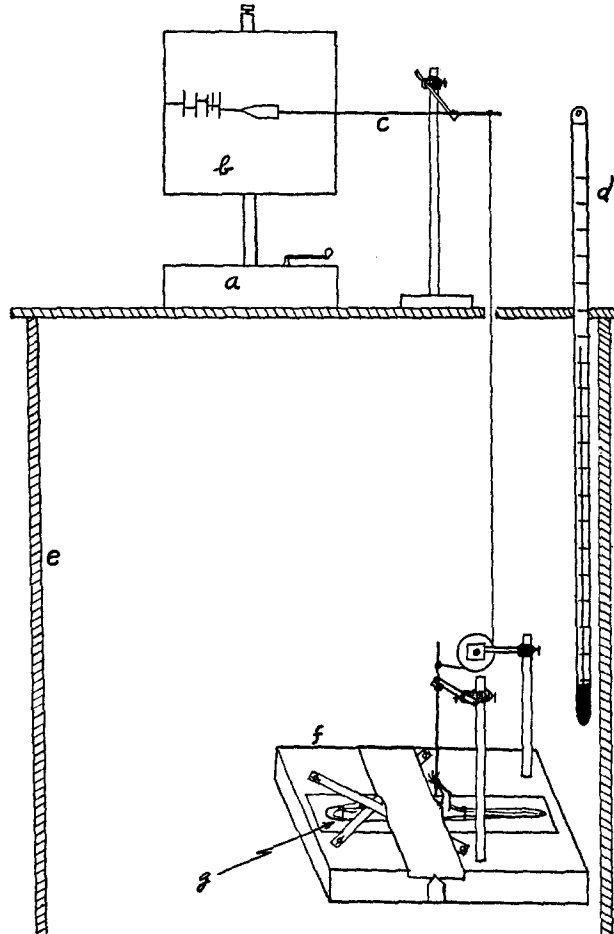


FIG. 1. Apparatus for obtaining records of spontaneous emergence from tonic immobility: *a*—kymograph, *b*—drum, *c*—writing lever, *d*—thermometer, *e*—wall of thermostat, *f*—animal holder, *g*—animal.

consisting of a block of wood with a depression gouged out of it. This depression was filled with paraffin and hollowed out in such a way that *Phrynosoma* would fill it when placed in it dorsally. In order to prevent escape, straps were placed

across the body of the animal, one running directly across from side to side and two others passing diagonally across the thorax. One of the lizard's legs was fastened by a strip of adhesive tape to a vertical lever supported by the holder block. A thread was fastened to this lever and after passing under a pulley was run upwards out of a hole in the top of the thermostat in which the lizard was placed. This thread was then directly connected to a writing lever, and in this way the movements of the animal's leg were recorded on a slowly revolving smoked drum.

It may be objected that the animal might fail to move the recording leg when it recovers. From many observations of the phenomenon, the recovery process has been observed to be constantly the same in certain particulars. It is always characterized in the cases of both *Anolis* and *Phrynosoma* by immediate struggles to turn the ventrum downward. This reaction on the part of the animal seems to be at a reflex level and is invariable. Moreover, the act of turning over requires the utilization of the legs and in all observed cases the legs are moved vigorously.

Although a number of kymograph records were obtained with *Phrynosoma* this animal was discarded for *Anolis carolinensis* owing to the excessively long periods of immobility occurring at intervals in the case of the former. The method developed for *Phrynosoma* obviated the difficulties of hyperexcitability formerly encountered with *Anolis* and at the same time the shorter periods of *Anolis* made it possible to obtain more data. It was found that the property of reimmobilization by preventing the animal from turning over is also characteristic of *Anolis*. Certain minor modifications of the animal holder were the only changes necessary in the foregoing method (see Fig. 1).

179 records of an approximate average duration of 4 hours each, and comprising 12,000 to 15,000 mobilizations were made over a period of 5 months. In all, six animals were used, four of which lived in a healthy condition throughout the experiments. Of the two that died one was killed by accident and the other died a "natural" death, but in both cases after a significant amount of data had been obtained.

The low temperature thermostat was adapted by the immersion of a cylindrical can 8 inches in diameter and 9 inches deep in the water bath. The animal holder was placed in this can and the thread connecting the leg with the writing arm was run out through a hole in the top. The kymograph was placed on a platform across the bath above the can and a calibrated thermometer was suspended inside to record the air temperature near the animal. The incubator set-up used for work above room temperature is diagrammed in Fig. 1.

The calibrated kymographs turned approximately once in 8 hours, corresponding to about  $\frac{1}{4}$  mm. of record per minute. Individual animals were not tested twice on the same day but only after at least 24 hours in the terrarium. The records were taken as intervals of  $2\frac{1}{2}^{\circ}\text{C}$ . from  $5^{\circ}$  to  $35^{\circ}\text{C}$ . In the cases of the four surviving animals at least two and in some cases three and four runs were made at each of the temperature intervals.

## III.

Fig. 2 is a sample set of records. The three numbers on the record at the left hand side reading from left to right designate the number of the run, the number of the animal, and the mean temperature.

The records show a rhythmical distribution of recovery periods in most cases, these rhythms in general shortening with rise of temperature. Certain other records, illustrated by the three sample records on the left side of Fig. 3, show a definite decrease in the number of recovery periods with time until finally the animal may remain continuously immobile for hours.

The data of Fig. 2 were all taken with the same animal and the effect of temperature on the duration of immobility is clear. The vertical marks on the record are caused by the movement of the leg connected with the writing lever. (Owing to the slowness of rotation of the drum, the few seconds that the animal is active during each recovery are not recorded, the up stroke representing a recovery and struggle period during which the pointer moves up and down in essentially the same track).

In computations made from the records the 1st hour of the run was not considered, in order that the temperature conditions should come to equilibrium and the effect of general excitement, incident to starting the experiment, should be minimized.

Run 48 at 5.9°C. demonstrates a long set of rhythms, the average duration, made from the last five, being 39 minutes. Run 74 at 10.7°C. shows a marked decrease in the duration of the rhythms, just one recovery period in each case marking the limit of the cycle (average = 12.3 minutes). In this case there is observed a characteristic, sudden cessation of recoveries at the end of several hours with only one or two struggles after irregular intervals. Run 167 at 14.5°C. is an excellent sample of the sort of result obtained at this temperature. Here are periods of immobility each representing a rhythm varying at remarkably regular intervals (mean = 4.5 minutes). This distribution remains constant after 3½ hours of the experiment. Run 129 at 19.1°C. is of interest. In this case there occurs apparently a double rhythmical effect. The period of immobility which at 14.5°C. was 4.5 minutes long has now become reduced to 2 minutes while another

