THE INTRINSIC RHYTHM OF THE TURTLE'S HEART STUDIED WITH A NEW TYPE OF CHRONOGRAPH, TOGETHER WITH THE EFFECTS OF SOME DRUGS AND HORMONES

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It is well known, since the time of Ringer, that an excised heart, if properly supplied with oxygen in a physiological salt solution, will beat for several days, in fact until the tissue begins to disintegrate. Snyder[1] in connection with his studies on the effect of temperature on the heart beat of the Pacific terrapin (*Chemmys marmorata*) counted rates at intervals over periods of 11 hours and observed considerable changes in rate under fairly constant conditions. At 25°±1°C. the heart of the terrapin did not beat longer than 6 hours, due probably to the fact that this species is adapted to cold water.

The question arises as to the constancy with which a heart can beat over a period of many hours or several days, if all external factors affecting rate are kept quite uniform. For this type of investigation some sort of *continuous automatic* recording mechanism is a necessity. The Loomis chronograph, a preliminary description of which has already been published,[2] is specially designed to record rates of periodically recurring processes.

Anything which will actuate a relay will run the chronograph whose mechanism is essentially as follows:

The time for ten pulsations to occur is recorded by the length of a line of ink drawn by a moving pen on a revolving sheet of paper. During the next four pulsa-

[1] Snyder, C. D., *Univ. Calif. Pub. Physiol.*, 1905, **2**, 125.

[2] Loomis, A. L., and Harvey, E. N., *Science*, 1929, **70**, 559.

tions the pen is returned to its zero position and is then ready to record the average time for ten more pulsations. Each line therefore represents fourteen pulsations and tells the average time for ten pulsations to occur. The machine is driven by a synchronous motor running on the A.C. lighting circuit. By reducing gears and pulleys the paper is revolved at the proper speed and the pen, attached to a pulley string, is drawn across at a constant rate of ten units per second. The string pulley which draws the pen is actuated by an electro-magnetic clutch in connection with a fourteen toothed wheel and an escapement, also worked magnetically. Each pulsation to be recorded is made to open an electric circuit that magnetizes the electro-magnet moving the toothed wheel one tooth. While the wheel revolves ten teeth the magnet clutch moves the pen at constant speed across the paper, and during the next four teeth the magnetic clutch is out and a spring pulls the pen back to the first position. If ten pulsations occur in 10 seconds the unit lines on the paper (ten to a second) represent a 1 per cent difference in rate and the paper can be calibrated by recording pulses of current at a rate of one per second. By mere inspection of the paper record one can observe any change in rate and very quickly calculate the percentage constancy of the rhythmic process.

Later models of the chronograph have been modified by (1) introducing an easily changeable gear box for driving the chronograph at different speeds; (2) returning the pen to its 0 position by revolving pulley wheel instead of by a spring, thereby shortening the recovery to two beats instead of four; (3) replacing the toothed wheel and escapement with an improved type of rachet mechanism that cannot miss a beat; (4) placing a small electro-magnet in connection with the moving pen so that it is lifted off the paper with each beat, recording not only the average time for ten beats, but the interval between beats as well; (5) introducing a device for recording the time between single pulses or series of pulses without missing the time interval involved in the return of the pen to its zero position.

A complete description of the mechanical details of the chronograph will be published elsewhere.

In its new form the chronograph will record rates of 300 per minute to 1 beat in 6 minutes with absolute fidelity, giving a record which shows at a glance the change of rate without counting each individual beat. Figs. 2 and 3 give an idea of the records that can be made. The swing of a pendulum, manual beating of time, pulsation of a tambour, the fall of drops from a tube, the flashing of neon lamps, or the speed of rotation of any device can all be recorded by the proper electrical contact or relay mechanism. The growth of plants can also be recorded. It is only necessary to arrange a contact device of such a nature that, when the growing tip of the plant makes contact with a surface, the surface is automatically lifted a definite distance. A certain time interval will elapse before the plant grows sufficiently to make another contact. By knowing distance and time interval, which the chronograph records, we can determine (and record) the rate of growth. A continuous record of changes in light intensity can also be obtained by a very simple accessory mechanism.

The heart offers the best known case of a biological rhythm whose rate change is of fundamental importance. Action currents of the human heart or of animals picked up by the cardiotachometer of Boas[3] or by any properly designed amplifying device make beautiful records. For recording the fundamental heart rhythm we wished to avoid cardioaccelerator and cardio-inhibitory effects, and the following procedure, illustrated in Fig. 1, was followed.

