A CONTRIBUTION TO THE TECHNIQUE OF POTENTIAL DIFFERENCE MEASUREMENT ON SINGLE-CELLED ORGANISMS

by

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INTRODUCTION

A. Potential Difference and its Significance in Biology

The concept of "difference of potential", or "potential difference", [1] is perhaps the most frequently employed concept amongst all those in the field of electricity. It plays a prominent role in practically any and all considerations involving electrical theory or electrical instruments. This concept demands for its formulation a comprehension of two more primary conceptions, namely, "charge" and "work". In terms of these primary conceptions, for adequate statements of which reference must be made to texts on mathematical physics, P.D. may be defined in the following statement:

> The P.D. between two points, A and B, is the work required to transport unit charge between point A and point B.

It might reasonably be supposed that the concept of P.D. can play no role in biological considerations, that the phenomena in which it takes origin make no appearance in living things. Such, however, is distinctly not the case. Rather, P.D.'s have been demonstrated in a host of animal and plant forms, as both 1) a normal accompaniment of metabolism, and 2) as a phenomenon accompanying the specific functioning of an organ system, an organ, or a cell. A complete exposition of the nature and range of these data is not within the proper scope of this paper, but the following tabular summarization is perhaps appropriate:

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1. Hereinafter, the abbreviation "P.D." will be employed in place of the phrases "difference of potential" and "potential difference".

TABLE I[1]

P.D.'s Occurring in Living Materials

Organism	Points between which P.D. is measured	Type of P.D.			Attendant Upon	Observer
		Constant	Varying	Temporary		
Man	2 pts. on cranium		✓[2]		Vision & other brain functions	Gerard ('37)
Man	2 index fingers			✓	Ovulation	Burr & Musselman ('36)
Rabbit	Vagina and abdomen			✓	"	Burr, Hill & Allen ('35)
Man and other forms	Fore-limb & opposite hind-limb		✓		Heart pulsations	Howell ('31)
"	2 pts. along nerve or nerve fiber		✓[3]		Passage of Nerve impulse	Adrian ('32)
"	2 pts. on a muscle			✓[4]	Muscular contraction	Howell ('31)
Paramecium caudatum	Cytoplasm & external medium	✓			Normal metabolism	Kamada ('34)
Ameba terricola	"	✓			"	Buchthal & Peterfi ('37)
Ameba sp.?	"	✓			"	Telkes ('31)
Hydroids	Apical & distal ends	✓			"	Barth ('34)
Fir tree	2 pts. on branch	✓			"	Lund ('32)
Halicystis	Cell sap & external medium	✓			"	Blinks ('29-30)
Nitella	"	✓			"	Osterhaut ('29-30)

1. This summary is not in any sense exhaustive. Thus, no attempt has been made to tabulate all known-biologically-manifested P.D.'s. Further, only one observer is recorded, though most of the P.D.'s have been observed by a number of investigators. The reference under "Observer" refers in some instances to a review article, or book.
2. The "Berger Rhythm". 3. The so-called "action current".
4. The "action current" of muscle.

Theoretical interpretation of the biologically-manifested P.D.'s, either constant or varying, such as are listed in Table I, is a topic upon which agreement has not been reached. Rather, interpretations based upon entirely distinctive conceptions have been advanced by various investigators of biological P.D.'s. Thus, Lund ('28) attempts to explain a constant P.D. between two points in a living system in terms of oxidation-reduction potentials, whereas Gerard ('37) postulates rhythmic discharge of masses of neurons as the cause of the "Berger rhythm". Dubuisson ('34) presents a critique of the various interpretations which have been advanced.

