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PHOTOGRAPHIC SCANNING AND ITS APPLICATION IN THE GENERAL PHYSIOLOGY OF MOTILE CELLS

by

Marion Lee Ferguson

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1959

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PREFACE

There can be no serious question about the indispensability of new instruments for scientific acvance; many current discoveries would have been quite impossible without use of the specially designed instruments made possible by modern technology, instruments that could not have been devised at an earlier stage in history. Improved instruments will surely be no less important for research progress in the future.

Graham DuShane, Editor
Science, 124:771
26 October 1956

I. INTRODUCTION

In paramecia and other motile microorganisms, a considerable portion of the energy liberated by cellular metabolism is regularly dissipated in the form of locomotor activity (Wingo and Browning, 1951). Indeed, under certain conditions, rate of movement is a sensitive indicator of physiological state. Measurements of locomotor velocity, therefore, may be considered quantitative expressions of the reactions of living systems to the physical and chemical influences of the environment. Such data have obvious utility to the physiologist. Their collection, however, has been rendered difficult by technical problems which remain unsolved even today.

Various techniques have been developed over the years for determining the rate of locomotion of microorganisms. In general, they are of three types. These will be discussed categorically as direct methods, photographic methods and electronic methods.

Direct methods are those in which an observer measures with a stop watch, clock, metronome or other timing device, the time required for a cell to traverse some known distance in the field of view. The distance traveled is ascertained

by reference to marks inscribed on the tube or slide containing the cells, by a millimeter scale placed alongside the trough in which the cells are moving, by a calibrated ocular micrometer scale whose image is superimposed on a microscopic field, or by other devices of similar nature. Swimming movements of the organisms may be restricted to paths more or less paralleling the axis of the distance-measuring scale by passing a weak, direct electric current through the suspending fluid, or, taking advantage of the negative geotactic properties of some forms, by introducing them at the bottom of a vertically-oriented container and observing them as they swim to the top. Temperature control is effected by placing the observation vessel in an appropriate environment, usually a thermostatically-controlled water bath, or by providing the vessel itself with channels through which water of the desired temperature may be circulated (Lee and Klain, 1945).

The stopwatch-micrometer method has serious shortcomings (Ferguson, 1957, pp. 208-209), but it has been employed with reasonable success by a number of workers, including Nagai (1907) in a study of the effects of narcotics and salts on the paramecium; Löhner and Markovits (1922) in a study of the oligodynamic effects of metal ions on the paramecium; Glaser (1924) in a study of the effects of temperature on the paramecium; Kamada (1928-31) in a study of the effects

of direct electric current on the paramecium; Chase and Glaser (1930) in a study of the effects of pH changes on the paramecium; Mills (1931) in a study of the effects of pH changes on Colpidium; Moeller and VanDemark (1955) and Baker, Cragle, Salisbury and VanDemark (1957) in studies of the motility of bovine spermatozoa; and many others.

The photographic method, as the designation implies, is an indirect observation technique in which the movements of cells are recorded on a photographic emulsion. Following development of the emulsion appropriate measurements of velocity are made. Two general types of photographic recording technique have been used. In the time exposure method a single photographic emulsion, or film frame, is exposed with intermittent or continuous illumination for a known period of time, during which the displacements of the cells in the photographic field are recorded on the emulsion as linear or sinusoidal traces. In the cinematographic method, a series of instantaneous exposures is made at some known constant rate by means of a motion picture camera, which, on each exposure, records the positions of the moving cells as points. By following the displacements of the points, frame-by-frame, as the developed film is projected, the rates of locomotion of the cells represented by the points can be determined. The principal advantages offered by photographic methods are scope, scanning speed and objectivity. Instead

of selecting and watching one cell at a time, as the human observer must, the emulsion of the photographic scanner watches and impartially records the movements of many or all of the cells in an experimental population simultaneously. By being able to furnish statistical information on the responses of entire populations as well as on all of the individuals in those populations, the photographic method is superior to any direct observation method.

The first use of photography in determining the rate of locomotion of microorganisms appears to have been that of Comandon (1917, 1919), who employed a time-lapse motion picture technique (one frame every six seconds) to study the chemotactic movements of phagocytes in parasitized blood. More recently, Schlenk and Kahmann (1939) and Rikmenspoel (1957) have used standard-speed cinematographic methods in studies of the locomotion of the sperm cells of the trout and bull, respectively.

The measurement of locomotor velocity from motion picture records is a tedious process, necessitating, in effect, a reprojection of the entire film record for each cell studied. The single-frame time exposure technique makes it possible to avoid this difficulty. Wense (1935), in a study of the effects of various neuropharmacological agents on the paramecium, was apparently the first to make use of this type of photography. In his photographs, made with one-

second time exposures and conventional photomicrographic illumination, the traces generated by paramecia appear as dark, wavy lines against a brightly lighted microscopic field. Wense, however, neither measured the tracks nor attempted to estimate the locomotor velocities of the cells generating them. He was interested only in qualitative differences in response and evidently failed completely to recognize the potentialities of his method. With light field illumination all parts of the photographic emulsion darken steadily as the exposure progresses. This tends to erase or obliterate the track images, since, under this type of illumination, they are merely shadows. The effect is somewhat like that produced by the evaporation of the conspicuous but ephemeral condensation trail of an aircraft. Subsequent workers have avoided this problem by employing dark field illumination. Under these conditions, no parts of the sensitive emulsion are affected by the exposure except those traversed by the point images of the brilliantly lighted cells. Consequently, lengthy time exposures can be made without obliterating tracks. The dark field photomicrographic method has been employed with varying degrees of success by several workers. Rothschild and Swann (1948) and Rothschild (1956) used it more or less incidentally in studies of the locomotion of sea-urchin spermatozoa. Wingo and Browning (1951), introduced a rotat sector disk into the dark field illumination

system to obtain crude, but usable, stroboscopic records from which the swimming speed of Tetrahymena was determined. Harris (1953), using continuous dark field illumination, made time exposures ranging in duration from five to fifteen minutes, from which records he calculated the rate of locomotion of granulocytes in blood clots. The effect of X-irradiation on the swimming velocity of ciliates was studied by Lengerová (1955) with the aid of dark field technique and one-second time exposures. A new type of recording system, employing macro-photographic apparatus and dark field illumination achieved through the use of polarizing filters, was described by Ferguson (1955, 1957) in connection with a study of the effects of temperature, pH, osmotic pressure and other influences on the rate of locomotion of paramecia. An improved version of this method and some of the results obtained with it are discussed in the following sections of this thesis.

Gebauer (1930) used dark field time-exposure photomicrography to study the galvanotactic behavior of Volvox, and Brokaw (1957, 1958) employed similar technique in investigations of the chemotactic and galvanotactic responses of bracken spermatozoids. However, inasmuch as locomotor velocity measurements were neither made nor sought in these studies, they will not be discussed further.

Electronic methods for determining the rate of locomotion of cells have great promise, but they are still very

much in the early experimental stages of development. In systems described by Rikmenspoel (1957) and by VanDemark, Salisbury and Moeller (1958), a light-sensitive electronic device (photoelectric cell or photomultiplier tube) is employed as the scanning element. The system is designed in such manner that the movements of cells past an orifice or through an observation volume result in variations in the amount of light reaching the scanning element, resulting in variations in the magnitude or frequency of its output. The electrical signals from this device can be displayed as curves or spikes on the screen of a cathode ray tube, which can be measured directly or photographically recorded, or processed in other ways to yield desired information on the mean rate of locomotion of the cells, the distribution of velocity in the population, and other statistics. Though costly and complex, electronic scanning systems of one kind or another are unquestionably destined to become important instruments of research in many biological laboratories.

In the following pages, the author reports on the further development and applications of a photographic method which, it is hoped, will prove amenable to electronic automation. As a technique for determining the rate of locomotion of microorganisms, the method is definitely superior to others of its kind which have been reported in the literature to date. It is a simple, versatile method of high pre-

cision, accuracy and resolving power. Unfortunately, however, it is an inefficient method. While the photographic emulsion sees and faithfully records the movements of tens, hundreds or even thousands of cells simultaneously, each track so recorded must be laboriously measured, one by one. Human hands and human eyes constitute, as in all the earlier methods, a bottleneck. Fortunately, however, this particular bottleneck seems susceptible to attack. The solution visualized by the author is a combination scanning system in which an electronic device automatically counts and measures the tracks recorded on a film record, analyzes the data, and prints out or oscilloscopically displays the results. Details of this proposal will be discussed later.

The research reported in the following pages was conducted with the above ideas in mind. Its purpose was twofold: (1) to assess the applicability of the method to a wide variety of physiological problems; and (2) to obtain certain types of information on the locomotor velocity response which the author considers indispensable to continued development and refinement of the technique.

II. APPARATUS

Components of the photographic scanner and their positional relationships are shown in Figure 1. The basic elements of this apparatus, described in earlier publications (Ferguson, 1955, 1957), are (a) an observation chamber, (b) a temperature regulating tank, (c) a recording camera, and (d) lamps for illuminating the photographic field. Recent additions to this setup, not previously described, include (a) an automatic control unit (not shown in Figure 1) and (b) an optical chronometer.

The observation chamber (Figures 2 and 3; see also, Ferguson, 1957, p. 213, for details of construction) is a vessel in which the cells are photographed. It is hung by its suspension arms in the water of the temperature regulating tank so that its front face, bearing two vertical rulings separated by a distance of 30mm, is visible to the recording camera through the rectangular window of the tank.

The photographic field, intensely illuminated by 100-watt Leitz microscope lamps, is caused to appear dark to the camera by rotating the polarizing filters in front of the lamps (Figure 5). Cells in the chamber, however, stand out under these conditions as brilliant points of light.

The central portion of the photographic field is occupied by the space image of a chronometer. This instrument, a World War II Air Force hack watch with black face and white numerals and hands, is mounted face downward at the top of a reflex optical system (Figure 4) and is illuminated from below by two small electric lamps. Light rays from the face of the watch are directed into the projecting lens of the system (Figure 5) by a 45° plane mirror (not shown). The projecting lens forms an image of the watch face in the central plane of the observation chamber (Figure 6). The brightness of the image is balanced against that of the field by rotating a polarizing filter mounted inside the baffle plate attached to the back of the temperature regulating tank (Figure 1). With this arrangement, the watch face and the movements of the cells in the chamber are simultaneously registered in each scanning photograph. The records are thus marked as to sequence, time, and approximate duration of exposure.

An automatic control unit was incorporated in the recording setup to regulate certain operations incidental to scanning and to insure uniform reproduction of the exposure interval. The timer of this unit (Figures 8 and 10), consisting of a synchronous electric motor, camshaft, cams, roller-type microswitches and relays (Figure 9), is based on a device described by Jones and Fields (1954). A circuit

diagram of the control system is presented in Figure 11, and the events of the control cycle are explained and summarized in Figure 10 and Table 1, respectively. As indicated in Table 2, the four-second exposure interval, on which the accuracy of the velocity determinations depends, is reproduced with considerable precision.

An Argus 35mm slide projector with a special film strip carrier (Figure 7) was used in the examination of the photographic records. The construction of the carrier is briefly described in the figure caption.

Figure 1. Photographic recording apparatus. Components include, from left to right, (a) reflex optical system for projecting image of watch face into observation chamber; (b) field illuminators (paired 100-watt Leitz microscope lamps) with adjustable Polaroid attachments; (c) light baffle; (d) adjustable Polaroid attachment for varying brightness of watch face image while maintaining constant aperture in lens of projecting system; (e) temperature regulating tank and observation chamber (suspension arms of which are visible above the rectangular front window of the tank); (f) a camera unit, consisting of Polaroid attachment, Leitz bellows focusing device, containing Elmar 50 mm f3.5 lens, and Focaslide with 5X wide field focusing magnifier (Leica IIIf camera box, not shown here, is mounted on the Focaslide when recording cell movements); and (g) an optical bench, on which all of the components are mounted and held in proper positional relationship.

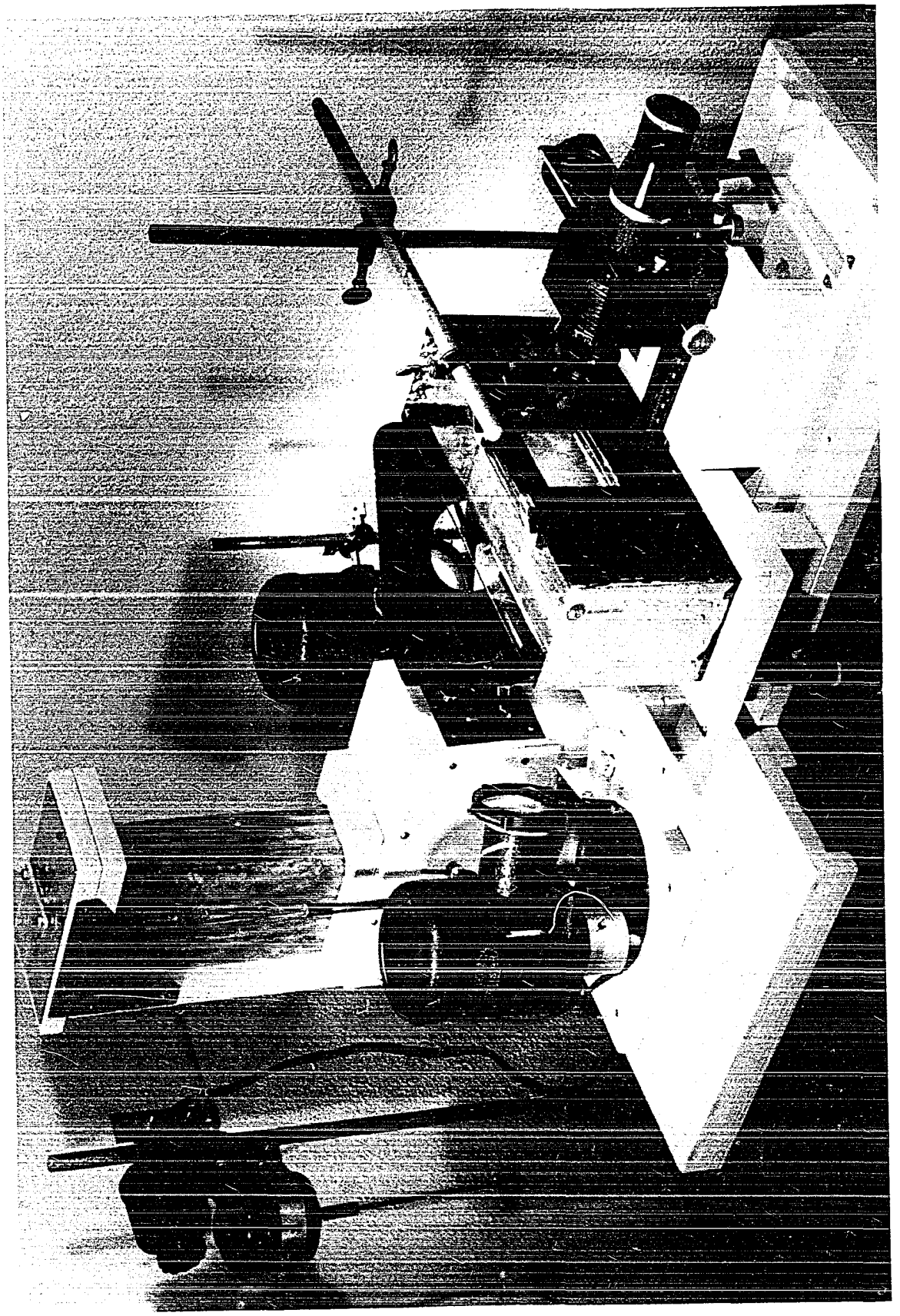
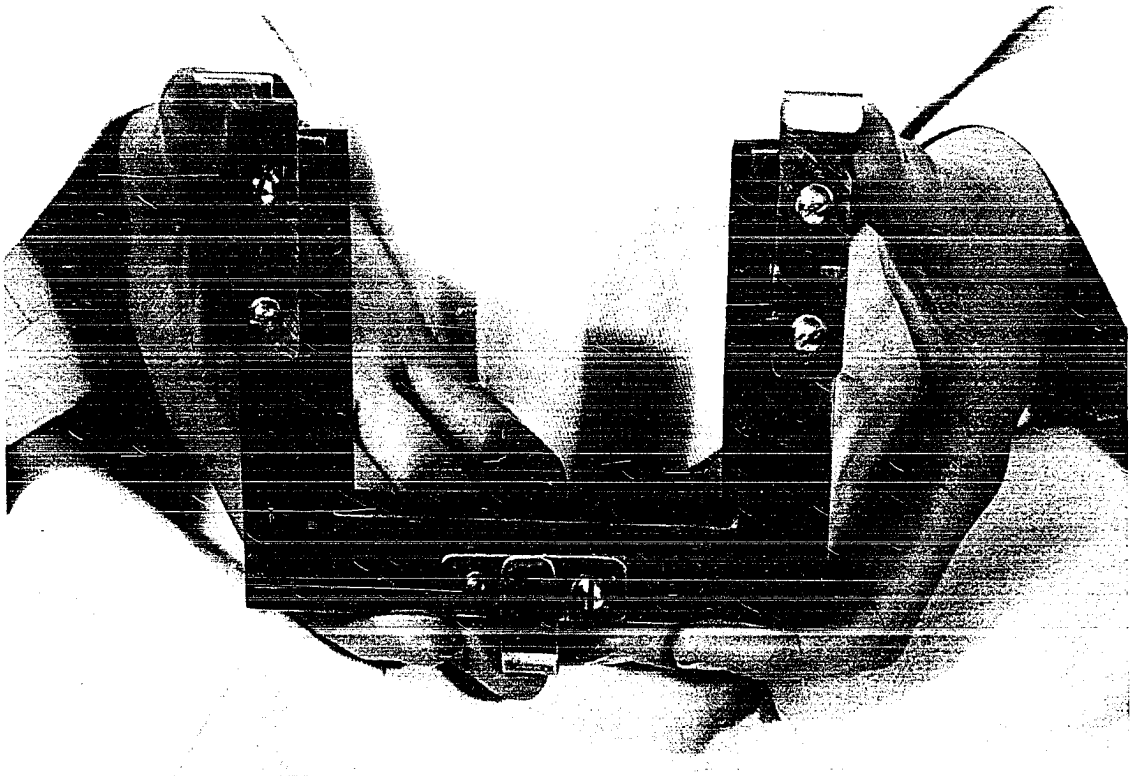


Figure 2. Observation chamber (assembled). Arms projecting from top of U-shaped frame suspend vessel in water of temperature regulating tank. Alignment arm at bottom maintains parallelism of chamber faces and film plane of recording camera. Face of chamber shown here is that presented to the recording camera. Vertical white line visible in shadow to right of little finger of holder's right hand is one of two rulings inscribed on front glass of chamber to indicate scale of photographic reproduction.

Figure 3. Observation chamber (partially disassembled). Components of chamber include milled frame, glass plates, neoprene gasket, pressure plate, suspension and alignment arms, and machine screws. Suspension and alignment arms, attached to opposite side of frame, are shown in Figure 2. All metal parts are of brass.