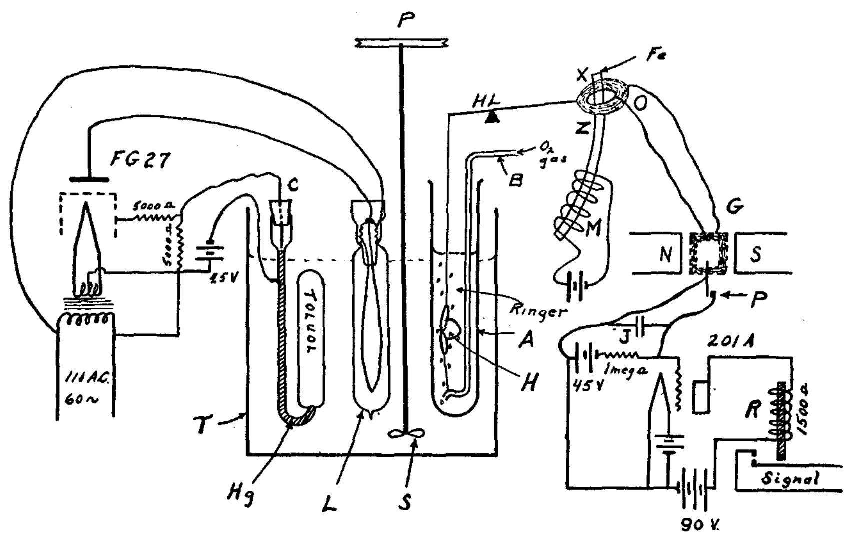

FIG. 1. Diagram of heart in thermostat showing wiring connections to chronograph. Explanation in text.

The heart of a turtle [either the painted (*Chrysemys picta*) or the slider (*Pseudyms rubriventris*) were used, and showed no characteristic differences] was removed, taking no special care to keep sinus intact, and mounted in a tube (*A*) of Ringer's solution[4] by tying threads to the tip of each auricle, one thread being wound around the end of an L-shaped glass tube (*B*) through which oxygen was bubbled into the Ringer, the other attached to the short arm of a heart lever (*HL*). It would seem that the easiest method of making an electrical contact would be through the long arm of the heart lever dipping into a pool of mercury. However, as is well known,

[3] Boas, E. P., *Arch. Int. Med.*, 1928, **41**, 403; Boas, E. P., and Weiss, *J. A. M. A.*, 1929, **92**, 2162.

[4] The Ringer's solution contained NaCl, 0.65 per cent; KCl, 0.014 per cent; $CaCl_2$, 0.014 per cent; $NaHCO_3$, 0.02 per cent; and sometimes glucose, 0.2 per cent.

the turtle's auricle contains smooth muscle fibers whose periodic contraction causes tone changes in the auricles which are often *greater than the heart muscle contractions*, and are, of course, indicated by a change in position of the heart lever. This means that at times the lever arm will never leave the pool of mercury, and at other times the heart will never relax enough for the lever to touch the mercury surface, consequently spoiling the record and involving continual adjustment. To obviate this difficulty, a coil of fine wire (O) was attached to the end of the heart lever which moved the coil over the end of an electro-magnet (M), thus cutting the field of force and inducing a small current in O, sufficient to actuate a galvanometer relay (G), the Weston No. 30 model. By this device we always induce a small current in O, whether tone changes cause the coil to be moving in position X or in position Z. Recording has always been perfect after the adoption of this device.

The contact made by the galvanometer relay actuates another vacuum tube (201A) relay, shown in Fig. 1, which is built into the chronograph. The advantage of the vacuum tube relay, which controls the flow of plate current by charging the potential of the grid of a vacuum tube, lies in the fact that no current need flow across the contact points. Only a change of potential occurs and slight oxidation of these contact points does not introduce enough resistance[5] to affect the working of the relay (R), a high resistance (1500Ω) type. The magnet is actuated by plate current whenever a negative voltage of 45 volts is removed from the grid of the tube (201A), by short circuiting the 45 volts through a megohm resistance. If the galvanometer relay contacts are very short in duration the plate current can be made to flow long enough to actuate relay R by introducing a condenser (J) of 0.1 to 1 mf. capacity in the position shown in Fig. 1.