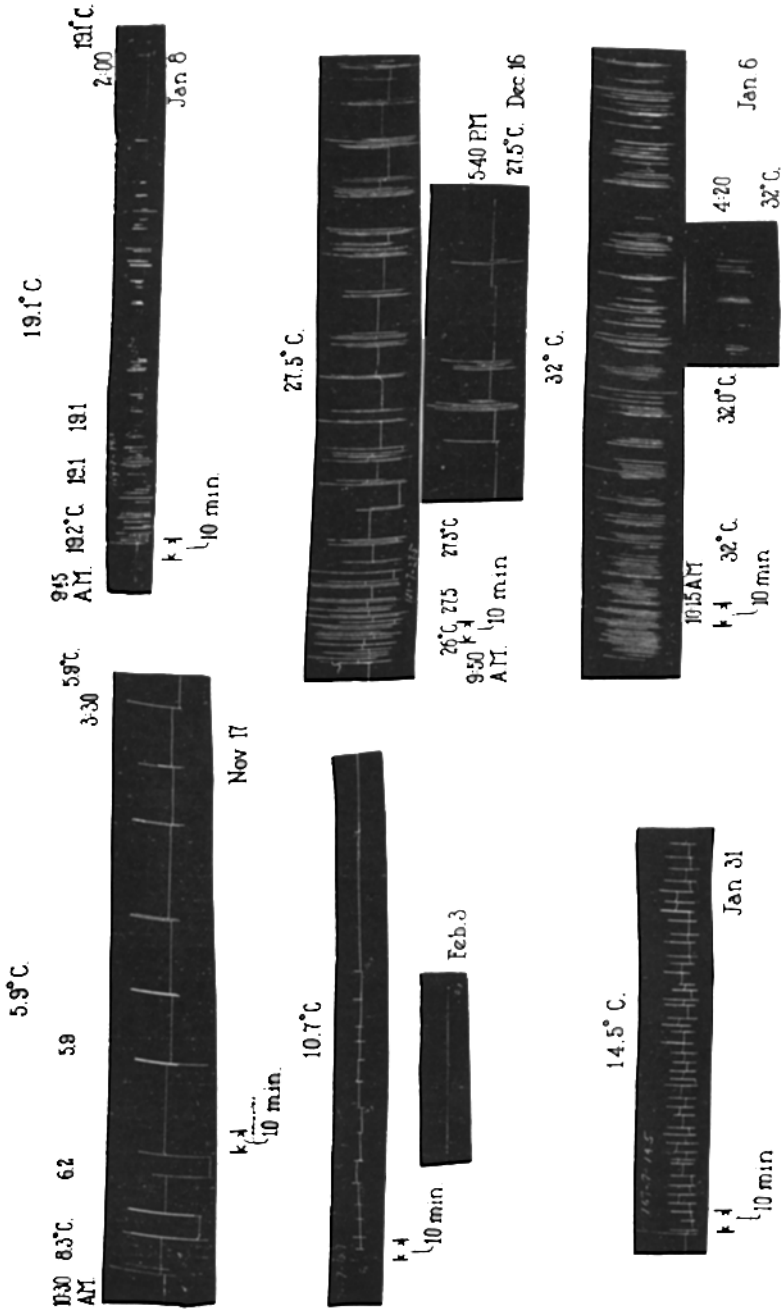


FIG. 2. Sample kymograph records from a single animal. The height of the vertical strokes of these records has no significance. The horizontal distances between strokes gives a direct measure of the duration of tonic immobility between spontaneous recovery and remobilization and succeeding spontaneous recovery and remobilization. The struggles of the animal actually occupy but a few seconds and so are only registered as a single up stroke on the slowly moving drum. The records illustrate the effect of temperature on the duration of immobility. The three numbers at the left of the records are respectively the number of the experiment, the number of the animal, and the temperature.



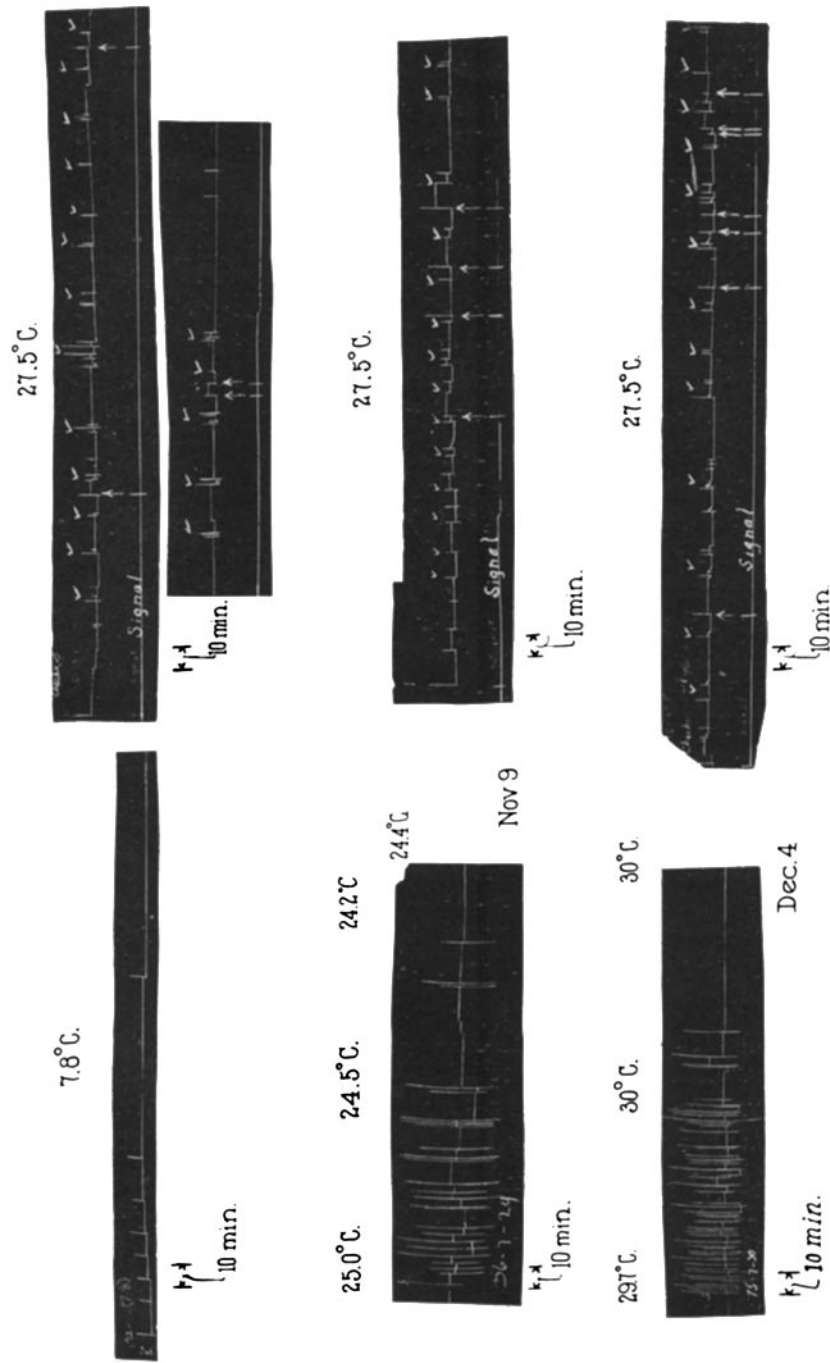


FIG. 3. The three sample records on the left of this figure show a definite decrease of frequency of recovery with time. The three records on the right demonstrate the essential functional independence of the rhythms. The check (V) marks indicate rhythms, and the signal below marks the place of forced recovery of the animal by shocking it with an inductorium. It will be seen that forced recovery has no effect on the rhythm or subsequent rhythms, the animal becoming reimmobilized and remaining so until its normal period of recovery.