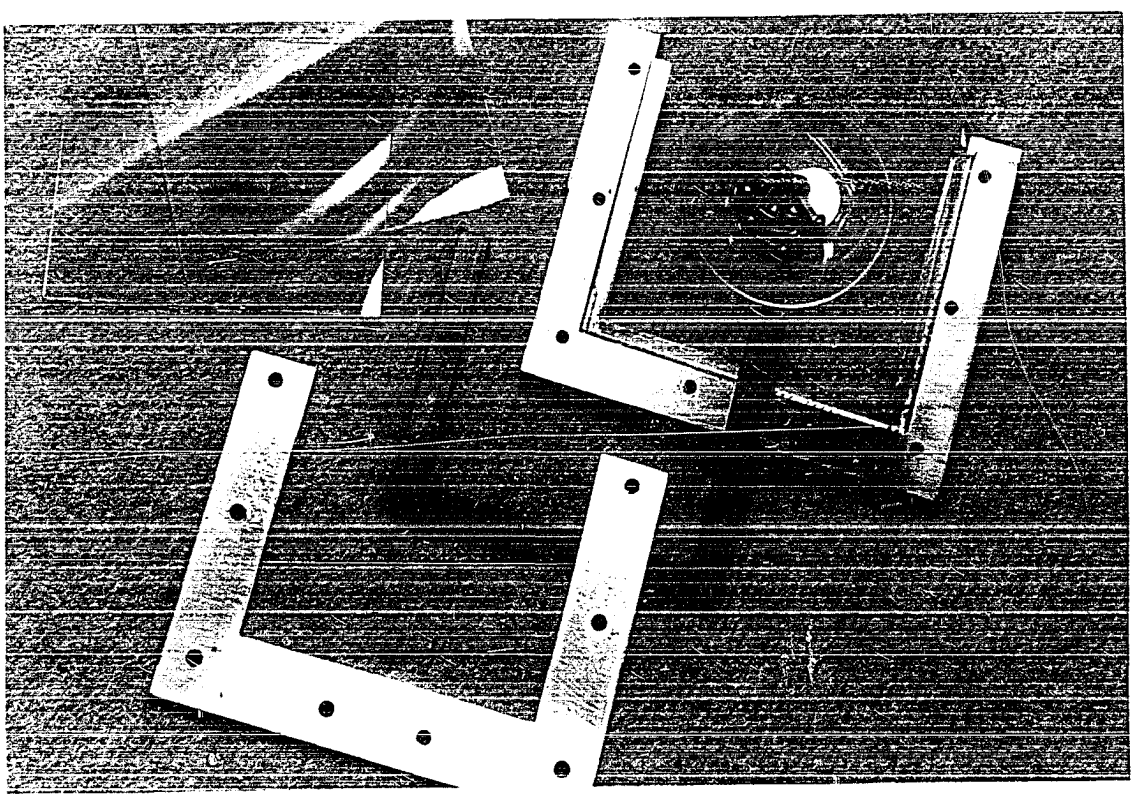
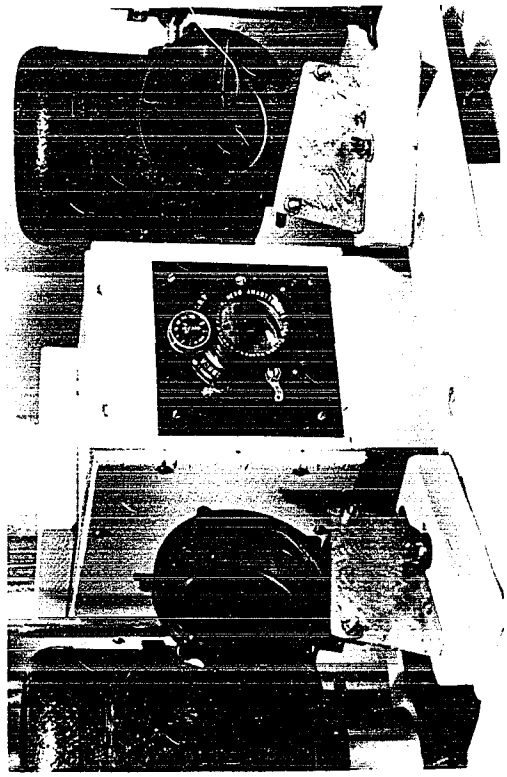


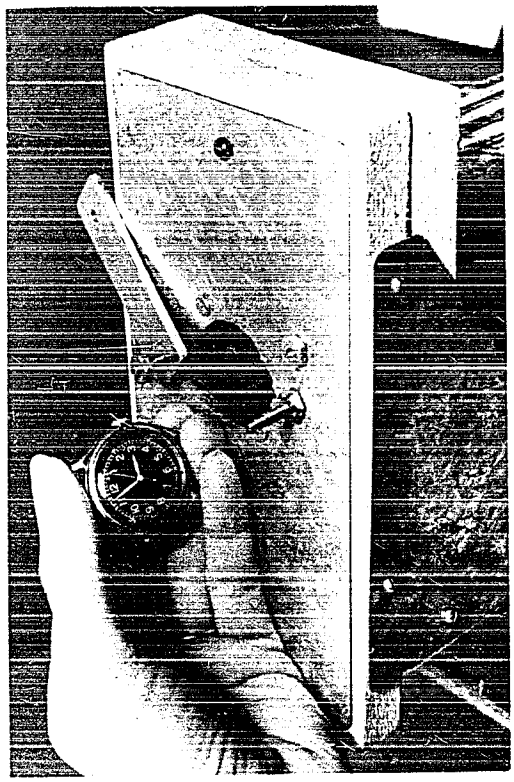
Figure 4. Watch and positioning mount at top of reflex optical system. Watch is placed face downward over hole in plate and is secured by metal bar (swung to right in this picture) slipped over vertical machine screws and tightened by nuts. Face of watch is illuminated by 7-watt lamps mounted to left and right of hole on underside of plate. An erect space image of the watch face is formed in the observation chamber when the watch is mounted with its 12 mark towards the recording camera.

Figure 5. Projection lens of reflex optical system. Lens is a 135mm f4.5 anastigmat used at full aperture. Forty-five degree mirror mounted behind lens and below watch directs rays from illuminated watch face into projection lens. Lens focuses rays to form space image of the watch face in the central plane of the observation chamber. Paired 100-watt Leitz microscope lamps to right and left of projection lens provide illumination for the photographic field. Polaroid attachments in front of lamps can be rotated to produce a dark field lighting effect.

Figure 6. Space image of watch face in observation chamber. Time and approximate duration of exposure, indicated by sector swept out by second hand, are integral parts of the track record. Circular halations at 9 and 3 positions, and lesser flares at 12 and 6, are reflections of lamps from surface of watch crystal. Small white points in field represent minute particles of debris suspended in the fluid of the observation chamber. Sinuous lines are tracks of small ciliates moving in the photographic field during the exposure.

Figure 7. Projector used in reading track records. Housing of lamp has been removed to show details of film strip carrier attached to standard 35mm slide projector. Carrier consists of frame holding spring-loaded glass plates separated at edges by thin metal strips. Film strip inserted between glass plates and metal strips is held flat and in proper position for projection.





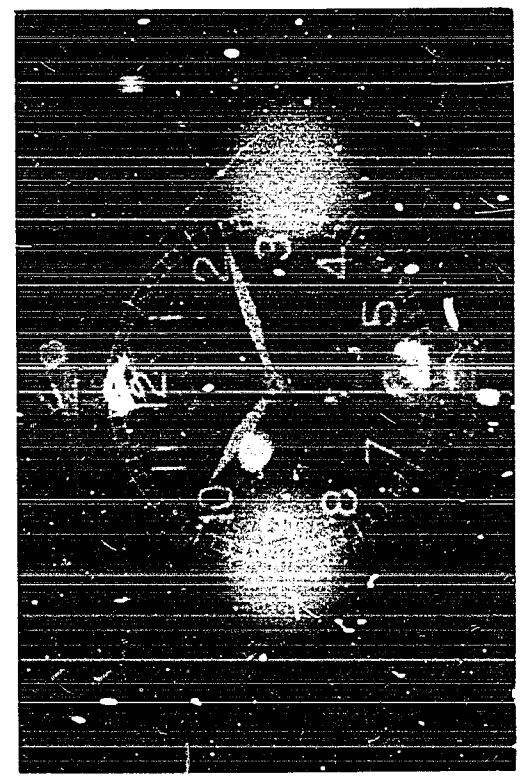
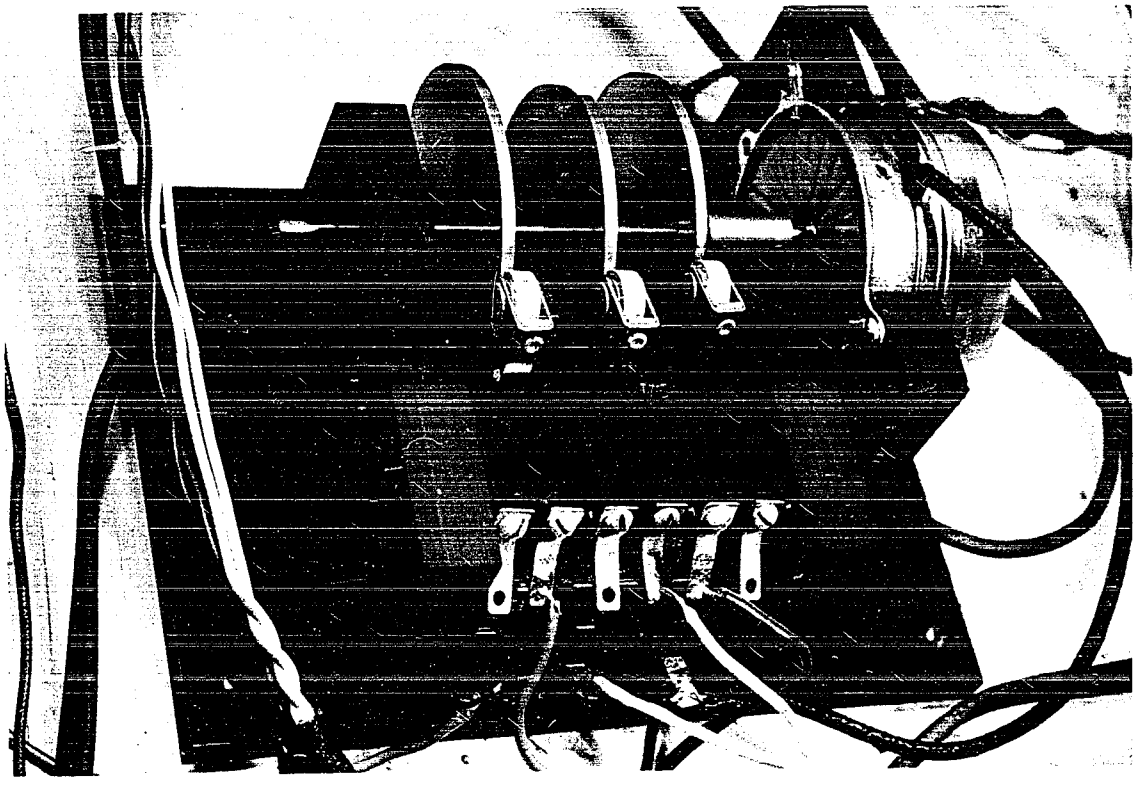


Figure 8. Automatic timing unit. Driving motor (3 RPM synchronous, 60 cycles, 110 volts AC) is at right end. Torque developed by motor is transmitted to camshaft by sleeve linkage. Timing cams A (right), B (center) and C (left) actuate roller-type microswitches.

Figure 9. Relays. Heavy duty relays in center, actuated by signals from microswitches, control 110-volt electrical apparatus operated in conjunction with recorder. Relays on extreme left and right are parts of other control systems and are not directly involved in operation of timing unit.



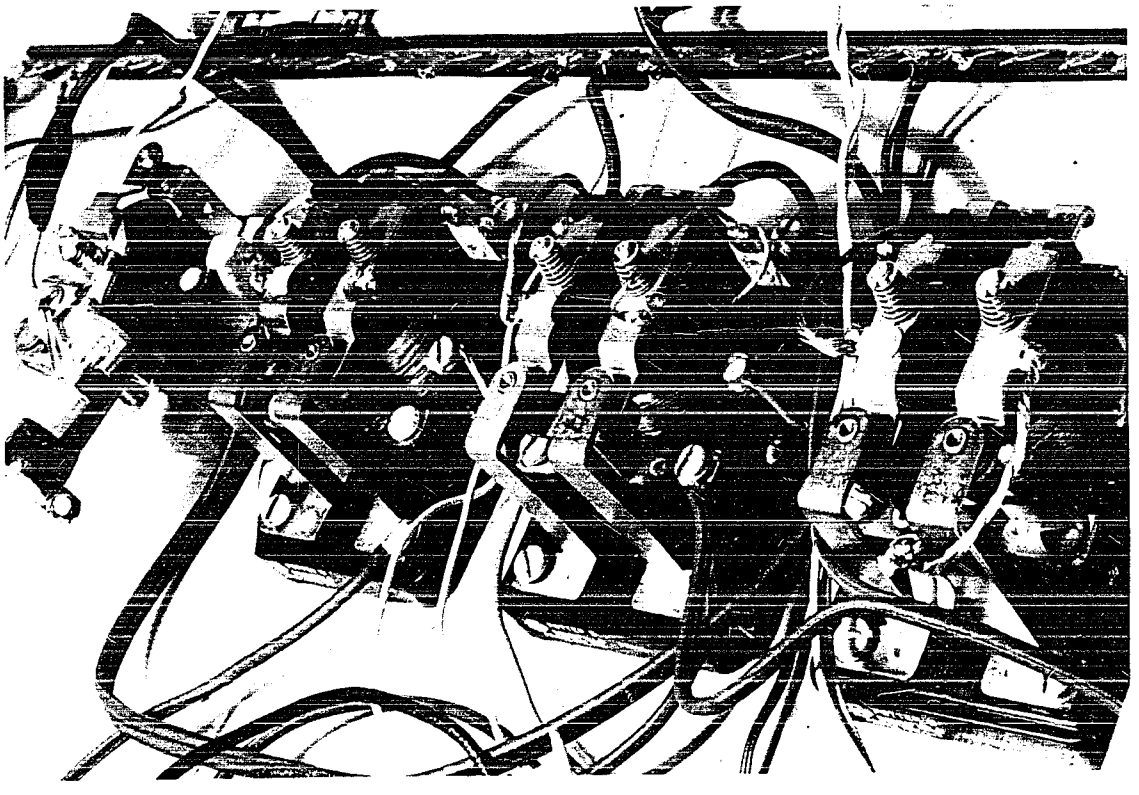
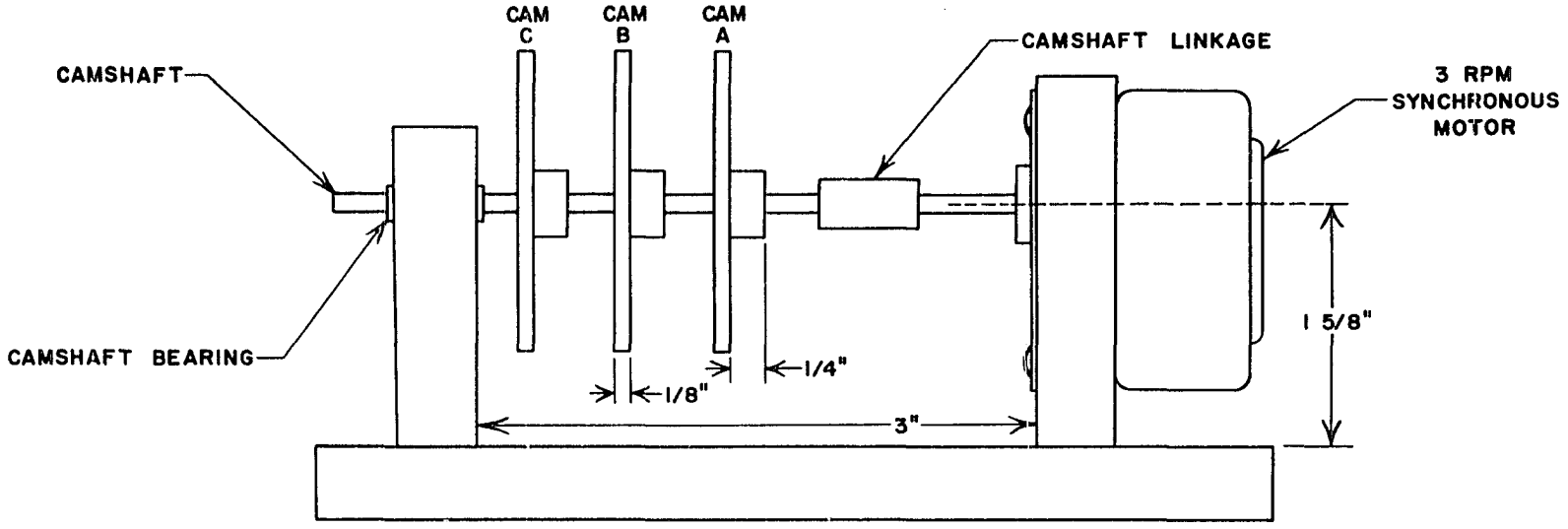


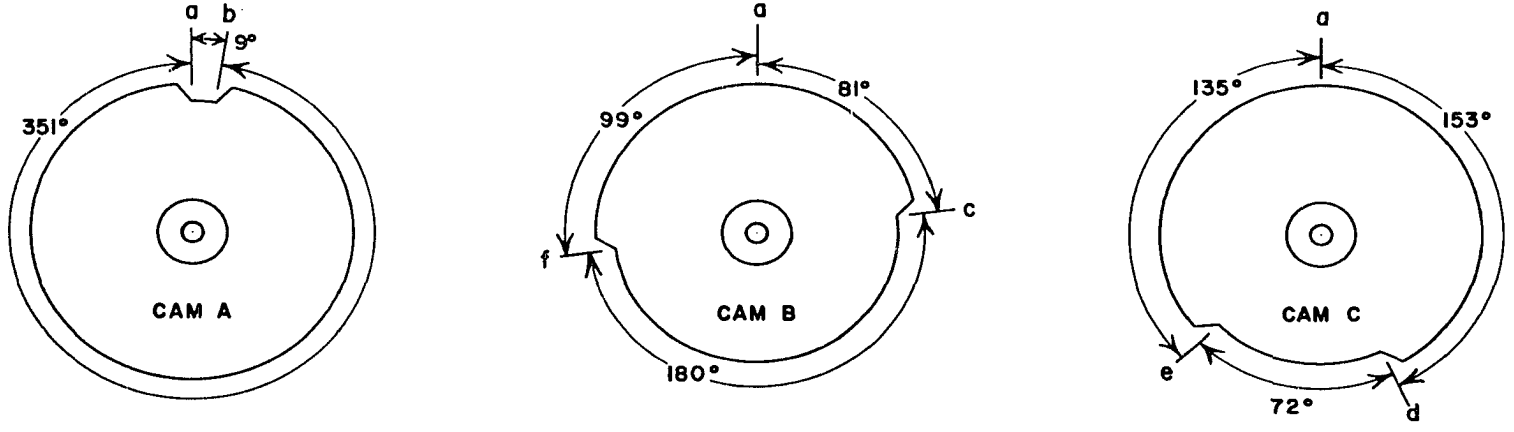
Figure 10. Automatic timing unit (diagram). Cam A is the system restoring cam. When roller contact of Microswitch A drops into cut ab, power to the synchronous driving motor is interrupted, stopping timing mechanism in a position of readiness to receive next recycling instruction. Driving motor may be re-energized at will by momentarily closing manual starting switches S3 or S4 (Figure 11), which are connected in parallel with Microswitch A. Rotation of camshaft resulting from brief closure of either of these switches lifts roller contact of Microswitch A out of the cut at point b, closing the electrical contacts of Microswitch A. During remainder of cycle, operation of timing unit is automatic. The camshaft continues to turn until it has rotated 351°. As point a passes beneath the roller contact of Microswitch A, the roller drops into the cut, opening Microswitch A and terminating action of the driving motor.