The same principle is adopted in the control of the thermostat (T) with the mercury-toluol regulator, the heating lamp (L) and stirrer S, with the pulley drive, P, except that a vacuum tube is used, a thyratron[6] (FG27) whose plate circuit is supplied by the 110 A.C. lighting mains with which the heating lamp is in series. The filament circuit is supplied by a 110 to 5 volt transformer and a negative grid voltage of $4\frac{1}{2}$ volts is applied whenever the mercury rises in the regulator and makes contact with the contact wire, C. This cuts off the plate flow and puts out the heating lamp which will light again as soon as the contact is broken and $4\frac{1}{2}$ volts negative removed from the grid. The outfit is an ideal regulating device for thermostats.

The temperature control of our hearts was 25°C. +0.02°C.; oxygen[7] was continually bubbled through at a constant rate; and the Ringer's solution was buffered so that constant pH was attained. As the heart was excised, cardio-motor reflex disturbances were eliminated. Everything worked automatically so that no

[5] The contact can be made through the body of an individual.

[6] We are deeply indebted to the General Electric Company for this thyratron.

[7] In some experiments air was used instead of oxygen, but no difference in the rhythmic behavior of the heart was noted.

mechanical disturbances accompanying adjustment could have had an influence on the rate recorded, which we believe represents the fundamental intrinsic rhythm of some periodic process within the heart muscle cells. Twenty-five hearts in all have been studied at 25°C., some of them recorded for as long as 36 hours.

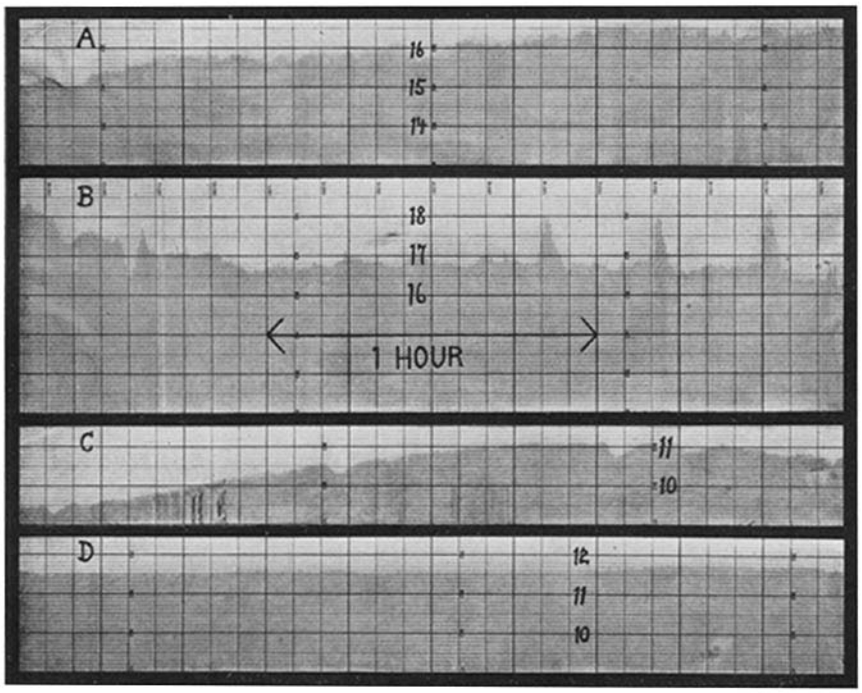

FIG. 2. Portions ($2\frac{1}{2}$ hours) of records of rate of turtle's heart in Ringer's solution without glucose under constant conditions at 25° ±0.02°C. *A*. Sept. 23, 1929, 4 hours after start, showing considerable rhythmic changes in rate. *B*. Same heart after 8 hours, showing marked rhythmic decreases in rate exactly 20 minutes apart. *C*. Sept. 22, 1929, 40 minutes after start, showing rhythmic increases in rate exactly 50 minutes apart. *D*. Same heart, 4 hours later, showing remarkably constant rate. Ordinates, seconds for ten beats. Abscissae, 15 minute periods. Each vertical line represents fourteen beats of the heart and records the average time for ten beats to occur. The longer the ordinate line the slower the heart.