The studies described herein relate to P.D.'s obtaining in a unicellular form, Chaos chaos. As is indicated in Table I, a number of studies on unicellular plants and animals have previously been reported. The significance attaching to such single cell studies is, therefore, a topic which may appropriately be discussed. The writer, it must be stated first of all, has not encountered any explicit statement of views on this matter in the literature of the field. However, it is apparent that significance attaches to P.D. studies on single cells because of the roles such studies play in the elucidation of fundamentally significant biological problems. In support of this view, the following specific citations are given:

1. Loeb and Cattell ('15) showed that eggs of Fundulus immersed in KCl solution took up sufficient amount of the salt to stop the heart beat, and that the heart beat could be restored by subsequent immersion of

the embryo in a solution of some other salt (Na halides, $NaNO_3$, NaCNS, and others), but that the heart pulsations could not be restored by immersion in distilled water. It is reasonable to postulate, as did Loeb and Cattell ('15), that the phenomena just described are dependent upon permeability characteristics of the envelopes of the Fundulus egg, especially upon permeability to various ions. Now, Michaelis ('29) has shown, by P. D. measurements as well as by chemical studies, that some natural membranes (e.g., apple skin) and some artificial membranes (e.g., dried collodion) are permeable for cations when cations on two sides of the membrane can be exchanged. Sumwalt ('29), by P.D. measurements between the chorion of the Fundulus egg and the external medium, has shown that the chorion behaves in a manner closely analogous to Michaelis' collodion membranes, and has thus provided an interpretation of the results of Loeb and Cattell ('15).

2. It has been abundantly demonstrated that

rhythmically varying P.D.'s are produced by aggregations of nervous tissue (entire brain, spinal cord, isolated ganglia, etc.) (Gerard, '36). The interpretation of such electrical variations which is at present most widely accepted is that they are due to synchronous electrical discharge of large numbers of neurones; in the words of Gerard ('36), "—— it is difficult to observe the perfectly smooth, regular and large potential waves of the frog olfactory bulb, obtained with a concentric electrode whose leads are not separated over 0.2 mm., without being strongly inclined to the belief that they correctly represent the potentials of nerve cells beating together in perfect synchrony". This statement obviously does not commit its author to any specific hypothesis concerning the nature of those changes occurring in a single neuron which may occasion the "beating", or rhythmic electrical discharge. However, it does clearly indicate the need for investigations on the electrical phenomena occurring in a single cell, namely, the neuron; this need is emphasized in Gerard's ('36) further statement, "The crucial evidence for the existence of slow cell potentials must come from studies on single or small numbers of

neurons isolated in space or by the conditions of the experiment".

The two foregoing citations sufficiently indicate, in the writer's view, that critical experimental data on the P.D.'s which may be manifested by single cells are, or in the future may be, of considerable significance as contributions toward the comprehension of distinctive and important biological phenomena.

B. Micrurgy and its Application to Electrical Studies on Single Cells

Development of micrurgical technique within the past thirty years has made realizable direct experimental attacks upon innumerable problems of cellular morphology and cellular physiology. For, by the use of micromanipulators, it is possible to obtain precise control of the movement of various micro-instruments in the microscopic field, and hence accurately to direct a micro-needle, micro-pipette, or micro-electrode upon or into a single cell. [1] The special application of this technique made herein to the problem of cellular P.D. measurements is described below (p. 42). At this point, however, it seems appropriate to remark at short length upon the marked advantages accruing in particular to the study of the electrical properties of cells as a consequence of the development of micrurgy. Studies on 1) protoplasmic conductivity, and 2) cellular P.D.'s will be discussed, as follows:

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1. An excellent discussion of the history and present status of the micrurgical technique, in its various aspects, is given by Chambers and Kopac ('37).

1. Gelfan ('27), by use of the micrurgical technique, inserted two electrodes into a single specimen, in the case of a number of protozoan cells, and, by measuring the electrical resistance between the two electrode tips, was able to obtain measurements of the electrical conductivity of the various cytoplasms. This is, obviously, a straightforward approach to the problem of cytoplasmic conductivity, and is one made possible, in its application to the vast majority of single cells, solely as a consequence of the development of means whereby electrical contact can be established with cytoplasm at some precise point within a microscopic cell. As regards directness and probable accuracy, it is decidedly preferable to an older technique of conductivity measurements, exemplified by the work of Osterhout ('22), in which a stack of discs cut from the fronds of Laminaria is pressed between the surfaces of two flat platinum electrodes.