Cam B is the appliance cam, regulating operation of all other electrical apparatus except the field illuminating lamps. From points a to c and from f to a, the roller contact of Microswitch B is in the raised position and the microswitch is closed. Through Relay 1 (Figure 11), power is supplied to room lights, water bath agitator motor and other electrical apparatus operating between exposures. At point c, as the roller contact drops into cut cf, these pieces of apparatus are turned off, eliminating vibration, extraneous light and other influences detrimental to the scanning operation. Ten seconds later, as the roller contact of Microswitch B is lifted at point f, Microswitch B closes and the appliances are turned back on.

Cam C is the exposure timing cam. Cut de on this cam (arc of 72°) represents a time interval of four seconds. When roller contact of Microswitch C drops into the cut at point d, Relay 2 (Figure 11) is energized and the paired 100-watt lamps illuminating the photographic field are turned on, initiating the scanning exposure. At point e, the roller contact lifts, turning the lamps off and terminating the exposure. The focal plane shutter of the recording camera is opened manually a few seconds before the lights are turned on and is closed immediately after they are extinguished.



TIMING CAMS AND DRIVING MOTOR ASSEMBLY (SIDE ELEVATION)



ANGULAR RELATIONS OF TIMING CAMS

Table 1. Time-angle relationship of events in one recording cycle of the automatic timing unit. Symbolism: E, event (cam symbol); T, cumulative time in seconds; R, cumulative angular displacement of camshaft in degrees; I, interval (cam symbols); A, interval in degrees of arc; S, interval in seconds. Cam symbols referred to in E and I are lower case letters in cam diagrams, Figure 10.

E	T	R	I	D	S	Description
a	0.0	0	-	-	-	Manual starting switch closed; camshaft rotation begins.
-	-	-	a-b	9	0.5	---
b	0.5	9	-	-	-	Microswitch A closes; control shifts from manual to automatic.
-	-	-	b-c	72	4.0	Manual starting switch opened.
c	4.5	81	-	-	-	Microswitch B closes; room lights turned off, other apparatus stopped.
-	-	-	c-d	72	4.0	Shutter of recording camera opened manually.
d	8.5	153	-	-	-	Microswitch C closes; field illuminating lamps turned on; scanning exposure begins.
-	-	-	d-e	72	4.0	Scanning exposure interval.
e	12.5	225	-	-	-	Microswitch C opens; field illuminating lamps turned off; scanning exposure ends.
-	-	-	e-f	36	2.0	Shutter of recording camera closed manually.
f	14.5	261	-	-	-	Microswitch B closes; room lights turned on; operation of other apparatus resumed.
-	-	-	f-a	99	5.5	---
a	20.0	360	-	-	-	Microswitch A opens; camshaft rotation ceases, leaving system in a condition of readiness to execute next recycling instruction.

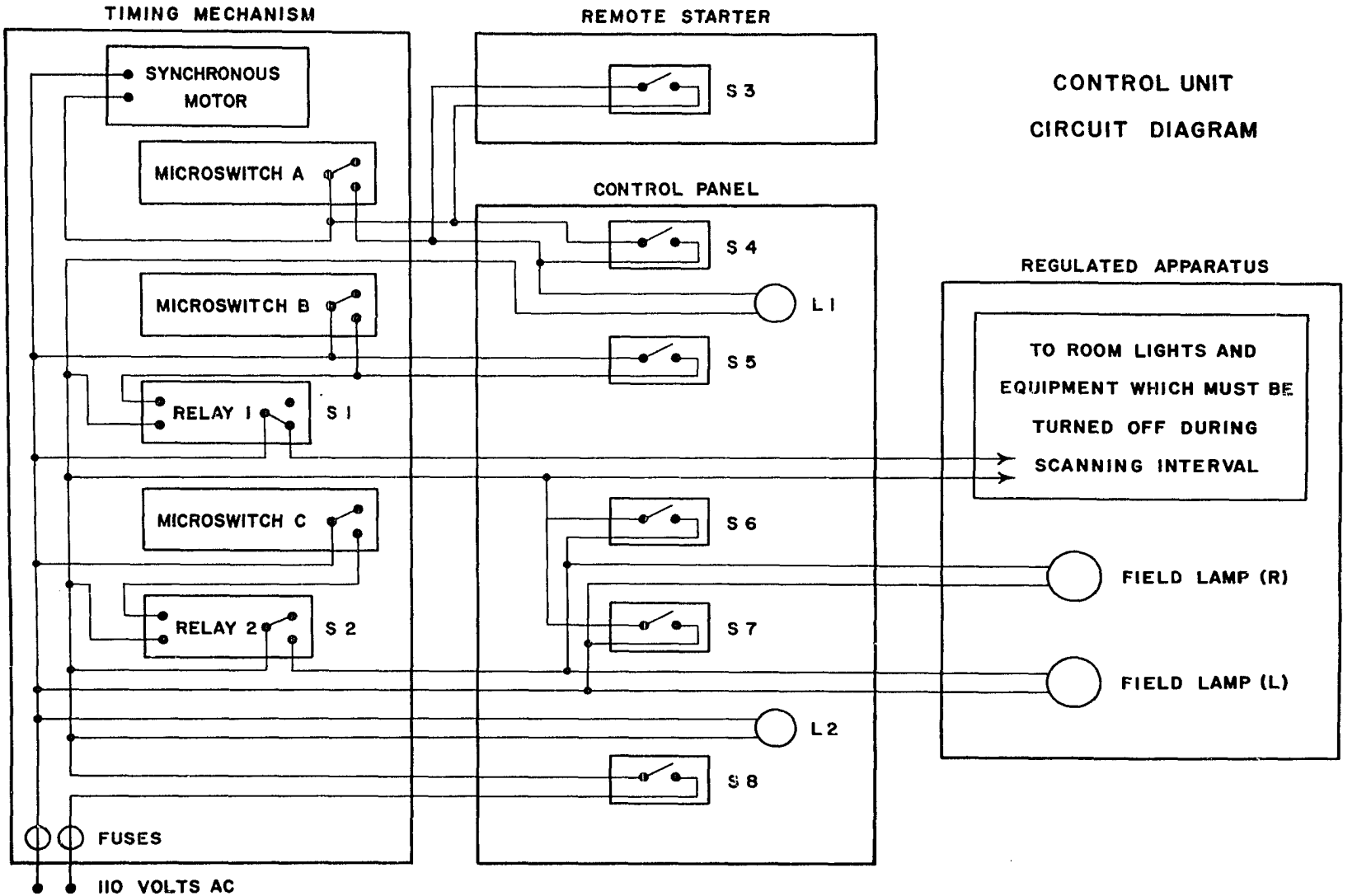
Table 2. Duration and reproducibility of scanning exposures regulated by automatic timing unit (measurements by electronic decade scaler).

Timing test	Duration of exposure in seconds	Deviation from mean
1	3.931	0.024
2	3.972	0.017
3	3.979	0.024
4	3.934	0.021
5	3.978	0.023
6	3.969	0.014
7	3.933	0.022
8	3.977	0.022
9	3.936	0.019
10	3.964	0.009
11	3.968	0.013
12	3.938	0.017
13	3.966	0.011
14	3.930	0.025
15	3.957	0.002
Totals	59.332	0.263
Means	3.955	0.018

Average deviation of exposure intervals =

0.018/3.955 x 100 = 0.5%

Figure 11. Control unit (circuit diagram). Electrical components labeled "timing mechanism" and "control panel" are actually parts of the same structural unit. Remote starter, S3, is a momentary switch on an extension cord which is connected in parallel with manual starting switch S4 on the control panel and Microswitch A of the timing mechanism. Switch S5, a toggle connected in parallel with Microswitch B, provides manual control of Relay 1 and, hence, of room lights and other electrical apparatus. Switches S6 and S7 are manually operated toggles controlling right and left field illuminating lamps. Switch S8, a tumbler, is the main power switch for the unit. Lamps L1 and L2 are jeweled panel lamps signaling, when lighted, "timer operating" and "power on," respectively.



CONTROL UNIT

CIRCUIT DIAGRAM

III. PROCEDURES

Except for the sequencing of scanning exposures, all experiments were performed in exactly the same manner. The routine was as follows: (1) the temperature of the water bath in the temperature regulating tank was adjusted to the desired value; (2) a 16-milliliter volume of a solution to be tested (distilled water in the case of the controls) was introduced into the observation chamber by means of a calibrated glass hypodermic syringe from which the metal needle had been taken; (3) the chamber was placed in the water bath and allowed four minutes to attain temperature equilibrium with its surroundings; (4) the temperature of the water bath was noted and recorded; (5) at the end of the four-minute equilibration period, one milliliter of culture fluid, containing from 200 to 400 paramecia, was forcibly injected into the solution in the observation chamber by means of a glass hypodermic syringe; (6) the chamber contents were immediately mixed by passing a disposable wooden stirring rod from one side of the chamber to the other four times; (7) exactly 20 seconds after introduction of the cells, an automatic control cycle was initiated by closure of the remote starting switch (S3, Figure 11), resulting in the series of

events outlined in Table 1; (8) at the end of the recording cycle, the water bath temperature was again noted and, if different from the previous value, recorded; (9) the chamber was removed from the water bath, emptied, thoroughly rinsed twice with distilled water and inverted on a special rack to drain; (10) the film in the recording camera was advanced, cocking the shutter.

The sequence of scanning exposures was varied from time to time in an attempt to ascertain optimum spacing and the minimal number of exposures needed to establish the form of the response curve. In every case, the initial exposure was begun one-half minute (actually 28.5 seconds) after introduction of the cells. Subsequent exposures were made at intervals of one-half minute, one minute, two minutes or longer periods of time, depending on the duration and nature of the experiment. The time sequence of exposures in a given experiment is indicated in the table summarizing its results.

Tracks were recorded with a camera lens aperture of f5.6 on Kodak Plus-X film (ASA tungsten rating of 64), which was developed in Panthermic 777 developer (manufactured by Sussex Chemical Corporation, Newton, New Jersey) kept at constant working strength by regular replenishment. The films were deliberately underdeveloped to produce negatives with minimal background darkening in which the tracks were sharply defined as black lines. To minimize emulsion

scratching in subsequent handling operations, the films were run through a chrome-alum hardening bath (Morgan, 1953, p. 77) prior to fixation.

Track records were examined by projecting the films on a ground-glass viewing screen with the slide projector shown in Figure 7. The distance between the screen and the projector was adjusted to provide an object-to-image enlargement ratio of one to ten. This adjustment was checked as the initial step in the examination of each negative by measuring on the viewing screen the distance between the images of the two vertical rulings inscribed on the front glass of the observation chamber (see Figure 2 and Ferguson, 1957, p. 213). The actual separation of the marks is 30 millimeters; in a 10X enlargement, the separation of their images is 300 millimeters.

In an earlier investigation (Ferguson, 1955, p. 90), greater variability in length was noted among tracks oriented more or less vertically than among those which were horizontal. In the present study, therefore, only those tracks were selected for measurement whose angle with the horizontal did not exceed 30°. Track length, as the term is used here, refers to a straight line distance from one end of a track to the other, rather than to distance along the sinusoidal curve itself.

Animals used in the experiments were identified with

the aid of a key by Wenrich (1928) as Paramecium caudatum. Clones were established by isolating the descendants of a single animal in separate, loosely-capped Mason jars containing bacterized lettuce infusion. Thriving populations were maintained at room temperature, which averaged around 26°C, by replenishing the cultures every 10 to 12 days. Replenishment consisted of discarding about one third of the fluid in each culture, adding several fragments of dried lettuce (Sonneborn's Dried Lettuce Medium, distributed by Difco Laboratories, Detroit, Michigan), and making up to the original volume with distilled water. The cultures were bacterized in the initial isolation by exposing freshly prepared lettuce infusion to the laboratory atmosphere about 48 hours before introduction of the paramecia. All cultures were regularly checked thereafter for protozoan contaminants. The few in which species other than P. caudatum developed were discarded. In performing experiments, animals were taken only from actively growing cultures which had been replenished within the preceding five to nine days.

IV. EXPERIMENTAL DATA

Experiments were performed to determine the relationship between rate of locomotion and the temperature, osmotic pressure and pH of the environment, and to assess the effects on rate of locomotion of several chemical agents, including vertebrate neurohormones (acetylcholine and adrenaline), a respiratory poison (sodium cyanide) and several anesthetics (urethane and an homologous series of alcohols).

The results of the experiments have been tabulated and are displayed graphically in figures. Curves in the figures have been fitted to point distributions by approximation methods. A guide to the experimental data is found in Table 3. Significant figures and probable errors of measurement are evaluated in Table 4, and symbols used as column headings in the velocity tables are explained in Table 5.

In all tables and figures in this section, rates of locomotion of cells are expressed in terms of the mean length, in millimeters, of the projected images of the tracks. Since the enlargement factor is ten and the exposure time four seconds, the mean velocity of the cells, in

millimeters per second, is equal to 1/40 the number expressing mean track length.

Concentrations of chemicals indicated in the tables and figures are those of test solutions prior to the introduction of the cells. As a consequence of mixing culture fluid and test solution in the volume ratio 1:16, cells were actually exposed to concentrations of the tested reagents which were 16/17, or about 94%, of the values specified. Except in the case of the buffer solutions (Table 10) used in the study of pH effects, each of the test solutions contained only a single solute dissolved in distilled water.

Table 3. Guide to experimental data.

Table 3. (Continued).

Table 4. Probable errors and significant figures.

	Probable errors		Values considered significant to
	Absolute	Relative	
Measurements			
Weight	50mg/10 grams	0.5%	---
Volume	1cc/100cc	1.0%	---
Temperature	0.3°C	-	1.0 degree
pH	0.05 pH unit	-	0.1 pH unit
Length of projected track image	0.5 mm	-	1.0 millimeter
Time intervals			
Scanning exposure	0.02sec/4sec	0.5%	0.02 second
Sequence of scanning exposures (variable)	1sec/interval	-	0.02 minute
Calculations			
Operations on 10" slide rule	1/1000	0.1%	3rd digit ± 1

Table 5. Special symbols employed in tables. Except where otherwise indicated, table symbols have the following meanings.

Symbol	Meaning
N	Number of tracks measured (or counted) on a given scanning photograph.
S	Sum of lengths (millimeters) of N tracks (projection enlargement ratio 10:1) measured on a given scanning photograph.
T	Cumulative time (minutes) of exposure of cells to a given experimental situation.
V	Velocity (millimeters per second) times 40.

V. DISCUSSION OF RESULTS

A. Temperature

Curves in Figures 12 and 13 show the relationship between temperature and rate of locomotion. In Figure 12, rate of locomotion is plotted as a function of time at several temperatures, while in Figure 13 it is plotted as a function of temperature at several times of exposure. The data in Table 7 are those plotted in Figure 13; they were obtained from the curves of Figure 12.

Responses of the cells in the physiological temperature range are shown in curves A through F of Figure 12. Curves A ($5^{\circ}C$-$7^{\circ}C$) through E ($25.5^{\circ}C$) are essentially straight lines paralleling the time axis; in the temperature range indicated, and within the period of observation specified, rate of locomotion appears to bear no relationship to time of exposure. Curve F ($30.5^{\circ}C$), representing response near the upper limit of the physiological range, shows stimulation and a transition to a depressed state of activity. Curves G and H are those of response at temperatures beyond the tolerable range. In G ($35.8^{\circ}C$), a brief period of heightened activity is followed by one in which there is a steady decline to death. In H ($40.0^{\circ}C$), inactivation and death occur almost at once. The tail on the right end of the curve in G and H is an arti-

fact due to convectional displacements of dead cells and does not actually represent locomotor activity.

The time-temperature-velocity data represented in Figure 13 are the same as those of Figure 12, but the curves of the two figures are strikingly different. The curves of Figure 13 are of interest for several reasons: (1) they have the same form; (2) they consist largely of two straight line segments, one of which has positive slope, the other negative slope; (3) corresponding segments of the curves, though differing somewhat in length, especially in the upper temperature range, all have essentially the same slope; (4) the 10°C-25°C portions of curves C (3 minutes) through H (10 minutes) are superimposable; (5) the curves in E (5 minutes) through H (10 minutes) are completely superimposable; (6) the only outstanding difference in the entire series of curves is a downward and leftward movement of the inflection point (peak), which occurs from A (1 minute) through D (4 minutes); (7) from E (5 minutes) through H (10 minutes) the inflection point remains relatively fixed in position, its x-coordinate being a temperature corresponding with that of the normal environment of the cells, viz., about 26°C (see page 29).

At least four generalizations concerning temperature and rate of locomotion can be made from the curves in Figures 12 and 13. First (illustrated by Figure 12, F), in

Figure 12. Effect of temperature on rate of locomotion (velocity versus time at several temperature levels). Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on Y-axes are minutes of exposure of cells to temperature indicated. Temperatures are as follows: A, 5°C-7°C; B, 10.0°C-10.5°C; C, 15.0°C; D, 20.0°C; E, 25.5°C; F, 30.5°C; G, 35.8°C; H, 40.0°C. Tail on right end of curves in G and H is an artifact produced by convectional displacement of dead cells in the observation medium.