There are several important observations which have come from these records. (1) A considerable variation in the behavior of hearts from different turtles (Fig. 4). The rate is by no means constant over a long time period. Neither does the rate continually decrease, although the general trend is in that direction. Usually the rate decreases during the first hour or two, but the rate may be the same at the

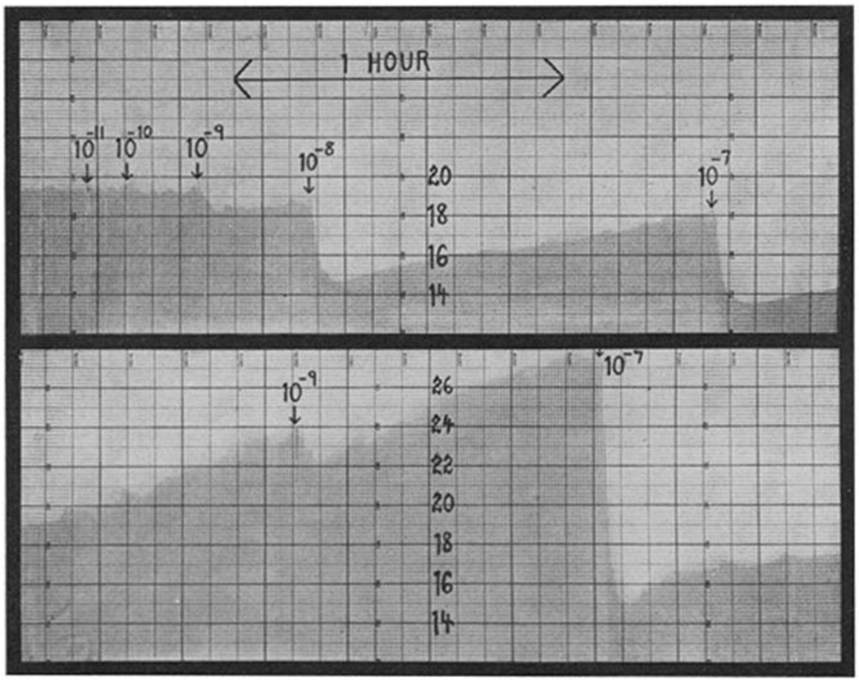

FIG. 3. Portions of a 32 hour record of the rate of a turtle's heart in Ringer's solution + 0.2 per cent glucose under constant conditions at 25°±0.02°C. Above—Fairly constant rate with slight rhythmic changes 3 hours after start, showing effect of adding increasing concentrations of adrenalin. Note first effect in increasing rate occurs with 10^{-9} gm. adrenalin per cubic centimeter and marked effect with 10^{-8} adrenalin. Below—Same heart with adrenalin effect gradually wearing off 90 minutes after end of *A* portion. Note again detectable increase in rate from adrenalin in 10^{-9} gm. per cubic centimeter and very marked effect of 10^{-7} adrenalin on a slowly beating heart. Abscissae, 15 minute periods. Ordinates, seconds for ten beats. The longer the ordinate line the slower the heart.

end of 12 hours, having made some fluctuations in the meantime. This initial decrease in rate may be connected with the seeping of adrenalin out of the tissue or the oxidation of adrenalin in the tissue. The amplitude of contraction always decreases and is very weak when the heart is about ready to stop. Often beats will be omitted under these conditions, that is, there will be two beats and one omitted, or three beats and one omitted, etc. The greatest variation in rate noted was about 33 per cent in a 5 hour period and 60 per cent in a 12 hour period, and