rhythm of 12 minutes duration seems to be superposed, as it were, on the original shorter rhythm. In the case of all animals examined this additional rhythm was found to occur at approximately 20°C. and to continue in more pronounced form with rise of temperature to about 32°C. Run 101 at 27.5°C. and Run 123 at 32°C. demonstrate the double rhythmical effect which is most pronounced at 27°C. In these cases one finds a fundamental unit of immobility time (2 minutes to  $\frac{1}{4}$  minute), shortening with rise of temperature, with superposed upon this a well marked longer rhythmical cycle. As will be seen presently, this longer rhythm also shortens statistically with rise of temperature.

Runs 172, 36, and 75 of Fig. 3 demonstrate the general exhaustion of the ability of the animal to recover from the immobile state. All of the samples of Figs. 2 and 3 are chosen because of the definiteness of the phenomena. In many cases the rhythms are not as obvious as in these and practice is necessary to enable one to identify them. The other records of Fig. 3 serve to demonstrate a point of interest. It was desired to learn whether tonic immobility was caused by the release of some mechanism independently at each inducing stimulus, or whether the initial stimulus in a sequence releases a series of events which proceeds independently of succeeding stimuli. The latter had been found to be essentially the case by Crozier in his work with arthropods. In order to test this with *Anolis* an animal was "awakened" at various stages in the immobility period and the effect on subsequent periods was observed. The right-hand records of Fig. 3 illustrate the results. The lower line on these records was made by a signal magnet which was connected in series with an inductorium. At intervals during the rhythms as indicated by the signal the animal was shocked until it recovered activity and struggled to escape. During the period of stimulation the thermostat was opened briefly and the animal watched to make certain that it had fully recovered and that the mark on the record was not merely made by a reflex twitch in response to the shock. The rhythms in the records have been accentuated for convenience by check marks. It is apparent that the stimulus merely broke in upon an already functioning process which had been started by the initial stimulus. The forced recovery, even when the animal was aroused twice in the same cycle, has no effect on the general rhythm.

It was desired to make certain that the internal temperatures of the animals were equal to those of their surroundings after adequate exposure, and to discover any possible increase in temperature during the recovery struggle. A calibrated thermopile was inserted in the cloaca of one of the animals and the preparation was placed in a thermostat, a thermometer near the animal giving the temperature of

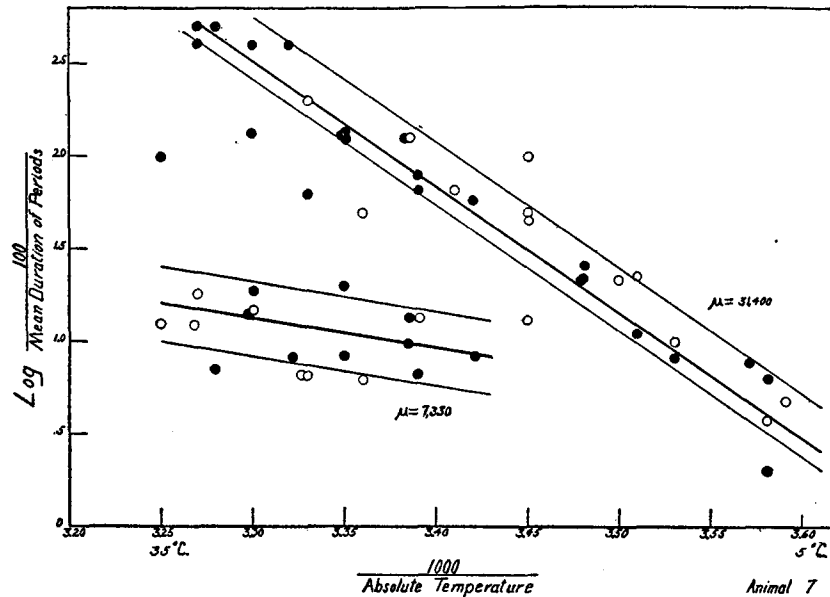


FIG. 4. This figure with Figs. 5, 6, 7, and 8 illustrates the effect of temperature on the duration of the rhythms of tonic immobility of *Anolis carolinensis*. The data were plotted according to the Arrhenius equation and  $\mu$  was calculated from the slopes of the lines. The points in general fall in bands between the parallel lines. The upper and steeper curve represents the rhythm in evidence at 5.9°C. (Fig. 2) which decreases throughout the temperature range. The lower curve corresponds to the secondary rhythm which becomes evident at approximately 20°C.

The latitude of variation of the data is large. This may be due to the fact that the experiments were made over a 5 months period while with most temperature work the data for one plot are taken within 24 hours. Moreover, in these cases one is dealing with an obviously complex process and points lying between the normal latitudes of variation may be accounted for by a confusion of the two rhythmical processes concerned. In the case of this figure the points lying between the bands were all obtained during approximately the first month of the animal's life in the laboratory and may possibly be correlated with the age of the animal.

Shaded points correspond to averages of at least five rhythms.

the surrounding air. It was found that the temperature of the animal was appreciably below that of its environment (temperature range of 8° to 30°C.) the difference being as great as 0.4°C. at 8°C. and decreasing with rise of temperature. This negative discrepancy is puzzling but may be due to evaporation of moisture with respiration. It is more or less consistent with similar results found by Buxton (1924) in the cases of certain desert insects.

No appreciable internal temperature change occurred when the animal struggled during the recovery periods.

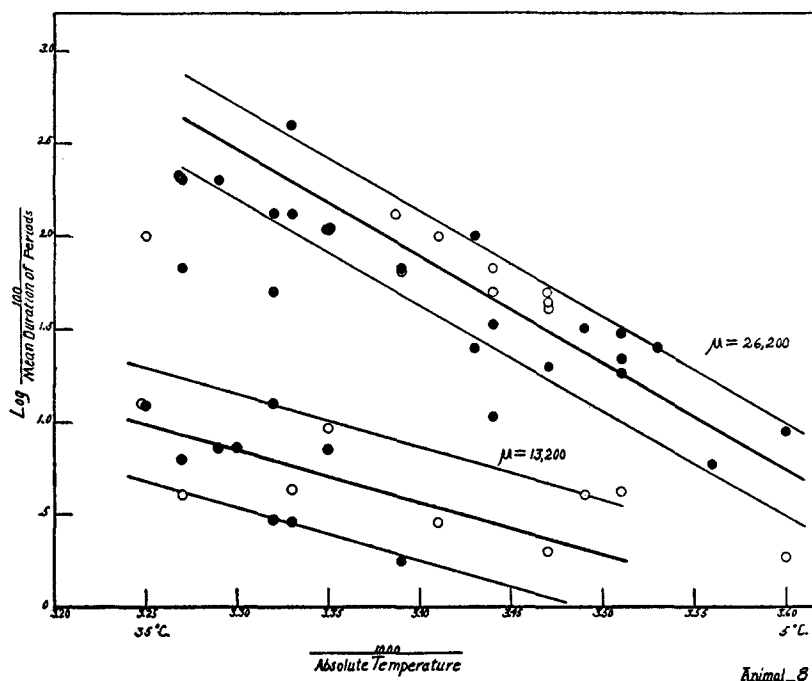


FIG. 5. Plot according to the Arrhenius equation illustrating the effect of temperature on the duration of tonic immobility for *Anolis carolinensis*.