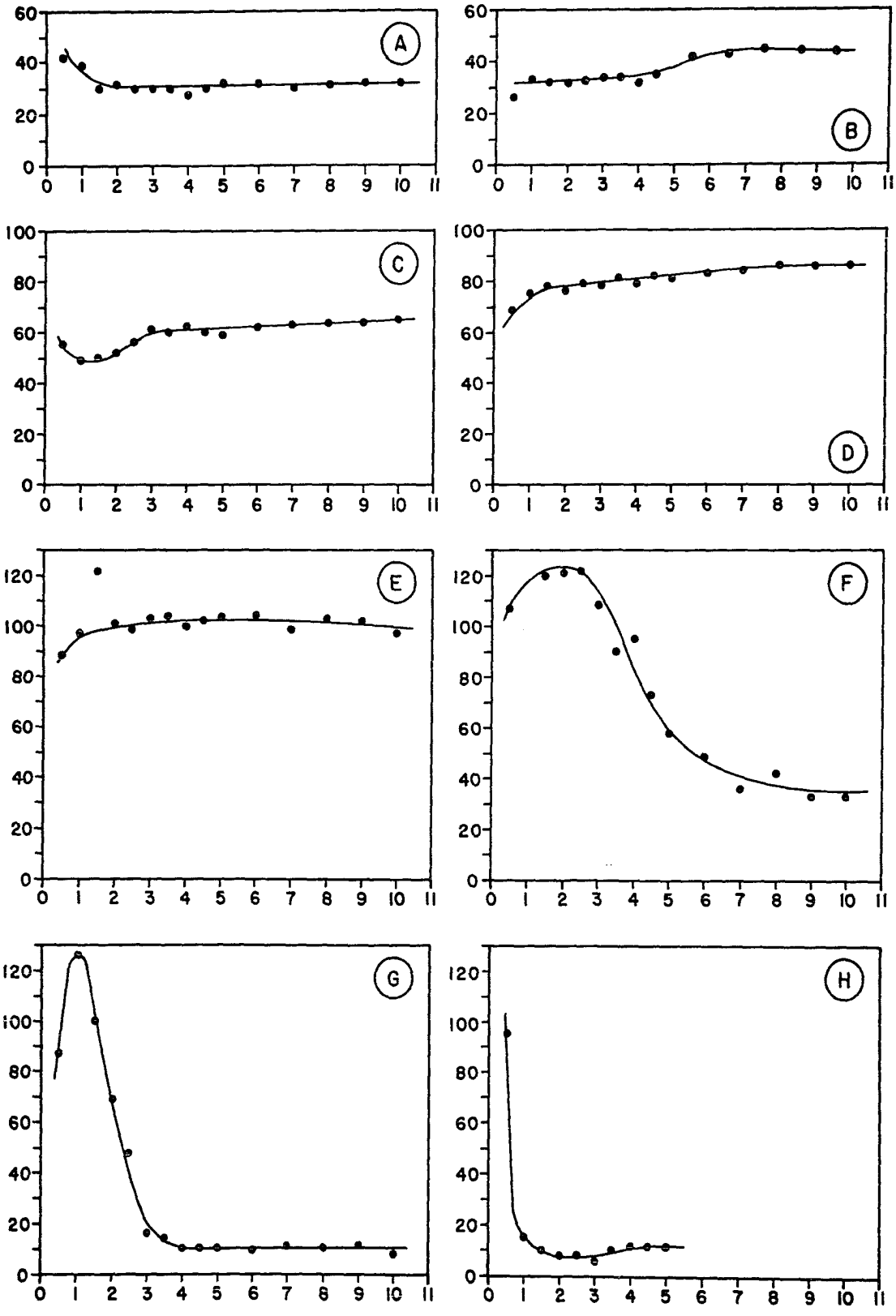

A
B
C
D
E
F
G
H

Figure 13. Effect of temperature on rate of locomotion (velocity versus temperature at several time intervals). Values on Y-axes, read from curves in Figure 12, are mean rates of locomotion (X40) in millimeters per second; values on X-axes indicate temperature in degrees Centigrade. Time intervals are as follows: A, 1 minute; B, 2 minutes; C, 3 minutes; D, 4 minutes; E, 5 minutes; F, 6 minutes; G, 8 minutes; H, 10 minutes.

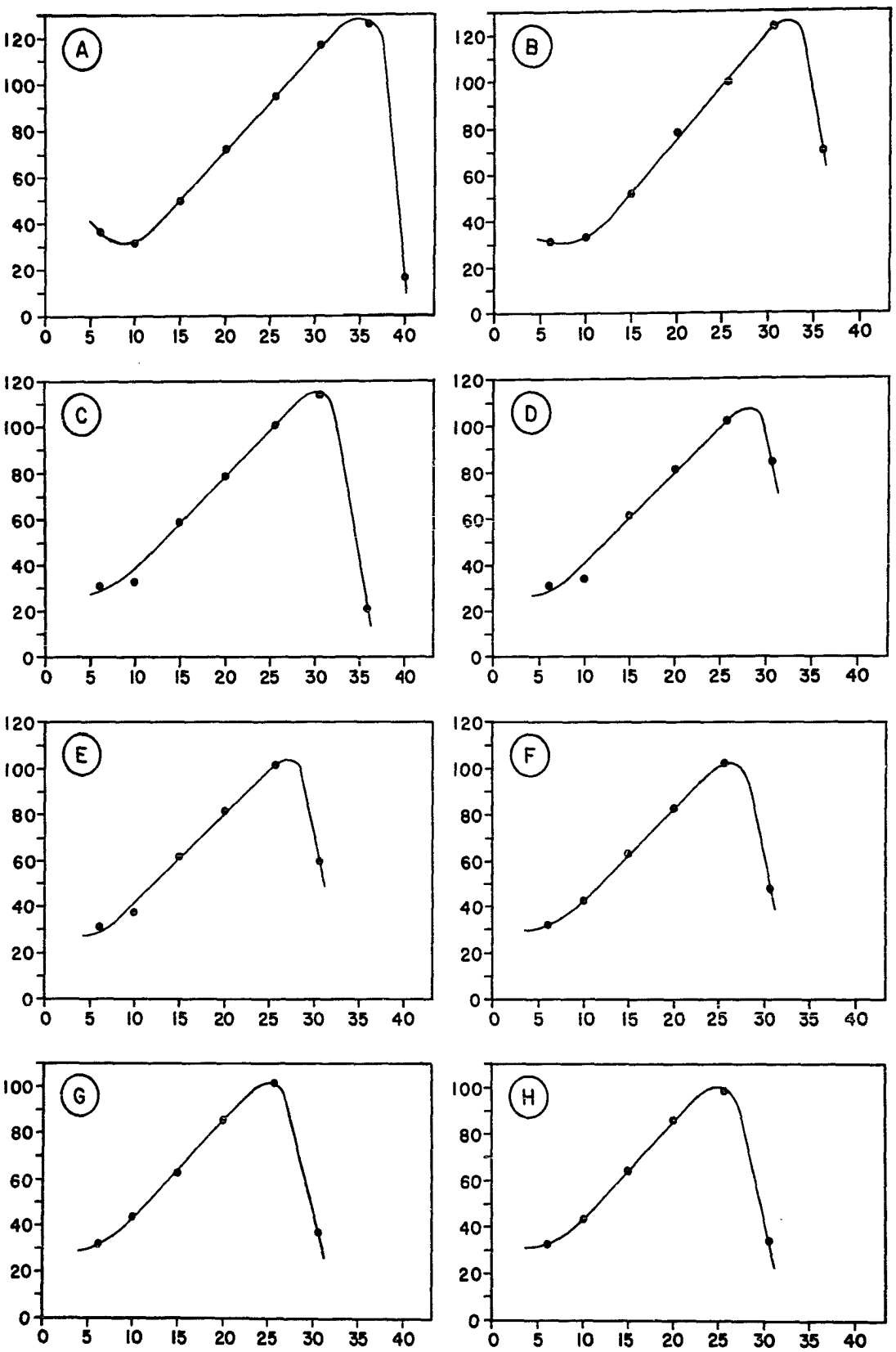

A
B
C
D
E
F
G
H
0 5 10 15 20 25 30 35 40
0 20 40 60 80 100 120

Table 6. Effect of temperature on rate of locomotion.

5.0°C-7.0°C

T	N	S	V
0.5	19	800	42.1
1.0	40	1542	38.6
1.5	47	1418	30.2
2.0	51	1608	31.5
2.5	47	1380	29.4
3.0	46	1365	29.7
3.5	51	1555	30.5
4.0	48	1352	28.2
4.5	45	1345	29.9
5.0	51	1622	31.8
6.0	54	1702	31.5
7.0	57	1713	30.1
8.0	57	1769	31.0
9.0	56	1767	31.6
10.0	55	1739	31.6

10.0°C-10.5°C

T	N	S	V
0.5	26	689	26.5
1.0	64	2091	32.7
1.5	57	1842	32.3
2.0	54	1750	32.4
2.5	51	1670	32.8
3.0	50	1691	33.8
3.5	52	1747	33.6
4.0	53	1638	30.9
4.5	52	1819	35.0
5.0	50	1874	37.5
5.5	39	1620	41.5
6.5	39	1673	42.9
7.5	37	1667	45.1
8.5	39	1741	44.7
9.5	33	1433	43.5

15.0°C

T	N	S	V
0.5	13	720	55.3
1.0	23	1127	49.0
1.5	27	1363	50.5
2.0	40	2079	52.0
2.5	44	2462	56.0
3.0	36	2197	61.1
3.5	40	2380	59.6
4.0	34	2104	61.9
4.5	45	2722	60.5
5.0	42	2492	59.3
6.0	43	2664	61.9
7.0	43	2714	63.1
8.0	41	2638	64.3
9.0	42	2695	64.2
10.0	44	2874	65.3

20.0°C

T	N	S	V
0.5	14	971	69.3
1.0	26	1951	75.0
1.5	40	3100	77.5
2.0	44	3362	76.4
2.5	45	3556	79.0
3.0	44	3439	78.2
3.5	46	3715	80.8
4.0	47	3720	79.2
4.5	38	3117	82.0
5.0	41	3327	81.2
6.0	41	3406	83.1
7.0	48	4007	83.6
8.0	41	3519	85.8
9.0	44	3784	86.0
10.0	47	4027	85.7

Table 6. (Continued).

25.5°C

T	N	S	V
0.5	29	2591	89.4
1.0	47	4561	97.2
1.5	39	4753	122.0
2.0	40	4037	100.9
2.5	41	4051	98.8
3.0	35	3616	103.3
3.5	39	4041	103.6
4.0	39	3893	99.8
4.5	39	3965	101.7
5.0	37	3854	104.2
6.0	35	3652	104.3
7.0	39	3848	98.7
8.0	40	4138	103.3
9.0	37	3762	101.8
10.0	38	3687	97.1

30.5°C

T	N	S	V
0.5	39	4153	106.7
1.0	--	--	--
1.5	39	4691	120.3
2.0	37	4480	121.1
2.5	38	4623	121.7
3.0	42	4574	109.0
3.5	48	4303	89.8
4.0	37	3525	95.3
4.5	22	1607	73.0
5.0	18	1039	57.7
6.0	9	444	49.3
7.0	8	292	36.5
8.0	6	250	41.7
9.0	5	165	33.0
10.0	4	133	33.3

35.8°C

T	N	S	V
0.5	22	1922	87.3
1.0	32	4026	125.9
1.5	34	3438	100.4
2.0	40	2749	68.7
2.5	24	1159	47.9
3.0	19	306	16.1
3.5	15	206	13.7
4.0	16	162	10.1
4.5	11	112	10.2
5.0	12	120	10.0
6.0	10	92	9.2
7.0	6	64	10.7
8.0	16	166	10.4
9.0	11	123	11.2
10.0	10	83	8.3

40.0°C

T	N	S	V
0.5	13	1238	95.3
1.0	16	238	14.9
1.5	14	134	9.6
2.0	16	123	7.7
2.5	17	136	8.0
3.0	18	103	5.7
3.5	18	180	10.0
4.0	19	230	12.1
4.5	27	311	11.5
5.0	17	200	11.8
6.0	--	--	--
7.0	--	--	--
8.0	--	--	--
9.0	--	--	--
10.0	--	--	--

Table 7. Relationship between rate of locomotion and temperature at various times of exposure (data from curves in Figure 12).

	Time of exposure of cells in minutes							
Temperature	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
5.0°C-7.0°C	37	31	31	31	31	32	32	33
10.0°C-10.5°C	32	33	33	34	38	43	44	44
15.0°C	50	52	59	61	62	63	63	65
20.0°C	73	78	79	81	82	83	86	87
25.5°C	95	99	101	102	102	102	102	99
30.5°C	117	123	114	84	60	48	38	35
35.8°C	126	70	21	--	--	--	--	--
40.0°C	16	--	--	--	--	--	--	--

the supra-optimal but tolerable temperature range the rate-time curve of response has the form of a reversed sigmoid whose major features, in chronological order, are (1) a shoulder, representing excitation and briefly-sustained hyper-normal activity; (2) a diagonal of negative slope, representing the transition from a state of abnormally high activity to one of abnormally low activity; and (3) a toe, representing completion of adjustment and attainment of a steady state characterized by depressed but continuing activity.

Second (illustrated by Figure 12, F-H), the rate-time sigmoid of response in the range above optimal becomes progressively compressed to the left and its toe progressively lowered as conditions approach the upper limits of toleration.

Third (illustrated by Figure 13), within the first ten minutes of exposure, rate is directly proportional to temperature in the range 5°C to 25°C.

Fourth (illustrated by Figure 13), within the first ten minutes of exposure, response is maximal at a temperature equal to or slightly above that of the normal environment.

In an earlier study of the relationship between temperature and rate of locomotion (Ferguson, 1955, pp. 61-64; 1957, p. 213 Figure 16), a population of Paramecium aurelia

was exposed to a temperature gradient of 0.4°C per minute from 9.0°C to 36.7°C. Under these conditions, the rate-temperature curve in the 10°C to 30°C range consisted of two straight line segments, that in the 19°C to 28°C range having a slope more than twice that of the segment in the 8°C to 19°C range. The temperature corresponding with the inflection in this two-segment curve, viz., 19°C, was that of the normal environment of the experimental animals.

Other studies of the relationship between temperature and the rate of processes in the paramecium include those of Jacobs (1919) on thermal death; Cole (1925) on pulsation of the contractile vacuole; Mitchell (1929) on division rate; Gaw (1936) on pulsation of the contractile vacuole; Lee (1942a) on the formation of food vacuoles; Pace and Kimura (1944) on respiration, and many others. The only study of the relationship between temperature and rate of locomotion, however, appears to have been that of Glaser (1924).

Linear relationships between temperature and rate, resembling that depicted in Figure 13, are evident in the data reported by Glaser (1924), Lee (1942a) and Cole (1925). Examples of such relationships in organisms other than the paramecium are cited by Bělehrádek (1935, pp. 9-10).

The data on respiratory metabolism presented by Pace and Kimura (1944) indicate that the paramecium utilizes carbohydrates almost exclusively at temperatures near the upper

limit of its physiological range. The respiratory quotient for Paramecium caudatum at 35°C is said by these authors to be 0.99, as opposed to 0.75 at 25°C.

Comprehensive surveys of the relationship between temperature and biological processes in many different types of organisms are found in Bělehrádek (1935); Precht, Christophersen and Hensel (1955), who include an entire chapter on microorganisms; and Johnson (1957).

B. Osmotic Pressure

Curves in Figures 14 and 15 show the relationship between osmotic pressure and rate of locomotion. In Figure 14, rate of locomotion is plotted as a function of time at several sucrose concentrations, while in Figure 15 it is plotted as a function of the concentration at several times of exposure. The data in Table 9 are those plotted in Figure 15; they were obtained from the curves of Figure 14. Osmotic pressures corresponding with the various concentrations of sucrose can be calculated by means of the van't Hoff relation, $P = iCRT$, in which P is the pressure in atmospheres, i the isotonic coefficient (which has a value of 1 for sucrose and other non-electrolytes), C the molar concentration of sucrose, R the universal gas constant (0.082 liter atmospheres/degree/mol) and T the absolute temperature (degrees Kelvin). In the tables and figures, however, the osmotic

pressures have been expressed only indirectly as sucrose concentrations.

With regard to colligative properties, the paramecium is said by Frisch (1937, p. 159) to have "...within limits, a control over its environment." It is apparently not a homoiosmotic animal (Kamada, 1935-38, p. 61), but certain homeostatic mechanisms do appear to function in this organism. Curves E through G of Figure 14 reveal the existence of such a mechanism and, at the same time, demonstrate its failure. The locomotor steady state of the paramecia used in this experiment seems to be that associated with the Y = 50 level of activity; the time during which this state can be maintained in a concentrated solution appears to be inversely related to the concentration of the solution. The duration of the steady state (measured from time zero to the point of abrupt downward deflection of the rate-time curve) is six, four and three minutes, respectively, in curves E (0.20 M), F (0.25 M) and G (0.30 M) of Figure 14. In this restricted range, the time required for failure of the homeostatic mechanism is roughly proportional to the reciprocal of the concentration. In curve H (0.35 M) and subsequent curves in Figure 14, a steady state is apparently never attained. Results similar to those shown by curves E through G were obtained in earlier experiments with Paramecium aurelia (see Ferguson, 1957, p. 214, Figure 18).

Figure 14. Effect of osmotic pressure on rate of locomotion (velocity versus time at several osmotic pressures). Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes are minutes of exposure of cells to solution indicated. Control curve is in A. Sucrose concentrations are as follows: B, 0.05 M; C, 0.10 M; D, 0.15 M; E, 0.20 M; F, 0.25 M; G, 0.30 M; H, 0.35 M; I, 0.40 M; J, 0.45 M; K, 0.50 M.

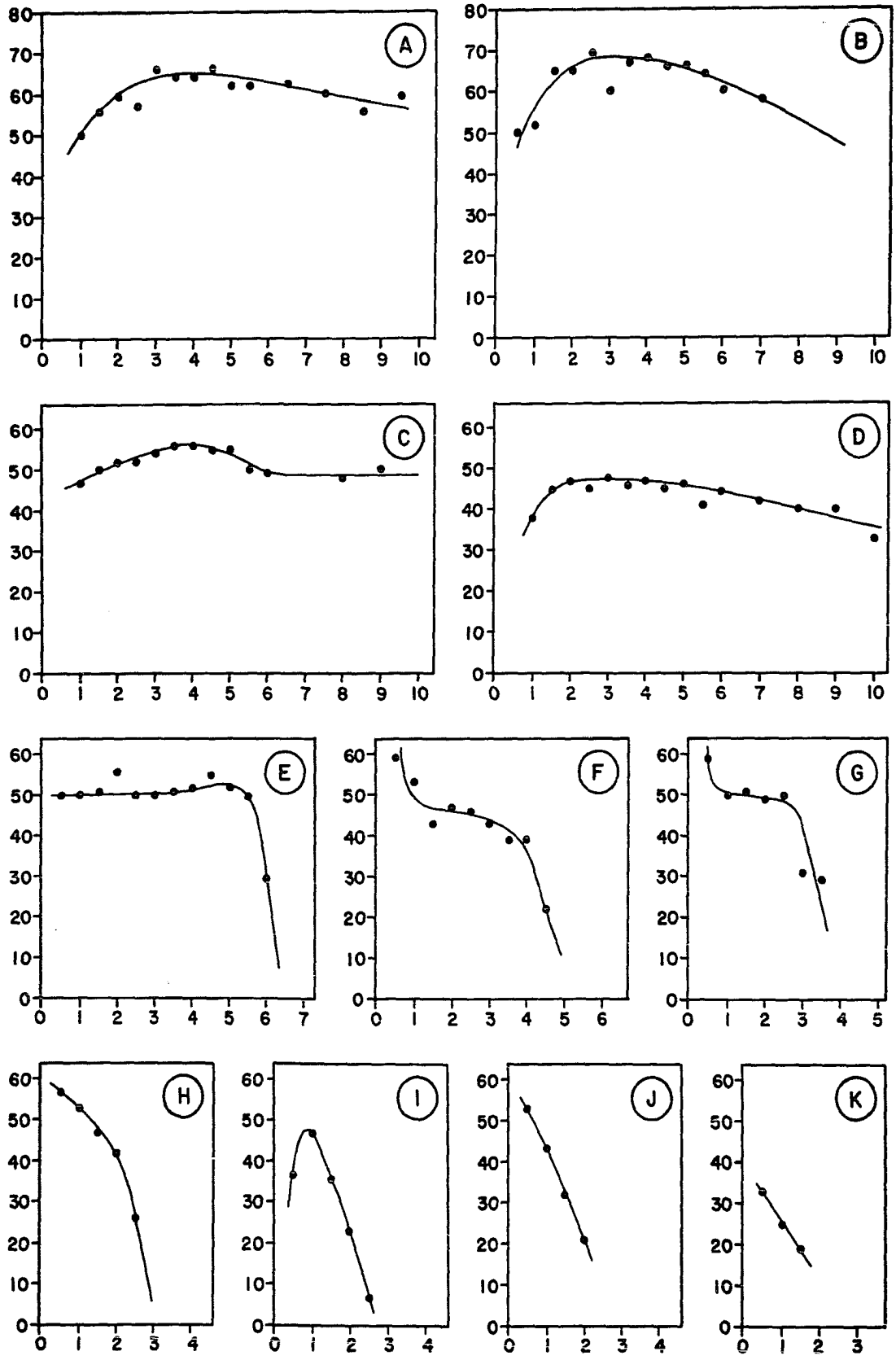

A
B
C
D
E
F
G
H
I
J
K

Figure 15. Effect of osmotic pressure on rate of locomotion (velocity versus sucrose concentration at several time intervals). Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes indicate concentration of sucrose solutions in moles. Time intervals are as follows: A, 1 minute; B, 2 minutes; C, 3 minutes; D, 4 minutes; E, 5 minutes; F, 6 minutes; G, 7 minutes; H, 8 minutes.