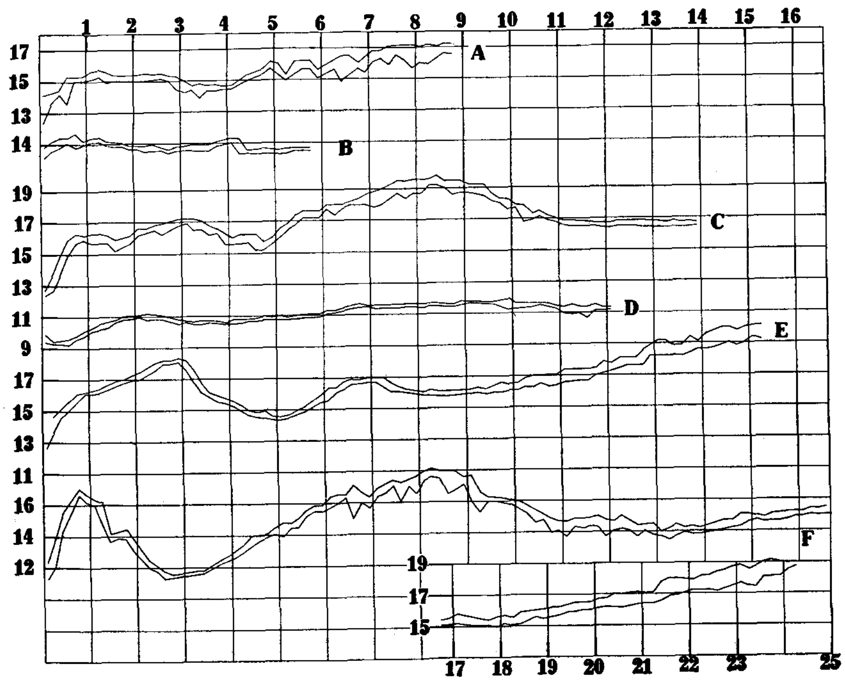

FIG. 4. Plots of rates of six hearts (*A* to *F*) Ordinates, seconds for ten beats. Abscissae, hours after heart removed and mounted in Ringer's solution at 25° ±0.02°C. All conditions constant, no drugs or hormones added. *A*. June 16, 1929. *B*. June 14, 1929. *C*. June 17, 1929. *D*. Sept. 22, 1929. *E*. Oct. 7, 1929. *F*. Oct. 6, 1929. Abscissae for latter part of this record at bottom of chart. The curves were drawn by plotting the slowest rate (upper line) and the most rapid rate (lower line) for each 10 minute period.

the least variation was 2 per cent in a 5 hour period and 1 per cent over half hour periods (Fig. 2, *D*). This is quite remarkable regularity as it means that a heart will beat 100 times with a change of only one beat.

(2) The occurrence of rhythms in rate of beat which often recur at

rather definite intervals, that is periodic changes in the rate. A heart may show a 6 per cent decrease in rate lasting 3 minutes every 20 minutes for four 20 minute periods (Fig. 2, *B*), or similar 3 to 4 per cent increases in rate lasting a few minutes for three periods 50 minutes apart (Fig. 2, *C*). Such rhythmic changes in rate are not connected with the tone changes often observed in the auricles, whose

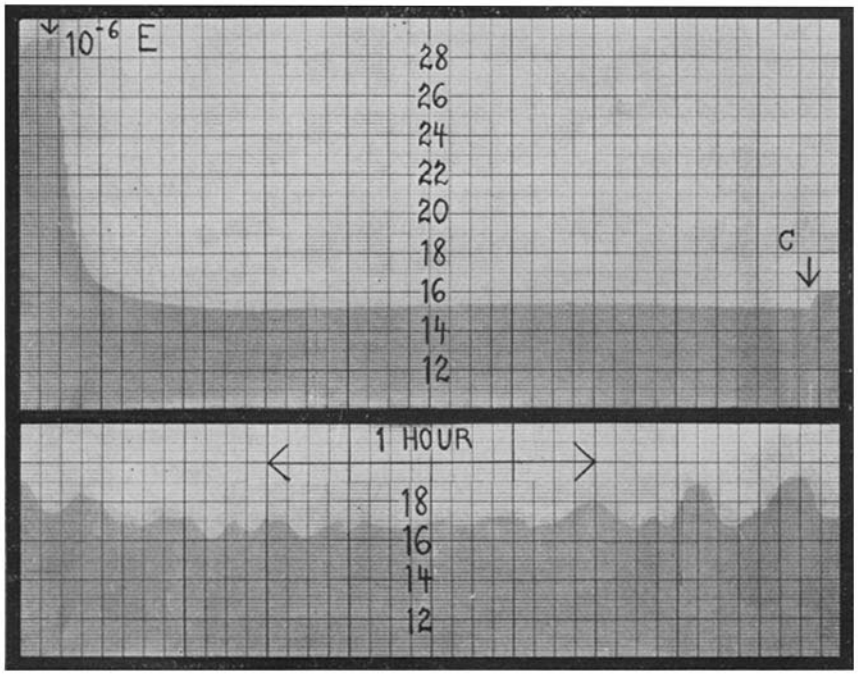

FIG. 5. Portions ($2\frac{1}{2}$ hours) of records of rate of turtle's heart showing: Above—effect of epehedrin ($1:10^6$) at *E* and effect of chloretone (1:20000) at *C*. Note remarkable regularity after ephedrin. Below—normal heart showing marked rhythmic changes in rate. Ordinates, seconds for ten beats. Abscissae, 15 minute periods. The longer the ordinate line the slower the heart.

period is 40 to 50 seconds, nor can they be traced to any change in the environment. They are true variations of the fundamental rhythm. Most frequently the maximum of these rhythms occur at intervals of 5, 6, or 10 minutes, but the period of such superposed rhythms is continually changing (Fig. 5).