2. Telkes ('31), Kamada ('34), and Buchthal and Peterfi ('36-'37) have presented studies on the P.D.'s existing between one electrode

presumably [1] contacting the cytoplasm and a second electrode dipping into the external medium environing the cell. Both electrodes are manipulated by means of a micromanipulator. Accurate placement of electrodes here again makes possible a direct experimental approach in the measurement of the P.D. in question. As a technique of experimental study, it is distinctly to be preferred to an earlier approach to the problem of cellular P.D.'s which is exemplified by the work of Hardy ('15), in which P.D.'s between cell organelles were gauged on the basis of displacements (of cell parts) which occurred in cells exposed to electrical currents.

C. P.D. and Electromotive Force

"P.D." is commonly employed as though it were synonymous with "Electromotive Force", or E.M.F. It must be pointed out, however, that differentiation between the two terms is necessary, and that, furthermore, in the present investigation an attempt has been made to measure E.M.F.'s, rather than P.D.'s.

It is not within the proper scope of this paper to expound the basis for the distinction between E.M.F. and P.D.; full treatments of this topic are available in textbooks of physics. [2] The follow-

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1. See p. 14 for reason for this qualification.
2. See Mendenhall, Eve, and Keys ('35, pp. 345-47).

ing facts may appropriately be pointed out, however:

> In case current is being drawn from a battery, say, a voltmeter applied to the battery reads the P.D. between the battery terminals. This P.D. is a function of the current drain and can in no sense be said to characterize the battery. But if no current is drawn from the battery, the voltmeter applied to the battery terminals reads the E.M.F. of the battery, a quantity which is characteristic of the battery.

The smaller the current drain imposed upon the battery, the closer do the voltmeter's indications come to being indications of the true E.M.F. Obviously, evaluation of E.M.F.'s, rather then P.D.'s, is what must be sought in measurements such as those herein reported on single-celled organisms. As is shown in Appendix C, the voltmeter herein employed possesses such high input impedance that the current drawn from the specimen can be regarded as negligible. Nevertheless, since minute current is drawn from the specimen, it is best to speak of the voltmeter's indications as being those of P.D.

REVIEW OF LITERATURE

The studies described herein deal with P.D.'s existing between two electrodes placed in relationship to the protozoon, Chaos chaos, one electrode being in contact with the cytoplasm whereas the second electrode contacts the liquid medium environing [1] the organism. [2] Such P.D. measurements on this form have not heretofore been reported. However, studies on other unicellular forms have appeared, and these antecedent studies must now be considered.

Unicellular plants, particularly Valonia and Halicystis, have been studied rather extensively, due to their large size and ostensible simplicity of organization. Due, however, to differences in morphological organization as between such algae and protozoan cells, these studies are not of direct concern for the present study, and need not be discussed further. [3]

Studies on protozoan cells, in which one electrode presumably (p. 14) contacted the cytoplasm and the second electrode contacted the external medium, have been reported by Ettisch ('28) (Ameba terricola), Telkes ('31) ("an ameba", not identified), Kamada ('34) (Paramecium caudatum), and Buchthal and Peterfi ('36-'37) (Ameba terricola and Ameba proteus). In summarizing these studies, it is desirable first of all to state certain pertinent experimental requirements, fulfillment of which is requisite if the results of such studies are to be considered trustworthy, and to indicate to

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1. Hereinafter designated as "external medium".
2. P.D.'s existing between such two regions, regardless of the experimental organism in which they occur, will hereinafter be designated as "cytoplasmic P.D.'s". See p. 53 for analysis.
3. For a review of these studies, see Osterhout ('31).

what extent each of the above-mentioned studies fulfills the specified experimental requirements.

Requirement #1: The external medium must be known, in order that the results obtained may be susceptible of re-examination.

Ettisch ('28) and Kamada ('34) satisfactorily fulfill this requirement. Telkes ('31), however, does not do so, since the external medium employed consisted of unspecified volumes of various simple salt solutions added to unspecified volumes of an unspecified culture medium. Buchthal and Peterfi ('56-'57) likewise fail to fulfill this requirement, for they employ, as external medium, the same Knopp agar in which their specimens have been cultivated, the accumulated metabolites in which render the medium entirely unknown.

Requirement #2: The electrolyte in the micro-electrode tips must be identical with the external medium if an unknown liquid junction

potential, at the surface of contact of the external electrode [1] with the external medium, is to be obviated.