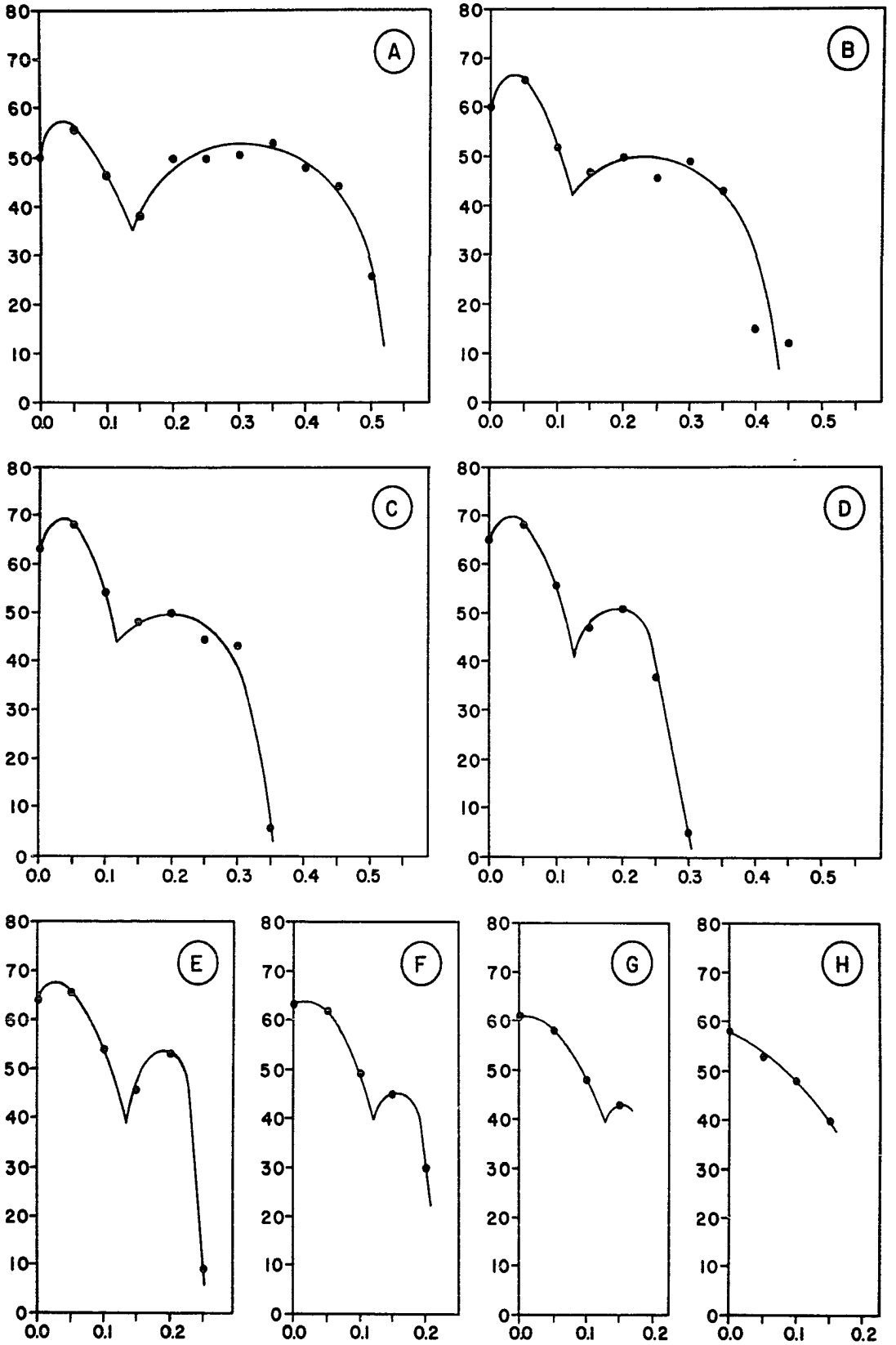

A
B
C
D
E
F
G
H
0
10
20
30
40
50
60
70
80
0.0
0.1
0.2
0.3
0.4
0.5

Table 8. Effect of osmotic pressure on rate of locomotion (sucrose solutions).

Control (27.3°C-27.0°C)

T	N	S	V
0.5	--	--	--
1.0	5	248	49.6
1.5	12	666	55.5
2.0	14	828	59.2
2.5	23	1319	57.3
3.0	18	1189	66.0
3.5	25	1598	63.9
4.0	31	1987	64.1
4.5	31	2031	65.5
5.0	31	1928	62.2
5.5	31	1938	62.5
6.5	23	1414	61.5
7.5	24	1426	59.4
8.5	30	1674	55.8
9.5	17	1002	58.9
10.5	21	1391	66.3

0.05M (27.0°C-26.8°C)

T	N	S	V
0.5	4	199	49.8
1.0	10	515	51.5
1.5	22	1427	64.9
2.0	33	2148	65.1
2.5	38	2615	68.9
3.0	39	2355	60.4
3.5	38	2552	67.2
4.0	40	2724	68.2
4.5	50	3280	65.6
5.0	44	2892	65.8
5.5	43	2756	64.1
6.0	40	2401	60.0
7.0	44	2536	57.7

0.15M (26.5°C)

T	N	S	V
0.5	9	221	24.6
1.0	18	691	38.4
1.5	23	1030	44.8
2.0	25	1178	47.1
2.5	29	1316	45.4
3.0	30	1429	47.6
3.5	36	1670	46.4
4.0	35	1637	46.7
4.5	40	1816	45.4
5.0	38	1756	46.5
5.5	32	1311	41.0
6.0	33	1450	43.9
7.0	31	1296	41.8
8.0	34	1356	39.9
9.0	20	790	39.5
10.0	8	267	33.4

0.10M (26.5°C-26.7°C)

T	N	S	V
0.5	--	--	--
1.0	13	614	47.2
1.5	21	1043	49.7
2.0	31	1612	52.0
2.5	29	1500	51.8
3.0	25	1339	53.6
3.5	45	2514	55.9
4.0	42	2368	56.4
4.5	41	2263	55.2
5.0	43	2348	54.6
5.5	46	2311	50.2
6.0	46	2265	49.3
7.0	--	--	--
8.0	40	1938	48.5
9.0	40	1985	49.6
10.0	--	--	--

Table 8. (Continued).

0.20M (26.4°C-26.5°C)

T	N	S	V
0.5	3	149	49.7
1.0	9	445	49.5
1.5	19	975	51.3
2.0	19	1070	56.3
2.5	36	1813	50.4
3.0	32	1606	50.2
3.5	39	1974	50.7
4.0	34	1765	51.9
4.5	31	1712	55.3
5.0	24	1235	51.5
5.5	19	935	49.2
6.0	4	117	29.3

0.30M (26.0°C)

T	N	S	V
0.5	4	237	59.2
1.0	25	1260	50.4
1.5	29	1476	50.8
2.0	37	1823	49.3
2.5	23	1156	50.3
3.0	7	220	31.4
3.5	1	29	29.0

0.40M (26.0°C)

T	N	S	V
0.5	5	183	36.6
1.0	22	1031	46.9
1.5	30	1081	36.1
2.0	14	322	23.0
2.5	8	54	6.7
3.0	--	--	--

0.25M (26.0°C-26.3°C)

T	N	S	V
0.5	5	294	58.8
1.0	15	800	53.3
1.5	18	780	43.3
2.0	23	1079	46.9
2.5	22	1004	45.7
3.0	28	1204	43.1
3.5	26	1018	39.2
4.0	14	554	38.8
4.5	3	66	22.0

0.35M (26.0°C)

T	N	S	V
0.5	7	399	57.0
1.0	37	1948	52.7
1.5	36	1700	47.2
2.0	21	892	42.5
2.5	8	205	25.6

0.45M (26.0°C)

T	N	S	V
0.5	9	476	52.9
1.0	25	1073	43.0
1.5	20	647	32.3
2.0	3	64	21.3

0.50M (25.8°C-26.0°C)

T	N	S	V
0.5	7	232	33.1
1.0	14	351	25.1
1.5	15	290	19.3

Table 9. Relationship between rate of locomotion and osmotic pressure at various times of exposure (data from curves in Figure 15).

Molar concentration of sucrose	Time of exposure in minutes							
	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
0.00	50	60	63	65	64	63	61	58
0.05	56	66	68	68	66	62	58	53
0.10	47	52	54	56	54	49	48	48
0.15	38	47	48	47	46	45	43	40
0.20	50	50	50	51	53	30	--	--
0.25	50	46	44	37	9	--	--	--
0.30	51	49	43	5	--	--	--	--
0.35	53	43	6	--	--	--	--	--
0.40	48	25	--	--	--	--	--	--
0.45	44	22	--	--	--	--	--	--
0.50	26	12	--	--	--	--	--	--

The curves in Figure 15 are bimodal with a pronounced and characteristic minimum at the 0.12 M coordinate. Below this value, the curves are remarkably alike; in fact, in the 0.00 M-0.12 M range, curves B (2 minutes) through E (5 minutes) are superimposable. In the concentration range above 0.12 M, however, the curves become progressively compressed towards the left as time of exposure increases. Finally, in F (6 minutes) through H (8 minutes), this portion of the curve disappears. A minimum similar to that displayed by the curves in Figure 15 was observed by the author at a slightly lower concentration, viz., 0.08 M-0.10 M, in the study of Paramecium aurelia cited above. Because the data taken in this experiment were relatively incomplete, the minimum was dismissed at that time as an artifact or error (see footnote at bottom of Table 11, p. 67, in Ferguson, 1955).

Several conclusions may be drawn from Figures 14 and 15. First, the cells are stimulated by abrupt exposure to an environment differing in physical and chemical properties, including colligative, from that to which the cells have become accustomed.

Second, within limits, the magnitude of excitation and the persistence of the state of excitation produced by such exposure are related to the magnitude, and probably also the character, of the difference between the environments.

Third, the physical, if not the chemical, conditions in a 0.12 M sucrose solution must approximate those of the normal environment of the cells, since minimal excitation (lowest rate of locomotion) was consistently associated with this concentration.

Fourth, the cells possess homeostatic mechanisms which tend to resist the changes associated with excitation and which, in the absence of excitation, and under a specified set of conditions, maintains the cells in a steady state of locomotor activity.

Fifth, these homeostatic mechanisms have the capacity to handle any demands placed upon them in solutions whose concentrations do not exceed 0.12 M.

Sixth, at concentrations in excess of 0.12 M, the mechanisms are inadequate, failing at a rate which depends upon the concentration to which the cells are exposed.

In the concentration range 0.00 M through 0.12 M, the cell is exposed to a hypotonic medium, in which the problem is that of flooding. Contractile vacuoles and other provisions enable the cell to solve this problem. In solutions having concentrations in excess of 0.12 M, on the other hand, the problem is one of desiccation. The cell is not immediately able to cope with this situation. Thus water is rapidly withdrawn from the cells, impeding the metabolic processes which provide the cilia with energy. Locomotor

activity declines at a rate which appears to depend on the magnitude of the osmotic differential between the cell and its surroundings.

Apparently no quantitative studies of the relationship between osmotic pressure and rate of locomotion of the paramecium, other than that cited above, have been made, although much is published on the relationship between osmotic pressure and the functioning of the contractile vacuole. Kamada (1935-38) found that the rate of contraction of the vacuole in paramecia which were transferred to concentrated solutions first dropped, then rose, to level off at a rate which was not related to concentration. He interpreted this to mean that the cells somehow are able to maintain, after a suitable period of time, a constant osmotic pressure differential between their own protoplasm and that of the environment. Gaw (1936), observing the rate of vacuolar pulsation in animals exposed for two hours to solutions ranging in concentration up to 0.10 M, found that the equilibrium rate in these dilute solutions was related to concentration. Studies by Frisch (1937) indicate that the pellicle of the paramecium is impermeable to water and that the rate of contractile vacuole pulsation depends, not on the external osmotic pressure, directly, but rather on the rate at which food vacuoles are formed. He believes that end-osmosis does not occur and that water expelled by the contractile vacuole enters the cell

only through the cytostome. That the rate of water influx can be regulated to an extent is indicated by his observation that vacuolar pulsation ceased for periods as long as five minutes in animals which were swimming actively. In an investigation of the relationship between osmotic pressure and the processes of respiration and growth in Astasia, von Dach (1950) found that concentrated solutions inhibited growth much more than they did respiration. In nutrient solutions having a freezing point depression of 0.4°C, both processes were inhibited only slightly. Growth was completely inhibited in those with a depression of 1.0°C, while respiration was still 15% of normal in those with a depression of 1.5°C. In those with a depression of 2.0°C, however, the cells were quickly killed.

C. Hydrogen Ion Concentration

Curves in Figures 16 and 17 show the relationship between pH and rate of locomotion. In Figure 16, rate of locomotion is plotted as a function of time at selected pH levels, while in Figure 17 it is plotted as a function of pH at several times of exposure. Both figures were drawn from the data in Table 11.

Buffers used in this experiment were based on Clark (1928). The composition of the buffer solutions, and the pH of 1:100 dilutions of these solutions before and after the

addition of culture fluid are indicated in Table 10. As in the preceding experiments, culture fluid and buffer dilutions were mixed in the volume ratio 1:16; it will be noted in Table 10 that mixing caused the pH of the buffer solutions to shift slightly towards that of the culture fluid, which was 7.50. The pH change was greatest at extremities of the range (for example, the shift from 9.80 to 9.67) and was, of course, zero in the case of the buffer whose pH already approximated that of the added fluid. The buffer solutions were used in high dilution to minimize osmotic pressure effects.

Living organisms apparently react to all types of ions. They react differently to different ions; they react to changes in the concentration of specific ions; and they even react to alterations in the proportions of different ionic species. Therefore, in any study of the relationship between pH and a biological process, the responses of organisms in buffered solutions must be interpreted conservatively. The curves of Figures 16 and 17 depict the reactions of cells in complex environments which obviously contain ions other than those of hydrogen. The effects shown, however, are believed to be mainly those attributable to the influence of the hydrogen ion.

The six curves shown in Figure 16 have been selected because they typify the responses of the cells in various

Figure 16. Effect of pH on rate of locomotion (velocity versus time at several H-ion concentrations). Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes are minutes of exposure of cells to solutions. The pH of each buffer solution was determined electrometrically after introduction of cells; values were as follows: A, 7.20; B, 8.10; C, 8.17; D, 8.53; E, 9.15; F, 9.67. Buffers in A, B, C were prepared from M/10 citric acid and M/5 disodium phosphate; those in D, E, F were prepared from M/5 boric acid and M/5 sodium hydroxide. Dilutions of 1:100 were used to obviate osmotic pressure effects.

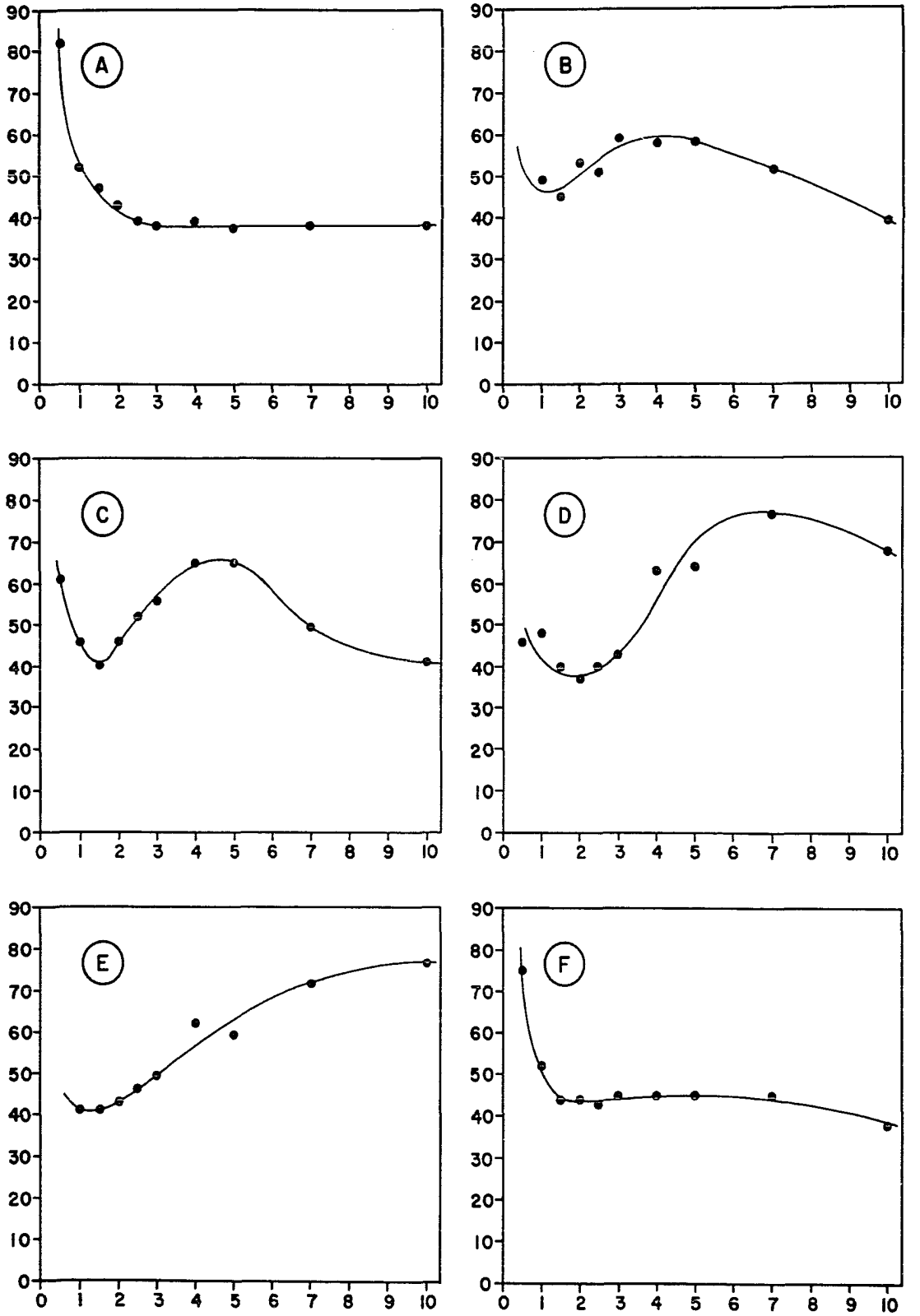

A
B
C
D
E
F
0 10 20 30 40 50 60 70 80 90
0 1 2 3 4 5 6 7 8 9 10

Figure 17. Effect of pH on rate of locomotion (velocity versus pH at several time intervals). Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes indicate pH of buffer solution following introduction of cells. Time intervals are as follows: A, 0.5 minute; B, 1.0 minute; C, 2.0 minutes; D, 2.5 minutes; E, 3.0 minutes; F, 4.0 minutes; G, 5.0 minutes; H, 10.0 minutes. Buffers in pH range 4.8 to 8.2 were prepared from M/10 citric acid and M/5 disodium phosphate; those in pH range 8.3 to 9.7 were prepared from M/5 boric acid and M/5 sodium hydroxide. Dilutions of 1:100 were used to obviate osmotic pressure effects.