These rhythmic changes are shown by practically all the hearts. Thinking that they might be connected with spontaneous activity of the postganglionic inhibitory nerve cells in the heart we tried adding atropin in concentrations of $1:10^6$, $1:10^5$, and $1:10^4$ to the Ringer's solution. These concentrations should paralyze the inhibitory nerve endings, but our records show no effect on the rhythmic changes in rate. The high concentration caused a progressive slowing of the rate.

Adrenalin or ephedrin added to the Ringer's does abolish the rhythmic rate changes, at the same time causing the well known increase in rate. One part of adrenalin in 10^9 parts of Ringer's causes a perceptible increase in rate (about 8 per cent) and $1:10^8$ adrenalin causes a 21 per cent increase followed by a very regular and progressive slowing presumably due to slow oxidation of the adrenalin (Fig. 3). During this progressive slowing the heart beats with remarkable regularity, that is, no indication of periodic changes in rate. The percentage increase in rate after adding adrenalin is greater if the heart is beating slowly when the adrenalin is added than it is when the heart is beating more rapidly. These effects are illustrated in Fig. 3. To keep a heart in good rhythmic condition over long periods of time our observations suggest that a slight concentration of adrenalin should be present in the medium bathing the hearts.

As is well known ephedrin has a physiological effect which is similar to adrenalin. Our records show that $1:10^6$ ephedrin added to Ringer's solution may double the rate of a slowly beating heart (10 beats in 30 seconds). The increase in rate takes 25 minutes for completion and the effect lasts for hours. Fig. 5 shows an ephedrin effect on a slowly beating heart. Note the difference in the form of the effect as compared with adrenalin. For greatest constancy in rate, a little ephedrin should be added to the Ringer's solution.

(3) Another characteristic of some hearts is a *sudden* change of rate. Both sudden increases in rate (15 per cent) and sudden decreases in rate (13 per cent) have been observed. They presumably indicate a sudden dropping out of one pace-making region or the sudden appearance of another. We cannot connect them with any changes in the environment.

α and β pituitary hormone[8] had no effect on the rate when freed of the chloretone which is added to preserve these solutions. The chloretone slows the rate slightly as shown in Fig. 5. The β hormone (pitressin) contained 10 pressor units per cubic centimeter and the α hormone (pitocin) contained 10 oxytocic units per cubic centimeter. 2 cc. of each one was added to 200 cc. Ringer's solution in testing. Insulin (Eli Lilly Company), 20 units per cubic centimeter also had no effect on the rate when 2 cc. were added to 200 cc. Ringer's solution.

Our results with thyroxin[8] were somewhat variable. In six experiments using $1:10^5$ thyroxin dissolved in a minimal amount of alkali, the increase in rate ranged from 0 to 26 per cent. The increased rate appears fairly quickly, but not as rapidly as with adrenalin, and is prolonged. It is possible that increased alkalinity of the Ringer's had some influence on the heart, as this factor was not sufficiently controlled.

Nicotin ($1:10^4$) caused a progressive slowing, as did ergotamine tartrate ($1:10^5$). Adrenalin administered after ergotamine showed the well known slowing of rate instead of an increase. Our records show that this chronograph is admirably adapted for studying the effects of drugs.

In conclusion we wish to express our thanks to Mr. Charles Butt, Technical Assistant in Physiology, Princeton University, for help in taking many of the records.

SUMMARY

A chronograph is described for recording continuously the rates of many different kinds of rhythmic processes over long time periods. The rate is read directly from the length of a line of ink, drawn by a moving pen.

Rates of beat of excised turtle's hearts in Ringer's solution have been recorded at 25°C. under constant conditions of temperature, pH,

[8] We are deeply indebted to Parke Davis and Company for a supply of α and β pituitary hormones, and to E. R. Squibb and Sons for 100 mg. of crystalline thyroxine.

and oxygen supply for periods of 36 hours. Regular periodic variations in the fundamental rhythm are figured, as well as rates of extraordinary constancy. The effects of adrenalin, ephedrin, thyroxin, α and β pituitary hormone insulin, nicotin, and atropin are described in the text.