#### IV.

The Arrhenius equation was applied to the data of those records showing rhythmical distributions of immobility periods as demonstrated in Fig. 2. The mean durations of immobility are shown plotted as a function of the absolute temperatures in Figs. 4, 5, 6, 7, and 8.

In these figures two distinct distributions of points with characteris-

tic  $\mu$  values may be seen. These distributions are enclosed between parallel lines and an average central line is drawn through the field of points. This is permissible since the points do not merely form a fan-shaped band but group themselves in the indicated distributions (*cf.* Crozier and Stier, 1927). The solid points represent weighted averages of not less than five distinct rhythms during the experiment; the open circles correspond to less certain averages. The mean probable error of the durations of rhythm of some 28 experiments on one animal, throughout the temperature range, is  $\pm 4.9$  per cent.

The latitude of variation of the plotted data may seem large, but in later sections an attempt will be made to account for this scatter in terms of a theory of the mechanism of tonic immobility.

The results of the Arrhenius equation plots may be summarized in the following table:

Animal	$\mu_1$ , calories	$\mu_2$ , calories
7	7,330	31,400
8	13,200	26,200
11	*	35,000
13	9,210	31,800
14	*	30,700
Arithmetic mean.....	9,913	31,020
Probable error of mean.....	$\pm 1,310$	$\pm 868$

\* In the case of Animal 11 the data was insufficient for a determination of  $\mu$ . With Animal 14 the scatter of points is too great to permit an assignment of a " $\mu$ " value.

## V.

It is necessary to consider the type of record illustrated on the left in Fig. 3, in which there is a progressive increase in the durations of immobility. Many records show this phenomenon alone, but a few show a combination of rhythmicity with progressive prolongation of immobility.

Plots were made of the logarithm of the frequency of recovery per hour against the total time from the beginning of the experiment and in most cases surprisingly good linear functions were obtained. A sample curve of this sort is illustrated by Fig. 9.

The values of  $R$ , the slope of the lines, were plotted as a function of the temperature. This was done by expressing the values of  $R$  for each temperature as a percentage of the mean value of  $R$  for the particular animal, and on this basis averaging data from all of the animals together in one plot. Fig. 10 is the result, averaged for 5° intervals.

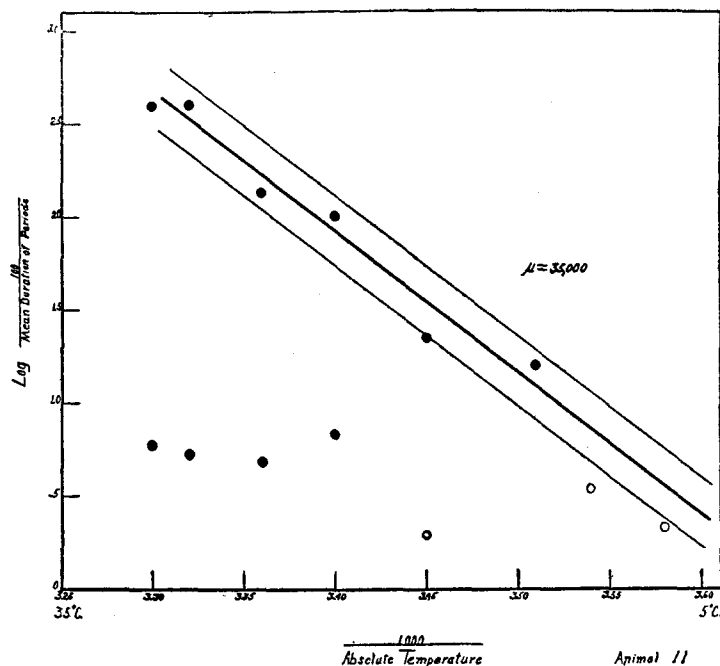


FIG. 6. Plot showing temperature effect on the immobility periods of *Anolis carolinensis*. In this case the animal died before enough data were obtained for a calculation of the lower  $\mu$ .

This plot clearly shows that the effect of temperature on the function in question is substantially negligible, the computed-value of  $Q_{10}$  being 1.04.

A value for  $Q_{10}$  of 1.04 is essentially not that characteristic for a chemical process ( $Q_{10} = 2$  to 3). The value of  $Q_{10}$  for diffusion processes in solution is of the order of magnitude of 1.0 to 1.3. Fig. 10 makes it seem likely that, in the cases under consideration, some physical event is determinative.

## VI.

The application of the Arrhenius equation to those records which show a rhythmical sequence of successive immobility periods suggests a basic chemical mechanism of control for the duration of tonic immobility. This is further borne out by the fact that if the animal is forced to recover in the midst of a period and is then reimmobilized (cf. Fig. 3, right) it remains immobile until that time when it would have recovered had the period not been interrupted. To account for

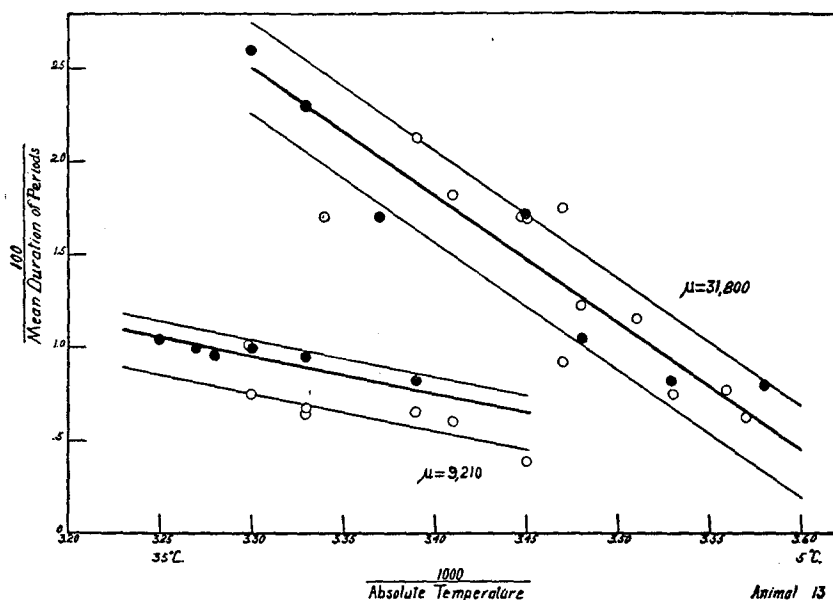


FIG. 7. This is perhaps the clearest illustration of the effect of temperature on the duration of tonic immobility of a single *Anolis*.

this last fact and for the suddenness and completeness of recovery the presence of a definite amount of substance inhibitory to the "higher centers" but allowing impulses from internuncial neurones at the "tonic centers" to pass to the muscles may be assumed. Accordingly the animal remains immobile until the decomposing substance, excreted perhaps from an endocrine organ in response to the immobilizing stimulus, reaches a definite threshold level when the animal recovers. This threshold is qualitatively suggested by inspection of the rhythmic kymograph records.