Ettisch ('28) fails to fulfill this demand, since the external media employed were "physiological KCl" and distilled water, whereas his micro-electrode tips were filled with either 0.1N KCl or 0.1N NaCl (both electrolytes being suspended in agar). Telkes ('31) fails to fulfill this demand, contacting various external media (largely unknown, as indicated under #1, above) with micro-electrode tips which are filled with 1 N KCl. Kamada ('34) nicely fulfills this requirement, employing micro-electrode tips filled with the external medium under investigation (and of which he employs a considerable variety). Buchthal and Peterfi ('36-'37) again fail to meet this requirement adequately. For, though they fill their micro-electrode tips with Knopp agar, the same medium upon which they culture their organism (Ameba

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1. "External electrode" designates that electrode contacting the external medium; this designation will hereinafter be consistently employed.

terricola), they report measurements in which the same culture medium in which the organism has been living serves as external medium (as mentioned under #1, above); and this is obviously objectionable because of the presence of accumulated metabolites in such an external medium.

Requirement #3: The electrolyte in the internal electrode [1] must be in direct communication with cytoplasm, obviously, if measurements of "cytoplasmic P.D.'s" are to be made.

The difficulty here involved is that an electrode tip stuck into a cell may actually not have ruptured the ectoplasmic wall (cell wall), but only have indented same, [2] so that the electrolyte in the microelectrode tip actually contacts ectoplasm rather than cytoplasm. It is impossible to determine,

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1. "Internal electrode" designates that electrode with which one seeks to contact the cytoplasm; this designation will hereinafter be consistently employed.
2. See Chambers ('22-'23), p. 189, who states : "Pushing a pipette, especially a comparatively large one, into an egg cell frequently causes the surface of the cell to become invaginated and thus forms a deep pocket. The tip of the pipette, even if it should finally break through the surface, is apt to be separated from the protoplasm of the interior by the formation of a new surface film continuous with the original surface of the cell."

on the basis of the published reports alone, in what measure the results of previous workers may be invalidated because of failure to rule out this possible source of error. However, Telkes ('31) and Kamada ('34) evince no awareness of this possible source of error, nor is the technique of either such as to obviate the possibility of its supervention. Ettisch ('28) and Buchthal and Peterfi ('36-'37) are aware of the difficulty, however, for Ettisch and Peterfi ('25) state:

> "Oft kommt es vor, dasz man beim Andrücken der Elektrodenspitze die Zellhaut nur einbuchtet, und obzwar man bei der geringen Dicke des Objektes im mikroskopischen Bilde den Eindruck erhält, wie wenn die Elektrode bereits in der Zelle wäre — da sie ebenso scharf eingestellt erscheint, wie der Zellkörper selbst — kann sie in Wirklichkeit noch auszerhalb der Zelle in einer Bucht der Zellmembran liegen, die sie vor sich hergeschoben hat. Eine in solchen Situation vorgenommene Messung hat natürlich keine wissenschaftliche Bedeutung, da ja beide Elektroden sich in demselben Medium befinden".

Nevertheless, neither Ettisch ('28) nor Buchthal and Peterfi ('36-'37) have employed any technical step which unequivocally makes for conformance with the experimental requirement set forth above.

Requirement #4: The current drain imposed upon the specimen by the voltmeter must be negligibly small if the "cytoplasmic P.D." measured is to approach the E.M.F. characteristic of the cell. (see pp. 8-9)

Ettisch ('28), Telkes ('31), Kamada ('34), and Buchthal and Peterfi ('36-'37) have all conformed with this requirement, employing either electrometers or vacuum tube voltmeters.

Requirement #5: The external medium must sustain the organism in its normal morphological and physiological state if the "cytoplasmic P.D.'s" measured are to be considered as characteristic of the organism.

This requirement seems to the writer to be adequately fulfilled if the organism manifests its characteristic morphology and physiology after an exposure of considerable duration (say, 1-2 hours) in the given external medium; but if the external medium

is a fresh preparation of the same solution in which the organism has been cultivated, then there can be no doubt concerning complete fulfillment of this experimental requirement.