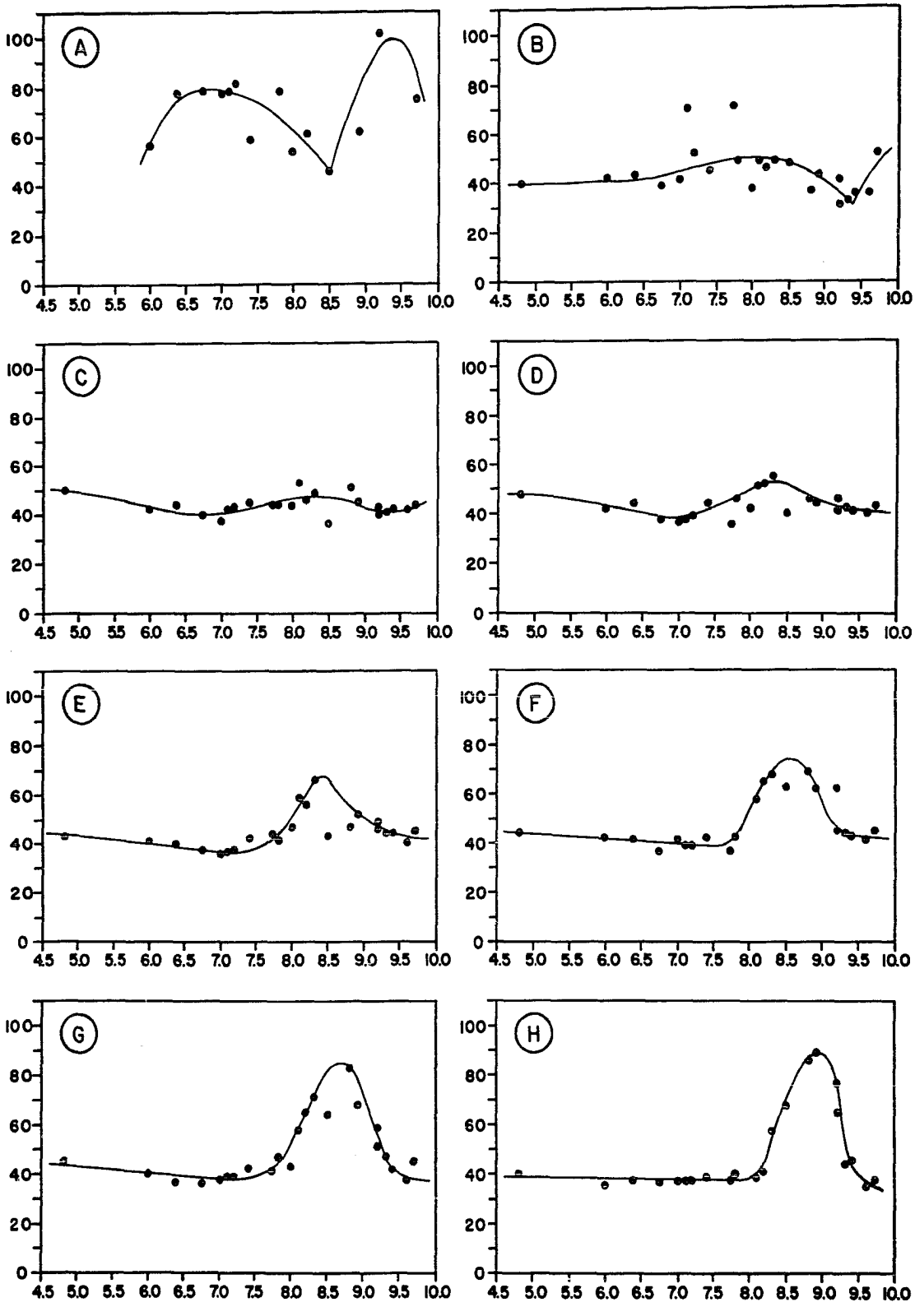

A
B
C
D
E
F
G
H
100
80
60
40
20
0
4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

Table 10. Composition and pH of buffer solutions.

Composition of buffer solution volumes in milliliters				pH of buffer solution diluted 1:100	
M/10 citric acid	M/5 disodium phosphate	M/5 boric acid	M/5 sodium hydroxide	Before addition of cells	After addition of cells
24.6	15.4	--	--	4.63	4.83
19.4	20.6	--	--	5.82	5.96
16.8	23.2	--	--	6.33	6.37
14.0	26.0	--	--	6.73	6.75
12.7	27.3	--	--	7.00	7.02
11.7	28.3	--	--	7.10	7.11
10.8	29.2	--	--	7.18	7.20
8.5	31.5	--	--	7.40	7.40
4.9	35.1	--	--	7.76	7.75
3.8	36.2	--	--	7.86	7.82
2.5	37.5	--	--	8.04	8.00
1.8	38.2	--	--	8.20	8.10
1.1	38.9	--	--	8.42	8.17
--	--	50.0	5.9	7.40	8.31
--	--	50.0	8.6	8.55	8.53
--	--	50.0	12.0	8.80	8.77
--	--	50.0	16.4	9.03	8.92
--	--	50.0	21.4	9.24	9.15
--	--	50.0	26.7	9.37	9.17
--	--	50.0	32.0	9.40	9.28
--	--	50.0	36.9	9.56	9.39
--	--	50.0	40.8	9.80	9.60
--	--	50.0	43.9	9.80	9.67

Table 11. Effect of pH on rate of locomotion.

pH 4.83 (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	5	202	40.4
1.5	18	859	47.7
2.0	22	1090	49.6
2.5	18	867	48.1
3.0	20	852	42.6
4.0	20	880	44.0
5.0	28	1258	44.9
7.0	26	1120	43.1
10.0	25	987	39.5

pH 5.96 (29.0°C)

T	N	S	V
0.5	4	229	57.3
1.0	13	543	41.8
1.5	34	1450	42.7
2.0	21	876	41.7
2.5	15	624	41.6
3.0	20	824	41.2
4.0	24	999	41.6
5.0	24	963	40.1
7.0	22	890	40.5
10.0	22	803	36.5

pH 6.37 (29.0°C)

T	N	S	V
0.5	3	235	78.3
1.0	15	761	50.8
1.5	32	1366	42.7
2.0	34	1500	44.1
2.5	32	1407	43.9
3.0	28	1115	39.9
4.0	30	1230	41.0
5.0	34	1256	36.9
7.0	35	1396	38.8
10.0	31	1195	38.5

pH 6.75 (29.0°C)

T	N	S	V
0.5	4	316	79.0
1.0	5	194	38.8
1.5	12	484	40.3
2.0	14	552	39.5
2.5	16	616	38.5
3.0	12	453	37.8
4.0	12	439	36.6
5.0	15	545	36.3
7.0	18	651	36.2
10.0	23	852	37.1

pH 7.02 (28.8°C)

T	N	S	V
0.5	3	235	78.3
1.0	7	288	41.2
1.5	15	622	41.5
2.0	22	841	38.3
2.5	14	516	36.9
3.0	9	322	35.8
4.0	9	369	41.0
5.0	15	570	38.0
7.0	18	657	36.5
10.0	20	736	36.8

pH 7.11 (28.8°C)

T	N	S	V
0.5	3	237	79.0
1.0	7	491	70.2
1.5	13	526	40.4
2.0	14	581	41.6
2.5	25	942	37.7
3.0	26	963	37.1
4.0	26	1019	39.2
5.0	26	1010	38.9
7.0	28	1056	37.7
10.0	40	1483	37.1

Table 11. (Continued).

pH 7.20 (28.8°C)

T	N	S	V
0.5	2	165	82.5
1.0	14	725	51.8
1.5	16	747	46.7
2.0	25	1067	42.7
2.5	17	659	38.7
3.0	15	574	38.3
4.0	16	620	38.7
5.0	15	585	39.0
7.0	24	906	37.7
10.0	23	878	38.2

pH 7.40 (29.0°C)

T	N	S	V
0.5	7	416	59.4
1.0	7	312	44.6
1.5	19	806	42.4
2.0	28	1248	44.6
2.5	28	1220	43.6
3.0	29	1226	42.3
4.0	29	1210	41.7
5.0	26	1080	41.6
7.0	23	907	39.4
10.0	32	1241	38.8

pH 7.75 (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	4	284	71.1
1.5	4	173	43.3
2.0	10	436	43.6
2.5	11	392	35.7
3.0	15	653	43.6
4.0	14	516	36.9
5.0	14	573	40.8
7.0	15	601	40.1
10.0	15	563	37.5

pH 7.82 (29.0°C)

T	N	S	V
0.5	3	236	78.7
1.0	8	394	49.3
1.5	13	584	44.9
2.0	15	655	43.7
2.5	17	780	45.9
3.0	15	614	40.9
4.0	19	796	41.9
5.0	21	983	46.8
7.0	22	921	41.8
10.0	34	1373	40.4

pH 8.00 (29.0°C)

T	N	S	V
0.5	2	108	54.0
1.0	2	77	38.5
1.5	7	294	42.0
2.0	13	569	43.8
2.5	19	790	41.6
3.0	15	704	46.9
4.0	--	--	--
5.0	25	1071	42.8
7.0	16	719	44.9
10.0	--	--	--

pH 8.10 (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	3	148	49.3
1.5	6	269	44.9
2.0	16	854	53.3
2.5	20	1024	51.3
3.0	24	1425	59.4
4.0	23	1334	58.0
5.0	23	1345	58.5
7.0	22	1123	51.1
10.0	20	780	39.0

Table 11. (Continued).

pH 8.17 (29.0°C)

T	N	S	V
0.5	1	61	61.0
1.0	9	414	46.0
1.5	10	401	40.1
2.0	25	1154	46.2
2.5	27	1406	52.1
3.0	30	1686	56.2
4.0	34	2213	65.2
5.0	30	1949	65.0
7.0	31	1516	48.9
10.0	25	1017	40.7

pH 8.31 (28.3°C)

T	N	S	V
0.5	--	--	--
1.0	10	493	49.3
1.5	25	1228	49.0
2.0	31	1529	49.3
2.5	27	1491	55.3
3.0	26	1714	66.0
4.0	31	2096	67.6
5.0	32	2262	70.7
7.0	32	2324	72.7
10.0	27	1562	57.8

pH 8.53 (28.3°C)

T	N	S	V
0.5	2	93	46.5
1.0	2	95	47.5
1.5	6	237	39.5
2.0	10	373	37.3
2.5	14	553	39.5
3.0	16	688	43.0
4.0	12	757	63.1
5.0	18	1147	63.7
7.0	9	696	77.3
10.0	8	541	67.7

pH 8.77 (28.1°C)

T	N	S	V
0.5	--	--	--
1.0	3	110	36.7
1.5	5	195	39.0
2.0	7	357	51.0
2.5	16	730	45.6
3.0	14	664	47.4
4.0	9	620	68.9
5.0	8	666	83.3
7.0	7	531	75.9
10.0	3	258	86.0

pH 8.92 (28.2°C)

T	N	S	V
0.5	2	125	62.5
1.0	12	519	43.3
1.5	17	720	42.3
2.0	21	946	45.1
2.5	26	1155	44.5
3.0	26	1341	51.6
4.0	30	1873	62.5
5.0	23	1566	68.1
7.0	18	1202	66.8
10.0	11	979	89.1

pH 9.15 (28.3°C)

T	N	S	V
0.5	1	102	102.0
1.0	4	164	41.0
1.5	9	365	40.6
2.0	14	599	42.7
2.5	20	919	45.9
3.0	21	1035	49.3
4.0	16	989	61.9
5.0	16	943	59.0
7.0	17	1217	71.6
10.0	19	1469	77.4

Table 11. (Continued).

pH 9.17 (28.3°C)

T	N	S	V
0.5	--	--	--
1.0	2	62	31.0
1.5	6	232	38.7
2.0	8	323	40.4
2.5	18	742	41.3
3.0	19	872	45.9
4.0	27	1212	44.9
5.0	17	860	50.6
7.0	14	989	70.6
10.0	13	841	64.7

pH 9.28 (28.3°C)

T	N	S	V
0.5	--	--	--
1.0	1	33	33.0
1.5	11	466	42.3
2.0	25	1023	40.9
2.5	28	1168	41.7
3.0	32	1410	44.1
4.0	26	1153	44.4
5.0	22	1024	46.6
7.0	16	737	46.1
10.0	16	706	44.1

pH 9.39 (28.3°C)

T	N	S	V
0.5	--	--	--
1.0	3	108	36.0
1.5	11	447	40.6
2.0	23	960	41.7
2.5	27	1105	40.9
3.0	26	1140	43.8
4.0	22	951	43.2
5.0	16	676	42.3
7.0	10	543	54.3
10.0	9	409	45.5

pH 9.60 (28.5°C)

T	N	S	V
0.5	--	--	--
1.0	3	108	36.0
1.5	10	364	36.4
2.0	18	750	41.7
2.5	24	954	39.7
3.0	24	948	39.5
4.0	16	651	40.7
5.0	10	385	38.5
7.0	4	140	35.0
10.0	9	312	34.7

pH 9.67 (28.5°C)

T	N	S	V
0.5	1	75	75.0
1.0	4	207	51.8
1.5	11	482	43.8
2.0	20	889	44.5
2.5	17	732	43.1
3.0	17	764	44.9
4.0	14	628	44.8
5.0	10	453	45.3
7.0	5	223	44.6
10.0	5	191	38.2

sections of the pH range, as follows: curve A, 4.83-8.00; curve B, 8.10 only; curve C, 8.17 only; curve D, 8.31-8.53; curve E, 8.77-9.17; and curve F, 9.28-9.67.

The basal or steady state level of activity under the conditions of the experiment seems to be that associated with the y = 40 ordinate. Throughout the entire acid range and at both extremities of the alkaline range, the rate-time curve in Figure 16 is sigmoidal, with (a) a shoulder representing excitation and briefly-sustained hyper-normal activity; (b) a diagonal of transition (which resembles an exponential adsorption curve); and (c) a toe leading into the steady state. In the range pH 8.1-9.2, however, the toe portion of the response curve is modified by development of a second peak. The activity represented by this feature is considerable (though never as great as that of the shoulder maxima in A and F), and, at least in D and E, is maintained for a relatively long time.

The rate-pH curves of Figure 17 show the development of this peak particularly well. The rise commences almost at once, in B (1 minute), and continues throughout the period of observation (10 minutes). Very broad at first, the peak narrows rapidly as it rises. The right margin of its base remains relatively stationary at about pH 9.5 (marked in B by the minimum); narrowing results from a progressive shift of the left margin up the pH scale.

The position of the peak in Figure 17 bears no obvious relationship to the pH of the culture from which the cells were taken. Neither does it seem to be the consequence of a change in buffer type from citrate-phosphate to borate-NaOH, which occurred between pH 8.17 and pH 8.31. There is likewise no reason to believe that it is a manifestation of an osmotic pressure change; the buffer solutions were used in hypotonic concentrations. In addition, the cells displayed essentially the same locomotor velocity in dilutions containing widely differing concentrations of the buffer components (e.g., at pH 7.40 and pH 9.67 in F). Whatever the ionic conditions associated with the peak may be, they result in reactions or physical changes which promote the action of the cilia.

The results presented in Figure 17 are at variance with those obtained in other studies of the relationship between pH and the rate of locomotion in ciliates. The curves presented by Chase and Glaser (1930, p. 635) for Paramecium and by Mills (1931, p. 24) for Colpidium are conspicuously bimodal, with minima at the extremities of the pH range (about pH 4.5 to pH 10) and one at (or near) neutrality. Their M-shaped curves (similar to A in Figure 17) bear striking and suggestive resemblance to the time-pressure curves presented in Figure 15 in this thesis, and lead to the suspicion that the effects displayed may be due as much to other

factors, cited above, as they are to pH. In his own earlier study of the relationship between pH and rate of locomotion in Paramecium aurelia, the author (Ferguson, 1957, p. 213, Figure 17) obtained a 4-minute exposure time curve which, with the exception of a single high datum point at about pH 5.7, roughly resembles the pH 5.5-8.5 portion of curve F in Figure 17.

Rate-pH curves having the general form of an inverted V, symmetrical about a pH abscissa near neutrality, have been obtained in studies by Gaw (1936) on contractile vacuole pulsation in paramecia; Lee (1942b) on food vacuole formation in paramecia; and van Wagtendonk and Zill (1947) on "killer substance" (paramecin) inactivation.

D. Vertebrate Neurohormones

Curves in Figure 18 show the effects on rate of locomotion of acetylcholine chloride (A-C) and adrenaline chloride (D-F).

The curves are essentially alike and occupy corresponding positions on the coordinate field. There appear to be no important differences in response. It is concluded that acetylcholine and adrenaline, in the concentration ranges tested, have no specific physiological effects on the paramecium.

These results are at variance with those of Wense (1935),

who reported that the locomotor activities of paramecia were stimulated by acetylcholine and inhibited by adrenaline.

There is evidence that neurohormones and related substances occur in ciliates. They presumably have some physiological role in these organisms. Bayer and Wense (1936) were able to demonstrate acetylcholine-like and adrenaline-like effects of paramecium extracts on various types of muscle preparations. They observed that some of the extracts fluoresced apple-green under the ultra-violet lamp, a property also displayed by adrenaline. Seaman and Houlihan (1951) found that various agents which inhibit the action of acetylcholinesterase also inhibit locomotion in Tetrahymena, suggesting that this enzyme, and therefore, acetylcholine is indispensable to coordinated ciliary action.

It seems reasonable to suppose, therefore, that adrenaline and acetylcholine do have physiological roles in paramecia. Had higher concentrations of these agents been employed, the results of this experiment might have been more conclusive.