It was originally suggested, to account for the effect of temperature on the periods of immobility (Hoagland, 1927) that two independent autacoids might be excreted from endocrine organs in response to the immobilizing stimulus. One of these was regarded as active over a range of 5° to 35°C. and the others active from approximately 20° to 35°C. In the case of *Anolis* immobilization usually results after a struggle and, if this hypothesis is correct, one or both of the autacoids might be released as a result of the struggle. It was also originally

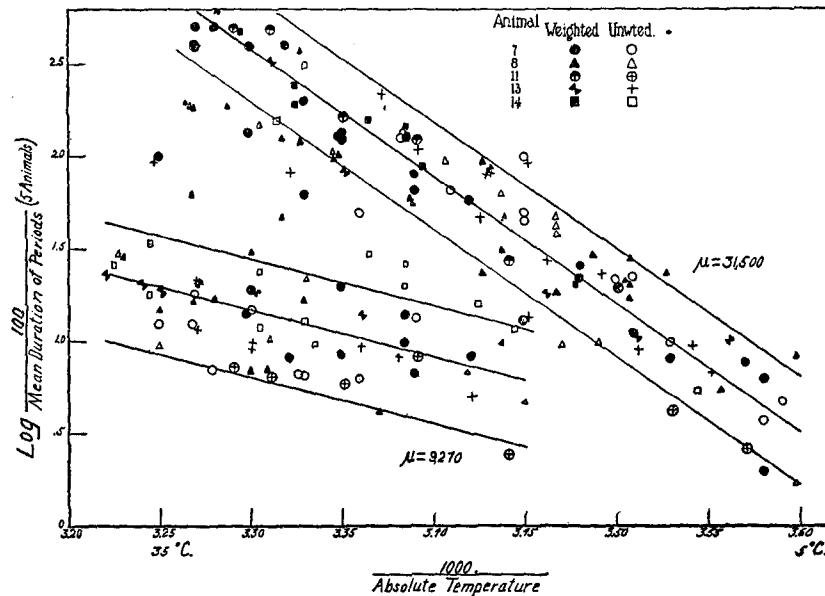


FIG. 8. Plot showing distribution of points for five animals according to the Arrhenius equation.

thought that the substances assumed,  $\alpha$  and  $\beta$ , were not themselves inhibitors but that they decomposed to Inhibitors *A* and *B* respectively, in this way giving the evidence of chemical control shown by the Arrhenius equation plots. This assumption, in the light of more recent experiments, seems unnecessary. It is more probable that the inhibitors are the initial substances,  $\alpha$  and  $\beta$ , which decompose to physiologically inactive substances, *A'* and *B'*.

To account for those records in which a progressive increase in the



durations of immobility are manifested (*cf.* Fig. 2) and which show the temperature effect characteristic of a physical change (*cf.* Figs. 9, 10), a diffusion process may be assumed controlling the amount of available catalyst in the decomposition of  $\alpha$  and  $\beta$ . This is consistent with the evidence of a logarithmic accumulation of the hypothetical inhibitory

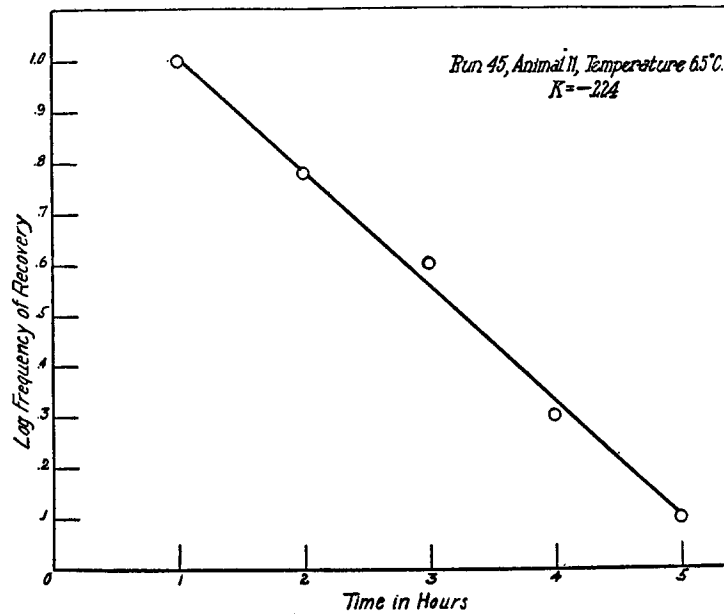


FIG. 9. Sample plot of the logarithm of the frequency of recovery of an animal per unit of time, arbitrarily chosen, against the total time from the beginning of the experiment. This is characteristic of those records in which there is an increase in the duration of succeeding immobility periods, *i.e.*, a decrease in frequency of recovery, as illustrated on the left of Fig. 3.

substance<sup>3</sup> as reflected in the logarithmic increase of the duration of immobilization. Recently, experiments have been made to ascertain

<sup>3</sup> It was observed in the course of the experiments that certain irregularities in the duration of tonic immobility periods served as an index of the approaching death of the animal. This was true for both *Phrynosoma* and *Anolis*, and it was also true for the isopods with which Crozier worked. It was found possible in several cases to predict the death of an apparently normal animal as much as 3 days in advance by irregularities in the rhythms. This fact suggests a possible relationship between the durations of immobility and metabolism.

the specific nature of the inhibitory "hormones" which might thus be involved. The injection of small amounts of adrenalin, in excess of a definite threshold amount, is found to prolong markedly the durations of tonic immobility in *Anolis*. The adrenalin is injected intraperitoneally (0.1 cc.), after diluting with Ringer's solution to concentrations varying with the experiments between 0.5:100,000 and 5.0:100,000. Fig. 11 shows typical samples of experiments demon-