Ettisch ('28) states that his Ameba terricola specimens become tensely spherical ("prall kugelig") in distilled water and (insofar as the text can be judged) likewise in physiological KCl and Knopp's solution, the external media he employs; such specimens are obviously abnormal, and the experimental requirement is not fulfilled. Telkes ('31) gives no information concerning the condition of the organism she studied, other than to state the organism was killed within various periods of time in various external media. Kamada ('34) deals adequately with this requirement, exposing Paramecium caudatum for 2 hours to any chosen external medium, and then observing each specimen for normalcy of morphology and physiology before attempting a measurement on same. Buchthal and Peterfi ('36-'37) fulfill this experimental requirement, employing the culture medium in which the organism

has been cultivated as external medium. (But see #1, above, for objection to this procedure.)

Requirement #6: The organism must be specified if the results are to be considered as a valid contribution.

Telkes ('31) alone fails to meet this requirement, stating merely that she studied "an ameba". Kamada ('34) studied Paramecium caudatum. Ettisch ('28) studied Ameba terricola. And Buchthal and Peterfi ('36-'37) studied Ameba terricola and, to a slight extent, Ameba proteus (= Chaos diffluens, Schaeffer, presumably).

It is apparent that non-fulfillment of one or more of the experimental requirements just set forth renders the results which are obtained of highly questionable value. It is further apparent that, except for the study made by Kamada ('34), the reports of all previous studies on "cytoplasmic P.D.'s" indicate non-fulfillment of one or more of the requirements; the work of Kamada ('34) is questionable only on the ground that he offers no assurance of the fulfillment of Requirement #3. It seems to the writer, therefore, that it is hardly worthwhile to discuss the experimental results heretofore announced at very great length, and that the following tabular summarization of these results is adequate:

TABLE II

Results of "Cytoplasmic P.D." Studies

Worker	Organism	External Medium	Cytoplasmic P.D. Polarity	Cytoplasmic P.D. Magnitude (Millivolts)
Ettisch ('28)	Ameba terricola	Distilled water Physiological KCl	--- ---	0 0
Telkes ('31)	Not specified	Not adequately specified	Cytoplasm ---	15 (an average valve)
Kamada ('34)	Paramecium caudatum	Various salt solutions, of 1 or more salts	Cytoplasm + in some solutions, - in others	28.7 in solution of KH_2PO_4, NaOH, and NaCl (Cytoplasm -) to 19.2 in N/40 $CaCl_2$ (Cytoplasm +)
Buchthal and Peterfi ('36-'37)	Ameba terricola	Culture medium (Knopp agar)	Cytoplasm + in 50% Cytoplasm - in 50%	1 - 1.5

MATERIAL

The organism employed in this study was the ameba, Chaos chaos (Type A) (Schaeffer, '36-'37 & '37). This is a multinucleate form which possesses many characteristics in common with Ameba proteus, but which differs from A. proteus notably in size; thus, an average figure for the length of A. proteus is 0.6 mm., whereas Chaos chaos often attains a length of 4-6 mm. This great size has made relatively easy the micromanipulative technique involved in this study.

Chaos chaos is a form which has been found very rarely in nature (Schaeffer, '36-'37). Schaeffer rediscovered the organism in a New Jersey marsh in 1936, and succeeded in his attempt at culturing, with the highly desirable result that the organism is now continuously available for experimentation.[1] Schaeffer[2] cultures the organism in hay infusion, adding Paramecium as food organism. Fortunately, this culture technique has proved modifiable in the sense that a dilute NaCl solution is exceedingly satisfactory as liquid medium in place of the hay infusion. (The significance for the present study of this simplicity of medium will be remarked upon below.) (p. 44) The culture technique which has accordingly been employed is as follows:

A watch glass (standard size, i.e., 2" internal diameter) is thoroughly cleaned and rinsed in double-distilled water.

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1. Supplied commercially by General Biological Supply House, Chicago.
2. Private communication, February 2, 1938.

0.001% NaCl, made up in double-distilled water from C.P. NaCl, is poured into the watch glass, to a depth, say, of 1/8 - 1/4 inch. The amebas are then pipetted from their original medium into the 0.001% NaCl.[1] Paramecia from a rich culture are then added, as little as possible of their culture medium being carried over. After 4 - 7 days the organisms must be transferred, presumably because of the accumulation of metabolites in the culture fluid; transfer is made, of course, into another watch glass prepared in the manner just described.