E. Sodium Cyanide

The effects of sodium cyanide on rate of locomotion are shown in Figure 19. In B through D, rate is plotted as a function of time at several concentrations; in E through G, rate of locomotion is expressed as per cent of control

Figure 18. Effect of vertebrate neurohormones on rate of locomotion. A-C, acetylcholine chloride; D-F, adrenaline chloride. Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes are minutes of exposure of cells to solutions. Upper curve (black symbols) in A and D is that of control. Acetylcholine concentrations: A (white symbols), 10.00 mg/ml; B (black symbols), 0.10 mg/ml; B (white symbols), 0.01 mg/ml; C, 1.00 mg/ml. Adrenaline concentrations: D (white symbols), 1:100,000; E, 1:10,000,000; F, 1:1,000,000.

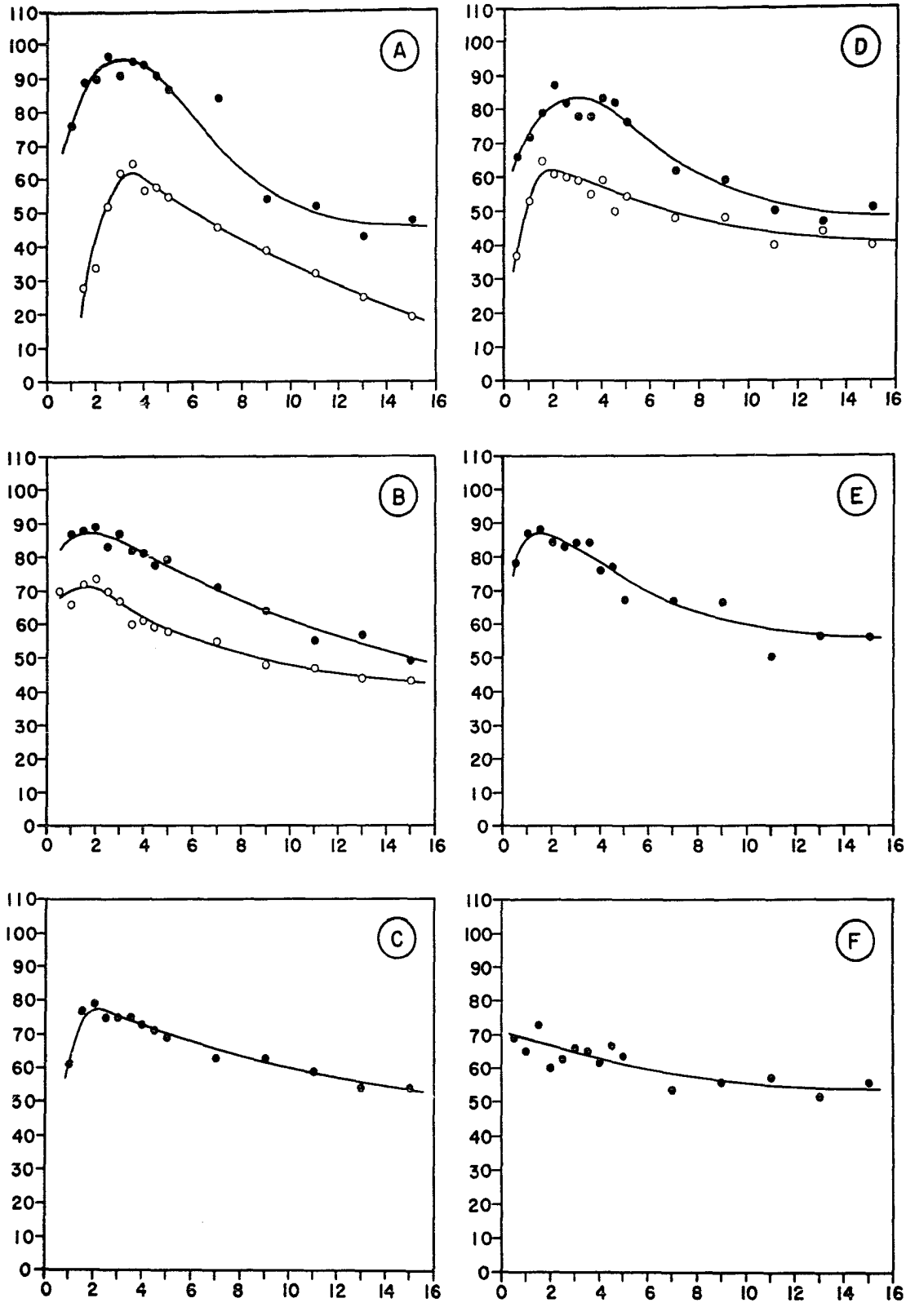

A
B
C
D
E
F
0 2 4 6 8 10 12 14 16
0 10 20 30 40 50 60 70 80 90 100 110

Table 12. Effect of acetylcholine chloride on rate of locomotion.

Control (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	24	1834	76.4
1.5	26	2308	88.8
2.0	32	2877	89.9
2.5	34	3300	97.2
3.0	53	4842	91.3
3.5	30	2850	95.0
4.0	38	3579	94.3
4.5	28	2551	91.2
5.0	28	2426	86.7
7.0	22	1847	84.0
9.0	14	762	54.4
11.0	15	778	51.9
13.0	7	298	42.6
15.0	10	476	47.6

0.01 mg/ml (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	30	1976	65.8
1.5	30	2155	71.7
2.0	28	2086	74.5
2.5	31	2180	70.3
3.0	26	1731	66.6
3.5	31	1853	59.8
4.0	33	2023	61.4
4.5	28	1659	59.3
5.0	31	1801	58.1
7.0	21	1155	55.0
9.0	26	1241	47.7
11.0	21	985	46.9
13.0	27	1176	43.5
15.0	33	1415	42.9

0.1 mg/ml (29.0°C)

T	N	S	V
0.5	11	775	70.4
1.0	21	1828	87.1
1.5	38	3335	87.8
2.0	33	2944	89.2
2.5	31	2582	83.3
3.0	27	2348	87.0
3.5	35	2885	82.4
4.0	37	2995	81.1
4.5	46	3569	77.7
5.0	39	3084	79.1
7.0	37	2615	70.8
9.0	37	2351	63.6
11.0	40	2204	55.0
13.0	33	1770	57.3
15.0	28	1368	48.8

1.0 mg/ml (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	40	2430	60.7
1.5	22	1697	77.2
2.0	34	2672	78.6
2.5	35	2609	74.6
3.0	31	2332	75.2
3.5	42	3129	74.6
4.0	39	2844	72.9
4.5	31	2196	70.8
5.0	37	2537	68.6
7.0	41	2567	62.7
9.0	41	2600	63.4
11.0	45	2648	58.9
13.0	52	2806	54.0
15.0	53	2889	54.5

Table 12. (Continued).

10 mg/ml (29.0°C)

T	N	S	V
0.5	--	--	--
1.0	--	--	--
1.5	2	56	28.0
2.0	8	274	34.3
2.5	10	517	51.7
3.0	11	686	62.3
3.5	14	915	65.3
4.0	27	1543	57.2
4.5	17	994	58.5
5.0	25	1373	55.4
7.0	24	1103	46.0
9.0	17	668	39.3
11.0	6	192	32.0
13.0	5	125	25.0
15.0	4	77	19.0

velocity and is plotted as a function of concentration at several times of exposure. In a concentration of 10^{-1} M, sodium cyanide caused immediate immobilization of the cells.

The rate-time curves B and upper C, for the low concentrations, are above that of the control, A, on the coordinate field, indicating stimulation; those of the high concentrations, lower C and D, are below it, indicating inhibition. The lowest concentration tested had the most marked stimulatory effect. In spite of a ten-fold difference, the high concentrations inhibited to virtually the same extent. From these results it may be concluded that (a) the physiological effects of cyanide (stimulation versus inhibition)

Table 13. Effect of adrenaline chloride on rate of locomotion.

Control (29.1°C)

T	N	S	V
0.5	6	398	66.3
1.0	26	1864	71.7
1.5	27	2134	79.1
2.0	24	2091	87.2
2.5	22	1798	81.7
3.0	24	1873	78.0
3.5	22	1726	78.4
4.0	25	2076	83.0
4.5	21	1724	82.1
5.0	21	1596	76.0
7.0	19	1183	62.3
9.0	18	1064	59.2
11.0	18	909	50.5
13.0	23	1082	47.0
15.0	20	1025	51.3

1:10,000,000 (29.3°C)

T	N	S	V
0.5	13	1101	77.8
1.0	21	1828	87.1
1.5	27	2382	88.2
2.0	23	1931	83.9
2.5	29	2396	82.7
3.0	21	1754	83.6
3.5	23	1935	84.2
4.0	28	2122	75.8
4.5	25	1917	76.7
5.0	23	1550	67.4
7.0	23	1532	66.6
9.0	28	1840	65.7
11.0	34	1689	49.7
13.0	35	1961	56.0
15.0	32	1804	56.4

1:1,000,000 (29.2°C)

T	N	S	V
0.5	11	756	68.7
1.0	17	1100	64.8
1.5	20	1466	73.2
2.0	25	1500	60.0
2.5	25	1575	63.0
3.0	21	1397	66.5
3.5	23	1492	64.9
4.0	25	1556	62.2
4.5	21	1399	66.7
5.0	24	1537	64.1
7.0	17	927	54.5
9.0	23	1281	55.7
11.0	19	1101	57.8
13.0	24	1258	52.4
15.0	24	1355	56.5

1:100,000 (29.2°C)

T	N	S	V
0.5	3	111	37.0
1.0	14	744	53.1
1.5	18	1163	64.7
2.0	21	1289	61.3
2.5	24	1427	59.5
3.0	25	1471	58.8
3.5	25	1385	55.4
4.0	26	1544	59.4
4.5	27	1349	50.0
5.0	27	1469	54.4
7.0	19	914	48.1
9.0	28	1330	47.5
11.0	24	957	39.9
13.0	19	833	43.8
15.0	21	846	40.3

depend on concentration; and (b) at least a portion of the metabolic mechanism which furnishes energy for locomotion in paramecium is cyanide-insensitive.

The first conclusion is in line with the Arndt-Schultz Law, which has been stated by Thimann (1956) as follows (p. 156): "Every poison causes either a reduction or an increase in physiological performance, corresponding to...its concentration." Commenting on this principle, he says (p. 146): "...if a substance typically inhibits a process it commonly (not always) stimulates it at sufficiently low concentrations."

Stimulation-inhibition effects similar to those shown in Figure 19 have been noted in connection with the action of many different types of chemical substances on a wide variety of plant and animal organisms. Niethammer (1927), for example, found that the salts of chromium, silver and lead in very low concentration promoted the growth of Aspergillus. Cole (1938), studying the effects of sodium and potassium salts on responses in the barnacle, found that low concentrations caused opening of the valves and high concentrations caused closure. The stimulatory effects of cyanide in very low concentrations has been reported by Johnson (1951, p. 583), in connection with the production of light by luminous bacteria, and by Arisz, Camphuis, Heikens and van Tooren (1955, p. 330) in connection with the secretion

Figure 19. Effect of sodium cyanide on rate of locomotion. A-D, velocity versus time of exposure at several concentrations. Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes are minutes of exposure of cells to solutions. Curve in A is that of control. Cyanide concentrations are as follows: B, 10^{-5} M; C (upper curve), 10^{-4} M; C (lower curve), 10^{-3} M; D, 10^{-2} M.

E-G, velocity versus concentration at several time intervals. Values on Y-axes are mean rates of locomotion (read from curves in A-D) expressed as per cent of control velocity; values on X-axes are logarithms of molar concentrations (M) of cyanide. Time intervals are as follows: E, 4.0 minutes; F, 9.0 minutes; G, 14.0 minutes.

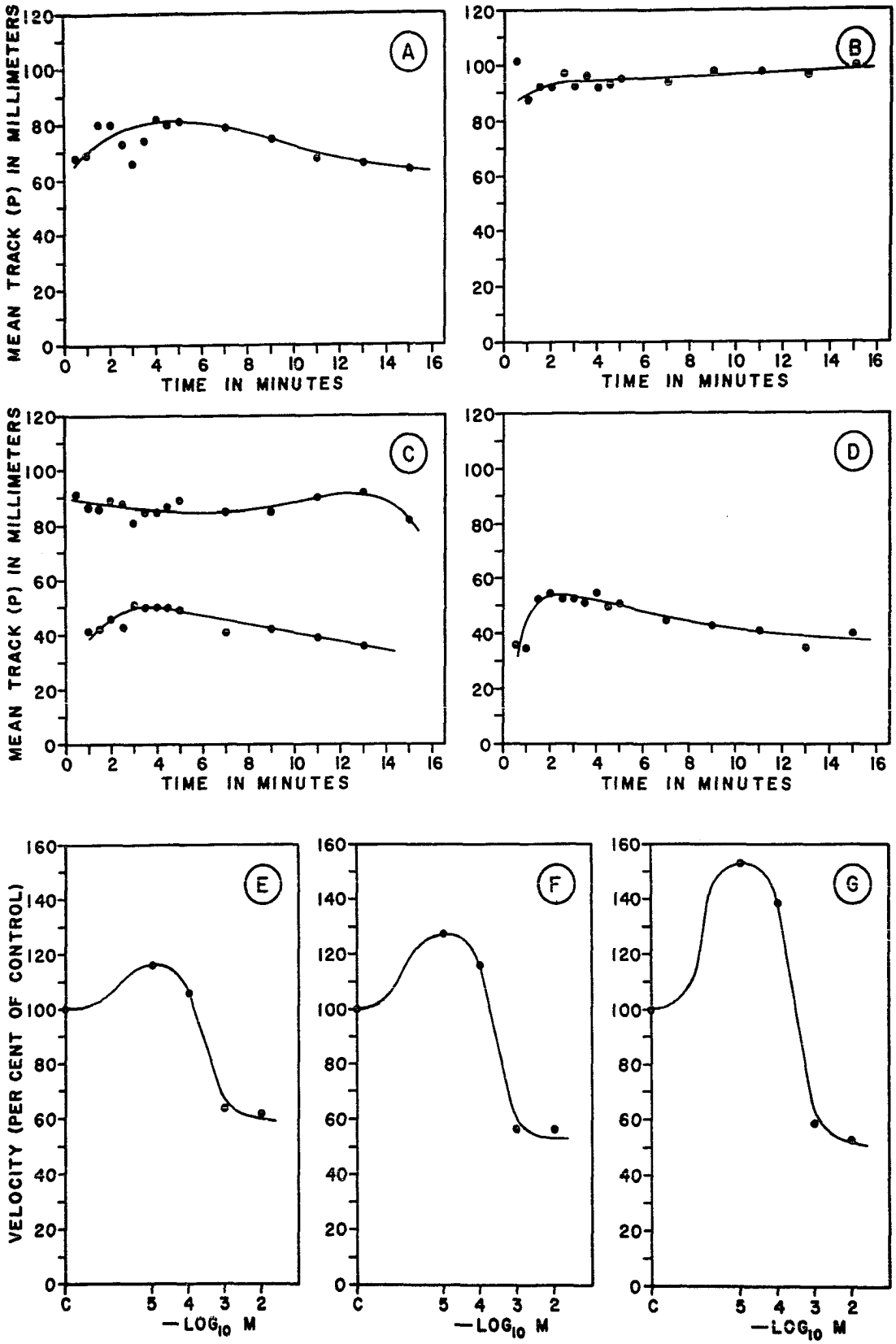

A
B
C
D
E
F
G
MEAN TRACK (P) IN MILLIMETERS
TIME IN MINUTES
VELOCITY (PER CENT OF CONTROL)
—LOG10 M

Table 14. Effect of sodium cyanide on rate of locomotion.

Control (28.0°C)

T	N	S	V
0.5	2	135	67.5
1.0	27	1858	68.8
1.5	28	2232	79.7
2.0	31	2477	79.9
2.5	31	2265	73.1
3.0	29	2210	76.2
3.5	33	2434	73.8
4.0	32	2623	82.0
4.5	33	2618	79.3
5.0	31	2513	81.1
7.0	37	2923	79.0
9.0	25	1882	75.3
11.0	28	1908	68.1
13.0	20	1315	65.8
15.0	31	1973	63.6

10^{-5}M (27.7°C)

T	N	S	V
0.5	16	1623	101.5
1.0	21	1838	87.5
1.5	28	2581	92.2
2.0	32	2942	92.0
2.5	28	2704	96.6
3.0	28	2583	92.2
3.5	34	3278	96.4
4.0	29	2667	92.0
4.5	36	3336	92.6
5.0	39	3714	95.2
7.0	29	2740	94.4
9.0	27	2642	97.8
11.0	32	3143	98.3
13.0	33	3163	95.8
15.0	33	3292	99.7

10^{-4}M (27.7°C)

T	N	S	V
0.5	14	1276	91.2
1.0	35	3035	86.7
1.5	35	3011	86.0
2.0	34	3024	89.0
2.5	41	3591	87.6
3.0	37	3000	81.1
3.5	37	3128	84.6
4.0	36	3069	85.3
4.5	36	3021	86.7
5.0	31	2746	88.7
7.0	38	3217	84.7
9.0	34	2896	85.1
11.0	36	3232	89.8
13.0	30	2752	91.8
15.0	31	2552	82.3

10^{-3}M (27.7°C)

T	N	S	V
0.5	--	--	--
1.0	13	531	40.8
1.5	21	872	41.5
2.0	16	740	46.2
2.5	19	809	42.6
3.0	24	1215	50.6
3.5	23	1155	50.2
4.0	22	1108	50.4
4.5	16	794	49.6
5.0	15	733	48.8
7.0	12	490	40.8
9.0	8	333	41.6
11.0	7	274	39.1
13.0	3	107	35.7
15.0	--	--	--

Table 14. (Continued).

10^{-2}M (27.7°C)

T	N	S	V
0.5	32	1139	35.6
1.0	23	814	35.4
1.5	34	1817	53.4
2.0	28	1531	54.7
2.5	27	1425	52.8
3.0	31	1633	52.7
3.5	19	975	51.3
4.0	18	998	55.4
4.5	14	705	50.3
5.0	13	667	51.3
7.0	10	452	45.2
9.0	7	302	42.9
11.0	4	163	40.8
13.0	2	70	35.0
15.0	2	81	40.5

of salt by the glands of certain tidal plants.

The second of the two conclusions drawn from the data in Figure 19 is supported in part by results obtained in other investigations. Lund (1918, 1921) was among the first to report cyanide insensitivity in the paramecium. He measured the rate of respiration of cells in KCN solutions for periods ranging up to fifty hours and concluded on the basis of his results that the respiratory process was entirely independent of the toxic effects of cyanide, even at concentrations strong enough to cause lysis of the cells. Similar observations were made by Gerard and Hyman (1931) and by Shoup and Boykin (1931). The latter workers found that

cells pre-conditioned in the absence of food in distilled water showed no further decrease in rate of respiration when transferred to solutions of KCN ranging in concentration from M/10,000 to M/200, although exposure times were as long as four hours. Working with luminescent bacteria, Strehler (1955) found that the mechanism of light production, though dependent on oxygen, was essentially unaffected by cyanide.

The possibility of alternate metabolic pathways, differing in sensitivity to cyanide and other toxic substances, is discussed by Ormsbee and Fisher (1943), and will be considered further in connection with the experiment on urethane. Thimann (1956), considering the contrasting effects of toxic agents in various concentrations, suggests that there are factors in living systems which normally retard, as well as promote, metabolic processes, and that the delicate balance between these influences may be shifted one way or another depending upon the relative sensitivities of the antagonists.