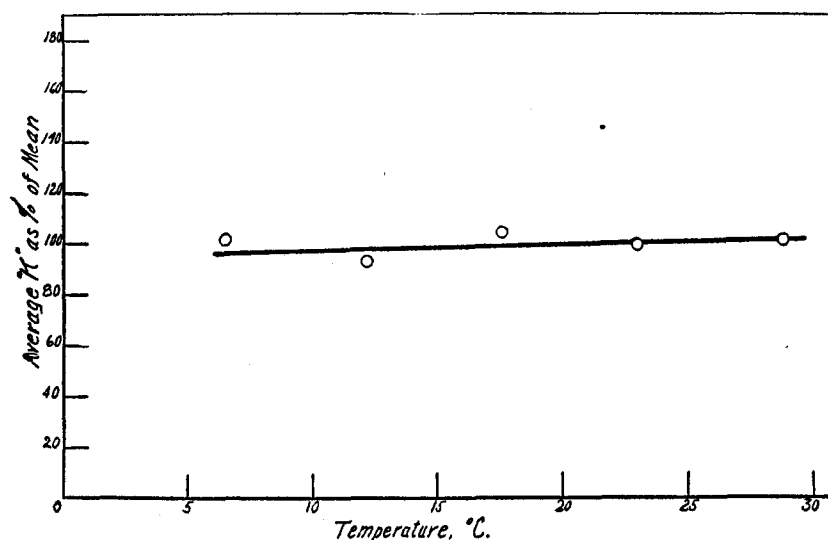
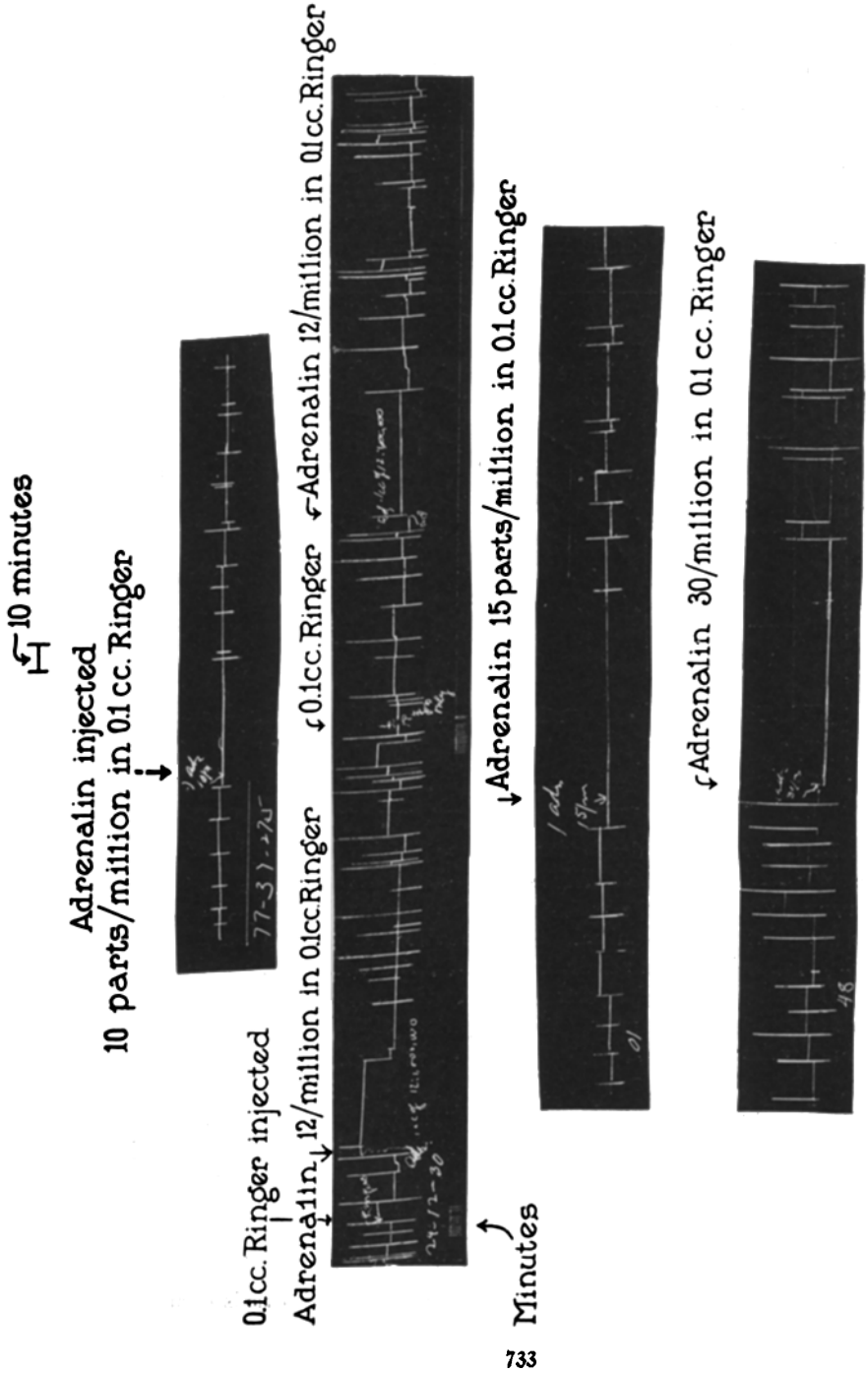


FIG. 10. This figure illustrates the effect of temperature on the frequency of recovery from tonic immobility in those cases in which the frequency decreases progressively with time from the beginning of the run. The constant  $K$  as ordinate is the slope of the lines measured from the frequency of recovery *vs.* time plots illustrated by Fig. 9. Each point of this figure represents an average of approximately five such slopes as that illustrated in Fig. 9.

strating the effect of adrenalin. Controls injected with 0.1 cc. of Ringer's solution show no prolongation of immobility. The injections were made with the animal in the recording mechanism at 27.5°C. after 1 hour from the beginning of the experiment. The animal was not removed from the holder or otherwise disturbed at the time of injection. Fig. 12 represents the *number of normal spontaneous recoveries missed* after adrenalin injection plotted as a function of the *amount of adrenalin injected*. The shaded circles are weighted aver-



27.5° C.

Fig. 11. Sample records from 80 experiments showing typical effects of adrenalin in Ringer's solution injected intraperitoneally in causing *Anolis* to miss normal recoveries. 0.1 cc. Ringer's solution without adrenalin does not prolong tonic immobility as may be seen in the second record.

ages of 80 experiments with many animals,<sup>4</sup> the unshaded circles are unweighted averages, and the crosses are points for one individual animal. Probable errors are indicated on the plot by lines through the points perpendicular to the abscissa. The curve may be a measure of the rate at which the adrenalin becomes inactive, since the number of periods missed is measured in time units; or it may de-