No accurate data on reproductive rate have been taken, but it appears that the organisms reproduce at about the rate Schaeffer finds[2] in hay infusion cultures, i.e., a trebling of number each three days, or so.

Plate I shows the culture vessel and the organisms, which appear as white threads.

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1. The amebas may appear unhealthy when first transferred into the dilute NaCl, but soon (1/2 - 1 hour) recover and appear perfectly normal.
2. Private communication, February 2, 1938.

APPARATUS

A. Microscopical, Micrurgical, and Electrical Apparatus

1. Inverted Microscope [l] [1]

Micrurgy has largely been performed by use of the "hanging drop" technique, i.e., the experimental material is placed upon a cover-glass in a drop of liquid medium, the cover-glass is then inverted over a hole in a "moist chamber", and the micro-instruments are then inserted into the sides (or front) of the chamber and up into the experimental material from below (Chambers, '17). From the outset, this technique appeared faulty in its application to the present research. For amebas placed upon a clean cover-glass for 15 - 30 minutes would, it was true, generally cling to the glass when it was inverted, and hence would be in favorable condition for insertion of a micro-instrument from below. But amebas once so pierced lost their hold on the glass and sank to the air-water interface, and could not be pierced a second time. For an ameba floating on the air-water interface of an inverted drop can hardly be pierced by a micro-tip, the organism rolling passively off the tip and, through no activity of its own, evading the micro-tip [m] in a most exasperating manner. It was obvious that re-measurements on a single individual might well be desirable, and hence recourse was had to a technique which involves use of an inverted microscope and which avoids the use of "hanging drop" preparations.

The inverted microscope, in which the objective approaches the

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1. Numbers or letters in brackets refer to pieces of apparatus, or parts thereof, and correspond with the numbers and letters employed in Plates II, III, IV, V, & VI, and Figures I, II, & III.

experimental material from below, has been introduced into micrurgical practice only in recent years, and is recommended on a number of grounds (Chambers and Kopac, '57). An instrument of this type is built by Leitz in accordance with the design of Chambers (Leitz pamphlet #7276). Since, however, this rather expensive commercial instrument was not available, construction of an instrument was undertaken. It is assembled chiefly of parts of a student laboratory microscope, plus two small prisms, as follows (Plate II, a & b):

> The arm [a] of a student laboratory microscope (of Spencer manufacture) is detached from its stage and stand, and affixed to a solid wooden base [b]. Attached to the body tube in place of the nosepiece, by means of an externally-threaded collar, is a metal prism chamber [p-c]; a lock-nut is of course provided in order firmly to fix the prism-chamber in proper position. The chamber [p-c] is 3 1/4 in. in length, and its internal cross-section measures 1 sq. in.; it is constructed of 1/8 in. sheet aluminum. The prisms are located, of course, at the opposite ends of this chamber. Each prism is held in place by screws projecting into the sides of the chamber.

The prisms are of good quality, the faces measuring 1 sq. in. Two objectives are immediately available, a 32 mm. lens [32] for scanning, and a 16 mm. lens [16] for the micromanipulations. Comparison of Plate II, a and Plate II, b shows the manner in which these two objectives are mounted on a sliding brass plate [b-p], which moves lengthwise over the upper surface of the prism-chamber [p-c]; both objectives screw into internally-threaded collars mounted on the sliding brass plate [b-p]. Either objective can readily be moved into position above the outer prism merely by moving the plate [b-p] forward or backward. The sides of the sliding plate [b-p] slide against guides, so as to insure its movement along a straight line.

Illumination of the objectives [16 & 32] is obtained thru use of a second 16 mm. objective [il], which is supported in a vertically- and horizontally-adjustable mounting [x], and which is itself illuminated by light reflected from a small mirror [M] mounted above it. The light source [L] is an ordinary 40 watt

bulb. (Such an arrangement cannot, of course, qualify as "critical illumination", but it is adequate for micro-manipulations in the low-power microscopic field.)