Relationships between cyanide sensitivity and such factors as cell age and state of nutrition have also been explored. Lund (1918) reported that young cells and those lacking food were more susceptible to the toxic effects of cyanide than those which were older and better fed. Pace (1945), in a similar type of study, found that the inhibitory effect of KCN on oxygen consumption was greatest on cells from newly-established cultures (5-7 days old) and

those in which there were high levels of carbohydrate (dextrose).

Cytochrome pigments have been reported in paramecia by Sato and Tamiya (1937), suggesting that at least a portion of the respiratory metabolism of this organism is of the usual type. This conclusion was reached by Boell (1942), who found that KCN, in the concentration ranges usually employed in studies of respiration, depressed oxygen consumption in Paramecium calkinsi by about 50%. Appraising his results, he says (p. 494):

> Insofar as susceptibility to cyanide and azide can be used as tests for the functioning in the cell of the cytochrome-cytochrome oxidase system, the results suggest that the respiratory mechanism of paramecium resembles that of most animal and plant cells.

F. Anesthetics

The effects of anesthetic agents on rate of locomotion are shown in Figure 20. Curves A through C represent the action of urethane (ethyl carbamate), while those of D through F represent the action of various alcohols. Rate of locomotion is plotted as a function of time for each of the several concentrations tested.

Relatively high concentrations of urethane were required to produce obvious anesthetic effects within the 15-minute period covered by the observations. Curves for the

Figure 20. Effect of anesthetics on rate of locomotion. A-C, urethane (ethyl carbamate); D-F, alcohol homologues. Values on Y-axes are mean rates of locomotion (X40) in millimeters per second; values on X-axes are minutes of exposure of cells to solutions.

Urethane concentrations are as follows: A (white symbols), 0.1 M; B, 0.001 M; C (black symbols), 0.01 M; C (white symbols), 0.2 M. Upper curve (black symbols) in A is that of control.

Alcohols and concentrations are as follows: D (white symbols), 0.4 M ethyl; D (black symbols, lower curve), 1.0 M methyl; E (white symbols), 0.08 M n-propyl; F (white symbols), 0.026 M primary iso-amyl; F (black symbols, lower curve), 0.064 M n-butyl. Upper curves (black symbols) of D, E are those of Control 1; upper curve (black symbols) of F is that of Control 2.

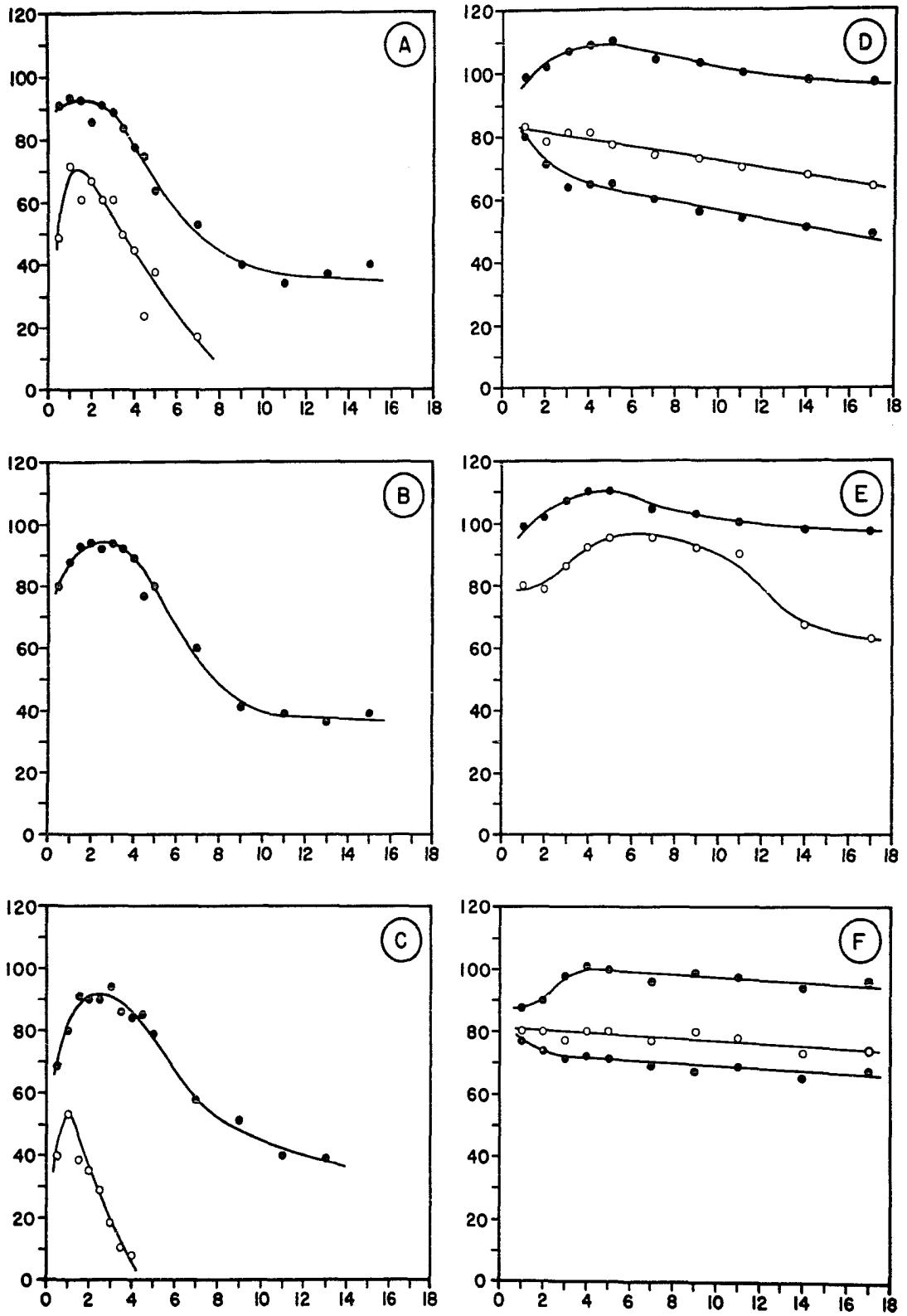

A
B
C
D
E
F
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18

Table 15. Effect of urethane (ethyl carbamate) on rate of locomotion.

Control (27.0°C)

T	N	S	V
0.5	10	909	90.9
1.0	30	2817	93.9
1.5	33	3079	93.3
2.0	33	2822	85.5
2.5	36	3279	91.2
3.0	38	3384	89.1
3.5	33	2769	84.0
4.0	40	3102	77.5
4.5	30	2258	75.2
5.0	25	1596	63.8
7.0	13	685	52.7
9.0	10	403	40.3
11.0	11	378	34.4
13.0	11	403	36.7
15.0	4	158	39.5

0.001M (26.4°C)

T	N	S	V
0.5	7	556	79.5
1.0	19	1672	88.0
1.5	28	2613	93.3
2.0	21	1981	94.3
2.5	25	2303	92.2
3.0	25	2344	93.8
3.5	30	2777	92.3
4.0	24	2137	89.0
4.5	20	1538	76.9
5.0	25	2013	80.5
7.0	15	907	60.5
9.0	14	558	40.6
11.0	16	617	38.6
13.0	14	498	35.6
15.0	8	311	38.9

0.01M (26.6°C)

T	N	S	V
0.5	6	415	69.2
1.0	14	1116	79.7
1.5	15	1359	90.5
2.0	22	1976	89.8
2.5	24	2159	89.9
3.0	26	2452	94.3
3.5	21	1809	86.2
4.0	27	2278	84.3
4.5	22	1869	84.9
5.0	19	1502	79.0
7.0	16	920	57.5
9.0	14	712	50.8
11.0	8	323	40.3
13.0	12	468	39.0
15.0	--	--	--

0.1M (27.0°C)

T	N	S	V
0.5	14	691	49.3
1.0	14	1012	72.3
1.5	17	1034	60.8
2.0	24	1598	66.7
2.5	26	1581	60.8
3.0	23	1396	60.7
3.5	20	992	49.6
4.0	9	402	44.7
4.5	6	144	24.0
5.0	5	192	38.4
7.0	3	51	17.0
9.0	--	--	--
11.0	--	--	--
13.0	--	--	--
15.0	--	--	--

Table 15. (Continued).

0.2M (26.5°C)

T	N	S	V
0.5	8	316	39.5
1.0	7	371	53.0
1.5	8	314	39.3
2.0	10	347	34.7
2.5	8	231	28.9
3.0	5	89	17.8
3.5	8	76	9.5
4.0	4	34	8.5
4.5	--	--	--
5.0	--	--	--
7.0	--	--	--
9.0	--	--	--
11.0	--	--	--
13.0	--	--	--
15.0	--	--	--

control (A, black symbols) and those of the two lowest concentrations tested (B, 0.001 M and C, black symbols, 0.01 M) are almost identical, although the slope of the tail (7-14 minute interval) in the 0.01 M curve is greater than that in the control. The curves for the two highest concentrations tested (A, white symbols, 0.1 M, and C, white symbols, 0.2 M) occupy much lower positions on the coordinate field and clearly indicate inhibition. In them, an abrupt peak is immediately followed by a steep, linear decline towards zero velocity.

Parallel and independent respiratory mechanisms, differing in sensitivity to urethane, may exist in some ciliates.

Table 16. Effect of alcohol homologues on rate of locomotion.

Control 1 (28.0°C)

T	N	S	V
1.0	47	4629	98.6
2.0	47	4798	102.1
3.0	47	5028	107.0
4.0	47	5154	109.6
5.0	47	5165	110.0
7.0	47	4911	104.4
9.0	47	4830	102.8
11.0	47	4707	100.2
14.0	47	4627	98.5
17.0	47	4575	97.4

Control 2 (27.5°C)

T	N	S	V
1.0	40	3511	87.7
2.0	48	4298	89.6
3.0	48	4699	97.8
4.0	49	4950	101.1
5.0	48	4814	100.5
7.0	49	4695	95.9
9.0	48	4730	98.7
11.0	48	4680	97.7
14.0	50	4696	94.0
17.0	50	4804	96.1

1.0M Methyl
(28.3°C-28.0°C)

T	N	S	V
1.0	47	3572	80.3
2.0	47	3320	70.7
3.0	49	3143	64.2
4.0	48	3202	64.7
5.0	45	2932	65.2
7.0	42	2516	59.9
9.0	42	2356	56.1
11.0	40	2176	54.4
14.0	35	1780	50.8
17.0	32	1560	48.8

0.4M Ethyl
(28.0°C)

T	N	S	V
1.0	46	3822	83.1
2.0	45	3567	79.3
3.0	41	3309	80.8
4.0	40	3252	81.2
5.0	31	2399	77.4
7.0	35	2595	74.2
9.0	21	1536	73.2
11.0	25	1742	69.7
14.0	17	1165	68.5
17.0	17	1079	63.5

Table 16. (Continued).

0.08M n-Propyl
(27.5°C)

T	N	S	V
1.0	16	1277	79.8
2.0	26	2058	79.2
3.0	47	4043	86.1
4.0	47	4336	92.4
5.0	47	4455	94.8
7.0	40	3789	94.8
9.0	20	1836	91.8
11.0	10	900	90.0
14.0	10	670	67.0
17.0	15	942	62.8

0.026M Primary iso-amyl
(27.5°C)

T	N	S	V
1.0	41	3267	79.8
2.0	41	3270	79.8
3.0	41	3142	76.7
4.0	41	3242	79.1
5.0	40	3175	79.4
7.0	41	3238	76.6
9.0	41	3263	79.7
11.0	42	3271	77.8
14.0	41	2998	73.2
17.0	48	3576	74.5

0.064M n-Butyl
(27.8°C)

T	N	S	V
1.0	34	2608	76.8
2.0	29	2141	73.8
3.0	25	1783	71.3
4.0	22	1595	72.5
5.0	40	2824	70.6
7.0	41	2808	68.6
9.0	42	2797	66.7
11.0	42	2879	68.6
14.0	44	2853	64.8
17.0	41	2775	67.7

Ormsbee and Fisher (1943) offer evidence which indicates that cell division in Tetrahymena (and probably also in Colpidium and Glaucoma) depends on a separate respiratory pathway which is relatively sensitive to urethane. They report that cell division is completely inhibited by concentrations not exceeding 0.1 M. As shown by the high concentration curves in A and C of Figure 20, locomotor activity in the paramecium is also completely inhibited in a corresponding concentration range (0.1 M to 0.2 M); however, it cannot be concluded from this fact alone that the metabolic pathway supplying energy to the cilia differs in any way from that which supplies energy for other cellular processes.

The curves in D through F of Figure 20 represent the anesthetic action of five homologous alcohols in five different concentrations. The test concentrations were selected with the hope of obtaining a set of superimposable rate-time curves, i.e., those having identical form and occupying comparable positions in the coordinate field. They were calculated by means of the relation $C_1 = C_2 a^n$ (Cole, 1938, p. 131; see also Traube, 1904, pp. 550-551), in which C_1 is the concentration of the lower member of a pair of homologues, C_2 the concentration of the higher member of the pair necessary to produce an effect equal to that of C_1, a the ratio of the successive concentrations, and n the difference in the number of carbon atoms in the homologues. In his studies of

the anesthetic action of alcohols on several animals, including the frog, the barnacle and the planarian, Cole found that the term a in the equation varied in value from about 2.8 to 3.0. On this basis, a value of 2.5 was arbitrarily assigned. C_1 for methyl alcohol was taken as 1.0 M. The calculated concentrations for the successive members of the homologous series were as follows: ethyl alcohol, 0.4 M; n-propyl alcohol, 0.16 M; n-butyl alcohol, 0.064 M; and primary iso-amyl alcohol, 0.026 M. The n-propyl alcohol proved in the tests to have much greater potency than was expected; cells exposed to it settled to the bottom of the observation chamber almost immediately. In order to obtain the response curve in F (white symbols) of Figure 20, the concentration was reduced 50%, to 0.08 M.

The rate-time curves in D and F of Figure 20 are unusual in that they are perfectly straight lines which almost exactly parallel their controls. With respect to linearity, they closely resemble the alcohol narcosis curves of Nagai (1907, p. 212). Except for that of n-propyl alcohol, they are very nearly superimposable, indicating that $a = 2.5$ rather closely approximates the correct value. In each curve set (control A, ethyl, methyl, n-propyl, and control B, n-butyl, primary iso-amyl), however, the curve representing the higher member of a pair of successive homologues is above its mate. From this it would appear that $a = 2.5$ is

somewhat excessive, i.e., that the successive concentrations were too dilute, rather than too concentrated.

According to Bills (1923, p. 55), the equi-narcotic concentrations (in per cent) of the first four members of the series methyl alcohol to n-propyl alcohol are, for the paramecium, 5.0, 3.3, 0.9 and 0.5, respectively; the ratios of the successive concentrations are 1.5, 3.7 and 1.8, respectively. An a-value on the basis of these figures would be in the neighborhood of 2.0, which is in line with the conclusion reached above. In the same paper, Bills states (p. 56) that the rate of increase of toxicity (as distinct from narcotic potency) of the alcohols reaches a maximum with propyl, which may explain the unexpected results with 0.16 M n-propyl described earlier. Both Bills (1923) and Macht (1920) report that iso-alcohols are less toxic than the corresponding normal primary alcohols.

VI. OPERATIONS ANALYSIS AND A PROPOSAL FOR AUTOMATION

Eight physiological studies have been presented which illustrate the potentialities of the photographic velocity scanning technique. In collecting the data for these experiments, nearly 800 scanning photographs were examined and over 19,000 individual tracks were measured. Nearly 300 hours were required for the execution of these studies, of which a total of 198 hours were allocated to four major operations as follows: (a) experimentation (including scanning), 12%; (b) film processing, 9%; (c) film inspection (track measurement), 63%; and (d) tabulation and graphic analysis of data, 16%. Operations involving measuring and data handling thus account for 80% of the time required for this research. Stated in another way, for every hour spent in performing actual experimental work, eight additional hours were spent in making track measurements and processing data. Fortunately, the two operations which are most tedious and time-consuming, and which greatly reduce the overall efficiency of the photographic scanning technique, are also those which are most susceptible to electronic automation methods. Proposals for automatic track measuring and data

processing will now be considered.

Seven main operations are involved in the preparation from a scanning photograph of a rate-time curve representing the locomotor activities of cells. These are: (1) observation (inspection of the photographic record); (2) mensuration (measurement of the length of individual tracks); (3) tabulation of data (recording of the length measurements and other numerical data); (4) enumeration (counting of tracks measured on each scanning photograph); (5) summation (adding the lengths of all tracks measured on a given scanning photograph); (6) computation (calculation of the mean track length from each set of measurements); and (7) presentation of results (preparation of a graph showing the relationship between mean track length and time of exposure). From the point of view of electronic automation, these operations may be categorized as scanning (1), computation (2 through 6), and read-out (7).

Fortunately, track images are sharply defined on photographic negatives as opaque (black) lines on a transparent background. As such they are susceptible to inspection by an electronic technique known as flying-spot scanning (Mansberg, 1957). The principal elements of a flying-spot scanner are (1) a high-resolution cathode ray oscilloscope; (2) a photomultiplier; (3) an optical system joining the oscilloscope and photomultiplier; and (4) some type of digital

electronic computer. The flying spot is produced by focusing the beam of the oscilloscope to a very small, bright point. Electrical controls cause the spot to move horizontally across the oscilloscope screen at a constant rate and in a single direction. Between successive traverses, the beam is advanced along the vertical axis of the screen a distance equal to its own diameter. The spot thus sweeps the screen much as the eyes of a reader move across and down the lines of type on a printed page.

By means of a system of lenses, the image of the oscilloscope screen is focused in the plane of the scanning field, which contains entities to be enumerated or measured. This field and the superimposed screen image are surveyed from the opposite side by the photomultiplier tube. As the image of the bright point on the oscilloscope screen moves across the scanning field it crosses and is occulted (blocked out) by objects in its path. The photomultiplier translates the resulting changes in light intensity into electrical impulses which are fed into the digital computer for processing and interpretation. The computer, in turn, prints out the results or displays them as traces on the coordinate field of an oscilloscope screen.

Such a system is more than a mere theoretical possibility; it is a practical, working reality. In view of the evidence which has been presented in preceding pages, there

is every reason to believe that a photo-electronic locomotion analyzer, combining the desirable features of photographic and electronic scanning and automatic electronic data processing, would have innumerable useful applications in experimental and applied biology. It is the author's hope that the development of such an instrument can be undertaken as an extension of the research reported in this thesis.

VII. SUMMARY

1. Rate of locomotion as an indicator of physiological state in microorganisms has been considered.

2. Direct, photographic and electronic methods for determining the rate of locomotion of microorganisms have been reviewed.

3. Apparatus and techniques of an improved photographic scanning technique have been described in detail.

4. Experiments illustrating research applications of the photographic scanning technique have been performed with Paramecium caudatum.

5. Experimental data have been presented which indicate (a) the relationship between rate of locomotion and environmental temperature, osmotic pressure and pH; and (b) the effect on rate of locomotion of various chemical influences, including acetylcholine, adrenaline, sodium cyanide, urethane and various alcohols.

6. Results of experiments have been interpreted and discussed in relation to published literature.

7. Photographic scanning operations have been statistically analyzed.

8. Development of an automatic photo-electronic locomotion analyzer has been proposed.

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