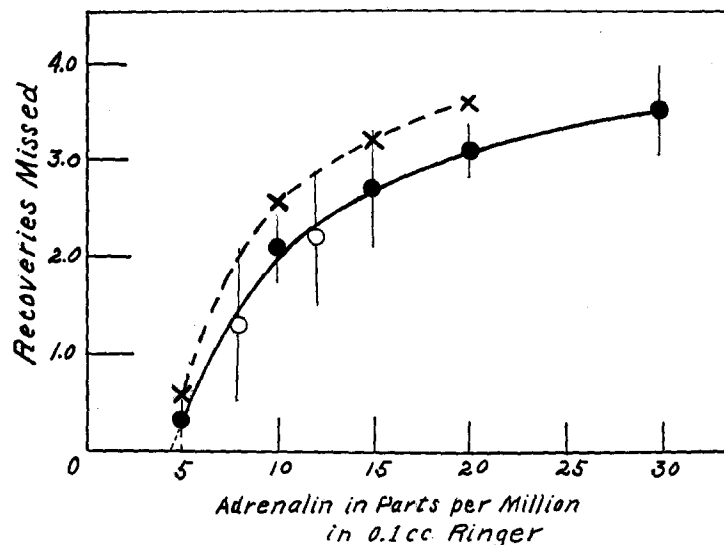


FIG. 12. Curve relating dose of adrenalin to number of normal spontaneous recoveries missed. The shaded circles are weighted averages of 80 experiments with many animals and the unshaded circles are unweighted averages. The crosses are points for one animal. The probable errors are indicated by lines through the average points perpendicular to the abscissa. A threshold for injected adrenalin is indicated by extrapolation. Controls injected with 0.1 cc. of Ringer's solution show no prolongation of periods of tonic immobility, *i.e.*, no normal recoveries are missed.

pend upon the diffusion of adrenalin from the body cavity. Moreover, the threshold previously suggested is found in the case of injected adrenalin by extrapolation of the curve to the abscissa. For the conditions of these experiments it is approximately four parts per million of adrenalin in 0.1 cc. of Ringer's solution. A lower and pos-

<sup>4</sup> Only those experiments in which definite rhythmicity of periods occur have been treated.

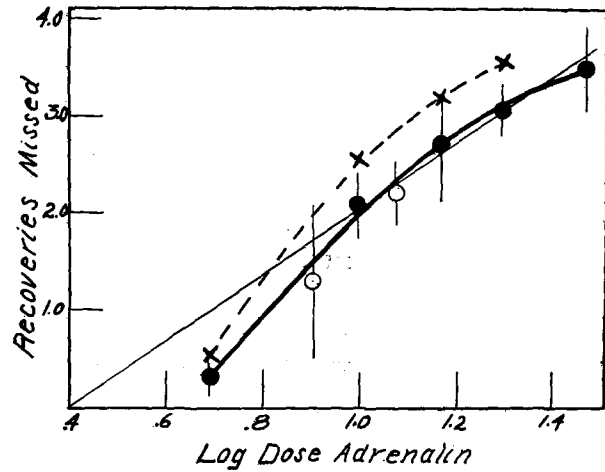


FIG. 13. Plot of logarithm of dose of adrenalin against the number of periods missed. The points are designated as in Fig. 12. The thin line cutting the abscissa at 0.4 corresponds to a threshold concentration of 2.5 parts per million of adrenalin in 0.1 cc. Ringer's solution. This line passes through the higher, more reliable points as described in the text.

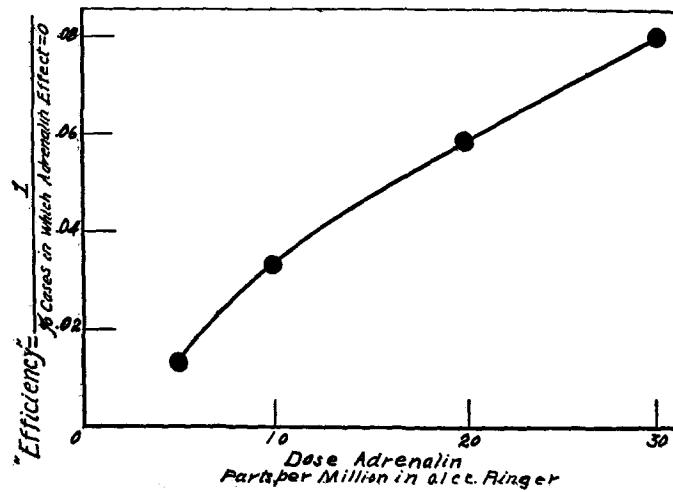


FIG. 14. Plot for principally weighted points of "efficiency" of adrenalin in prolonging tonic immobility against the dose of adrenalin in parts per million in 0.1 cc. Ringer's solution. The "efficiency" is measured by the reciprocal of the per cent of experiments at a given dosage in which the prolonging effect of adrenalin is zero.

sibly more significant apparent value for this threshold will be discussed presently.

Fig. 13 is a plot of the logarithm of the dose of adrenalin against the number of recoveries missed. The line is slightly curved but is closer to a straight line than any other simple curve which "makes sense" in terms of chemical dynamics. The departure from the logarithmic may readily be accounted for in terms of the rather large probable errors and probably has no theoretical significance.

It was found that sometimes adrenalin has no effect in prolonging tonic immobility. A given dose which, on the average, may cause the animal to miss several recovery periods, may sometimes have no effect at all, and at other times may have two- or threefold the average effect. The occurrence of the "zero" effects of adrenalin is more pronounced at low than at high concentrations. Fig. 14 shows the "efficiency" of adrenalin in prolonging tonic immobility plotted against the dose of adrenalin for the four principally weighted doses; the efficiency is here measured by the reciprocal of the per cent of experiments in which the effect of adrenalin is zero.<sup>5</sup> With the exception of the point at five parts per million the line is substantially straight indicating a direct proportionality between the amount of injected adrenalin and its efficiency. Since the lowest point on the curve seems to be an exception to this relationship, the results at higher concentrations may be regarded as more reliable. A line through these higher points (Fig. 13) cuts the abscissa at 0.4. The antilog of this is 2.5, which is probably a more significant threshold value (parts per million in 0.1 Ringer) for adrenalin than that obtained by the extrapolation of the curve of Fig. 12. This value corresponds to about 0.1 mg. adrenalin per kilo body weight.

The logarithmic function (Figs. 12 and 13) is what one would expect if adrenalin were decomposing according to a first order chemical process. The oxidation of adrenalin in the presence of excess oxygen

<sup>5</sup> The weighted point corresponding to the concentration of 15 parts per million is left out of Fig. 14. The per cent of cases in which the adrenalin effect is zero is abnormally high for this point. This abnormality may be explained by the fact that the data at this concentration were taken from two groups of animals of different ages and sizes at intervals of several months. The probable error for this point is also high in spite of many experiments. Because of this lack of homogeneity of material the point has been excluded.

would give such a function. The temperature analysis of tonic immobility, it will be remembered, yielded two values of the critical thermal increment,  $\mu$ , tentatively indicative of respiratory (oxidative) processes. It therefore seemed probable that if internally secreted adrenalin were one of the assumed autacoids which becomes inactivated through oxidation, that adrenalin first oxidized in the air and then injected into the animal should have no effect on tonic immobility. This was found to be the case. Adrenalin allowed to stand in the air until oxidized, as indicated by the solution turning pink, was found to have no effect whatever on tonic immobility.

Forced recovery in the midst of a period that has been lengthened by adrenalin injection shows the same result as is shown by forced recovery during a normal period (*cf.* Fig. 3). The animal if re-immobilized remains immobile for the remainder of the duration characteristic of the particular dose of adrenalin.