The purposes of a stage are fulfilled by an ordinary depression slide [S]. This is supported between the viewing and illuminating objectives by a slender rod which arises off of an ordinary mechanical stage [ms], which is itself affixed to the base [b] which supports the microscope arm [a].

A short vertical guide post [il] is convenient for alignment of the prism-chamber [p-c] with the illuminating objective [il], in the event the prism-chamber [p-c] becomes rotated on the body-tube of the microscope.

Use of this inverted microscope is probably obvious; however, a few explanatory remarks may possibly be in order. With an ameba on the stage [S] and the illuminating objective [il] lighted, the 32 mm. objective [32] is focused on the stage [S] (upper surface, of course) and the stage [S] then moved until the ameba is at the center of this low-power field. The micro-electrode tips [m] are then moved into this low-power field and brought fairly close to the specimen. Employment now of the 16 mm. objective [16] finds the organism in a field with the 2 micro-electrode tips [m], ready

for the establishment of contact by one or both micro-electrode tips [m] (Plate III, a, b).

2. Micro-electrode [2]

Electrical contact between the cell under investigation and the voltmeter (p. 30, and Appendix C) must obviously be established. The instrument directly contacting the organism is, in the event this latter is a microscopic form, commonly known as a "micro-electrode".[1]

Micro-electrodes have been employed by a number of investigators (Gelfan, '27; Ettisch and Peterfi, '25; Buchthal and Peterfi, '36-'37; Kopac, '36; Telkes, '31; Kamada, '34; etc.) All designs are of the "reversible" type, i.e., they are non-polarizable. Electrical contact with the cell is established by means of an electrolyte contained in a micro-pipette, and existing there either as pure electrolyte or as electrolyte stiffened by mixing with agar. For the present investigation, accurate control of the flow of a liquid electrolyte in the micro-electrode tip was desired, for reasons which appear below, (p. 29) and hence a combined micro-electrode and micro-pipette was designed. Kopac ('36) has likewise designed a combined instrument which is, however, considerably different from the present one, description of which now follows (see Fig. I): [2]

Pyrex tubing is used to fabricate the pipette body [p] and pipette shank [p'].

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1. But see Blinks ('30) on the preferability of the term "micro-saltbridge".
2. Figure I is natural size, and hence no measurements are stated in the written description.

A short side-tube [t] is welded to the pipette body [p] in order to receive a small rubber stopper [r]. Into the shank [p'], near its junction with the pipette body [p], a small rubber diaphragm [d] [1] is sealed, de Khotinsky cement being used to fasten the rubber to the glass. Filling the lumen of the pipette body [p] in front of the diaphragm [d] is the liquid electrolyte [el], namely, 0.001% NaCl. The shank [p'] fits closely onto the neck [h'] turned on a center-threaded brass head [h]. A screw [s] (32 threads/in.; 6 gauge) turns in this brass head [h] and contacts the rubber diaphragm [d]; this screw [s] is furnished with a hollow screw-head upon which a Sulfur [2] handle [H] has been molded. The brass head [h] is supported in Sulfur at the center of a sturdy bronze ring [R], roughening of the inner surface of the ring [R] plus knurling of the head [h] together serving

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1. This diaphragm is merely a section cut from a rubber stopper.
2. Sulfur is used here, as elsewhere thruout the assembly, because it is an excellent insulator, and because of the facility with which it may be poured into position or molded (in paper molds). It is poured when molten.

to prevent rotation of the head [h] when the screw [s] is turned against the diaphragm [d]. On its lower surface, the ring [R] receives a heavy screw, by means of which the entire assembly is securely mounted on the micromanipulator control unit [3]. Arising from the upper surface of the ring [R], a brass tube serves as carrier of a conducting wire [Cw] which leads off to the voltmeter [5]. The conducting wire [Cw] is, of course, imbedded in Sulfur. A 2-piece shield [S_f, S_r], fashioned of large-size brass tubing (plus flat end-pieces, of course) is likewise supported by the ring [R]. The end-piece of the rear section of the shield [S_r] allows egress of the Sulfur handle [H], whereas that on the front section of the shield [S_f] allows egress of the micro-electrode tip [m]. [1] Since the ring [R] is fixed securely to the micromanipulator control unit [3], and since, further, the control unit [3] is "grounded", the shield [S_f, S_r] is necessarily at ground potential and serves to shield the electrode completely

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1. For fabrication of micro-electrode tips, see Appendix A.

from external electrical fields.