While it is possible that adrenalin, on the basis of this evidence, may be one of the hypothetical autacoids which prolongs tonic immobility when present above a threshold concentration, this is not certain. The injection of ergotamine, in the case of mammals, is known to paralyze the endings of the sympathetic nervous system. If it has a similar effect on lizards and if adrenalin acts peripherally in producing tonic immobility one would expect that tonic immobility would be abolished by this drug. Exactly the opposite effect results.<sup>6</sup> Since it is not certain how ergotamine affects the sympathetic nervous system of *Anolis*, this experiment is not in itself determinative. The fact that spinal reflexes may be elicited during tonic immobility is a strong argument that the effect is central rather than peripheral, since if the inhibiting autacoid acted peripherally one would expect it to block spinal reflexes. If, in this case, adrenalin acts *centrally*, any sympathetic paralysis due to ergotamine would not necessarily shorten or abolish tonic immobility. The prolonging effects of ergotamine

<sup>6</sup> Not only does ergotamine prolong tonic immobility but an excessively large injection of Ringer's solution (0.2 cc.) may also prolong it slightly. These injections along with that of adrenalin may be assumed to increase the blood pressure. Amyl nitrite vapor, on the other hand, which presumably reduces blood pressure, abolishes immobility in *Anolis*, causing the animal to struggle violently. Both ergotamine and amyl nitrite are highly toxic and little can be concluded regarding isolated pressor and depressor effects in these cases.

may be due to general toxic effects which have nothing to do with the specific relations of adrenalin and the sympathetic nervous system to the mechanism controlling tonic immobility.

While the effect of temperature on the durations of immobility, the continuance of the rhythm after the animal has been aroused and reimmobilized, and the adrenalin effects, all point to a hormone (possibly adrenalin) controlling tonic immobility, there are other facts to be considered that at first seem difficult to reconcile with this general picture. The onset of the condition is very sudden. It follows immediately, with birds and mammals, on merely turning them quickly dorsum downward. This is hard to explain in terms of a process *initiated* by a hormone. Moreover, it is noteworthy that the mere injection of adrenalin does not produce tonic immobility; it only prolongs the state if it is already existing.<sup>7</sup>

The "shocking" effect on the centers of reflex tonus in the bulb and cord of suddenly removing an animal from the substratum and overturning it, or by allowing it to struggle on its back with limbs in the air so that normal afferent stimuli from the feet and from the stretched muscles are abolished, must be considerable. At all times the muscles controlling body posture are in a state of tonus determined by nicely graded reflexes afferently controlled by the amount of stretch of the muscles and by the mechanisms of the internal ear (*cf.* Cobb, 1925; Magnùs, 1924). The sudden overturning of an animal must result in a decided unbalancing, so to speak, of the central machinery of reflex tonus. In a very brief interval the tensions of most of the large muscles are changed, which may be understood to result in a disorganized volley of afferent impulses sent to the internuncial neurones at the tonus centers. Since the animal's limbs are removed from the substratum there is no possibility of rapid readjustment by way of normal afferent stimuli and it seems not unlikely that such a condition might result in a promiscuous discharge of the tonus centers effecting an immediate "locking" of the entire musculature.

Such a violent discharge of a group of internuncial neurones might also be expected to influence the adrenal glands. Adrenalin might,

<sup>7</sup> In some experiments *Anolis* was injected with adrenalin but not immobilized until some minutes later. In these cases the duration of immobilization was also found to be increased.



therefore, be excreted in excessive amounts and, once in the circulation, act as we know that injected adrenalin does to prolong the state of tonic immobility. According to this hypothesis, the hormone in question does not in itself initiate tonic immobility but serves to maintain the predominance of those tonic impulses already passing and to prevent impulses from higher centers from gaining access to the final common paths to the muscles. In the case of arthropods a similar mechanism may apply. Arthropods are often readily immobilized by merely lifting them from the substratum thereby producing pronounced changes in the stretch of the limb muscles. While it is not likely that adrenalin is the autacoid involved in these cases there is good evidence, as Crozier has pointed out, to suppose that some inhibitory substance controls the durations of immobility.

In addition to the question of the *mechanism* of tonic immobility, the condition itself offers opportunity for the study of tonus in skeletal muscle in the intact organism. The immobile animal resembles in many respects a decerebrate preparation. Certain aspects of deep reflexes are conspicuous, and experiments are in progress to measure under these conditions the frequency of discharge of certain stretch reflexes of *Phrynosoma* as a function of the tension in the muscles.

#### SUMMARY.

1. The durations of successive periods of induced tonic immobility in the lizard *Anolis carolinensis* was examined as a function of temperature. An automatic recording method was employed and observations were made of 12,000 to 15,000 immobilizations with six animals over a temperature range of 5° to 35°C. during 5 months.

2. The durations of the immobile periods were found to vary rhythmically in most cases.

The reciprocal of the duration of the rhythm, *i.e.*, the rate of change of the process underlying the rhythms, when plotted as a function of temperature according to the Arrhenius equation show distributions of points in two straight line groups. One of these groups or bands of points extends throughout the entire temperature range with a temperature characteristic of approximately  $\mu = 31,000$  calories, and the other covers the range of 20° to 35°C. with  $\mu$  equal to approximately 9,000 calories.

3. The initial stimulus in a series of inductions of immobility appears to set off a mechanism which determines the duration of the state of quiescence. Succeeding forced recoveries seem to have no effect on the normal duration of the rhythm.

4. These results are interpreted by assuming the release, through reflex stimulation, of hormonal substances, one effective between 5° and 35°C. and the other effective between 20° and 35°C. These substances are assumed to act as selective inhibitors of impulses from so called "higher centers," allowing impulses from tonic centers to pass to the muscles.

5. In some experiments a progressive lengthening in successively induced periods of immobility was observed. The logarithm of the frequency of recovery when plotted against time in most of these cases (*i.e.*, except for a few in which irregularities occurred) gave a linear function of negative slope which was substantially unaffected by temperature. In these cases it is assumed that a diffusion process is controlling the amount of available *A* substance.

6. The results are similar to those obtained by Crozier with *Cylisiticus convexus*. The duration of tonic immobility seems to be maintained in both arthropod and vertebrate by the chemical activity of "hormonal" selective inhibitors. The details of the mechanisms differ, but there is basic similarity.

7. Injections of small amounts of adrenalin above a threshold value are found to prolong the durations of tonic immobility of *Anolis*, by an amount which is a logarithmic function of the "dose." It is possible that internally secreted adrenalin, above a threshold amount, may be involved in the maintenance of tonic immobility.

8. The *production* of tonic immobility reflexly is a problem distinct from that of the *duration* of immobility. It is suggested that the onset may be induced by "shock" to the centers of reflex tonus causing promiscuous discharge of these centers with accompanying inhibition of the higher centers. Such a condition may result when an animal is suddenly lifted from the substratum and overturned, or when, as in the case of *Anolis*, it struggles with dorsum down. This reaction of the "tonic centers" may at the same time lead to discharge of the adrenal glands by way of their spinal connections thus prolonging the state.

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