The small rubber stopper [r], sealed with de Khotinsky cement into the side-tube [t], supports a most important item, namely, the Ag-AgCl reversible electrode [E]. This is simply a Ag wire chloridized [1] at one end. At this chloridized end, the electrode [E] projects into the electrolyte [el]; at its other end, it is soldered to the conducting wire [C_W].

The micro-electrode tip [m] is sealed with de Khotinsky into the lumen of the pipette body [p]. Its free end is, of course, that portion of the device which comes into immediate contact with the cell, or with the liquid medium environing the cell. The micro-electrode tip [m] may be fabricated with either an open or closed free end. If an open-end tip is sealed into the pipette body [p], capillary attraction generally suffices to pull the electrolyte [el] out into the free end; but this force of capillarity occasionally has to be supplemented by gentle pressure against the diaphragm [d] by means of the

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1. Chloridized 1/2 hr. at .05 ampere, in 0.1M HCl. The AgCl deposit is purplish-gray. The Ag wire is scraped and sand-papered before being chloridized, and is washed thoroughly in double-distilled water after chloridizing and before mounting in the side tube [t].

screw [s]. If, however, a closed-end tip [m] is sealed into the pipette body [p], its (sealed) end may be cracked off by rubbing gently against the glass stage of a low-power microscope, while under careful observation.

The device just described has already been designated as a combined micro-electrode and micro-pipette. It is an electrode in the sense that conduction of current [1] is possible via the dilute electrolyte [el], reversible electrode [R], and conducting wire [C_W]. It is also a pipette in that, by manipulation of the diaphragm [d], liquids may be forced out of, or drawn into, the micro-tip [m]. This last-named function of the instrument, namely its function as a pipette, has proved to be of utmost consequence, as is made plain below (p. 42)[2].

No correlation whatsoever has become apparent between the diameter of the mouth of the micro-tip [m] and the value of the "cytoplasmic P.D." indicated. Pipettes having mouths of 10 - 40 microns diameter have been employed in the present study.

Certain objections to the above-described instrument are dealt with in Appendix B.

3. Micromanipulator [3,4]

A micromanipulator [3] is an instrument by means of which micro-instruments (needles, pipettes, electrodes, etc.) may be

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1. Note, however, that current flow is negligible; see discussion of voltmeter in Appendix C.
2. Also, see Dubuisson ('34, p. 19) for statement on the desirability of this step.
3. For a complete list of references to micromanipulators, see Chambers & Kopac ('37).

manipulated in the field of a microscope with control adequate for the performance of precise operations on, say, single cells. Establishment of electrical contact with the ameba by means of electrodes obviously demands the use of a micromanipulator.

The micromanipulator employed is a Taylor instrument (Taylor, '23-'25).[1] Only two modifications worthy of mention proved to be necessary. Firstly, the control units [3] were removed from their original cast-iron base and mounted on a base of hard wood [4] (walnut) (see Plate V).[2] Secondly, there were fashioned two special brass plates which were clamped on the control units and which served, in turn, as supports for the micro-electrode assemblies (see Plate VI).

By use of the micromanipulator, it is possible to bring one or both of the micro-electrode tips into contact with the specimen of Chaos chaos with ease and precision (Plate III a, b).

4. Voltmeter [5]

A voltmeter is an instrument for the measurement of difference of potential. An instrument of this category, of adequate sensitivity and possessed of certain other qualifications to be specified below, was obviously necessary for execution of the present research.

The essential qualifications for an instrument applicable to an investigation such as the present have been stated by Burr, Lane, and Nims ('36) in the following words:

> "(a) The device shall have high input impedance, i.e., minimal current shall

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1. Taylor ('23-'25) describes general principles of employment of the instrument.
2. This change was made chiefly in order to avoid damaging the original base, since a number of holes would have had to be drilled in it in order to fit it for the present investigation.

be drawn from the specimen under test.

(b) The device shall be of high sensitivity. As a limit, a potential difference of 10 microvolts shall be measurable.

(c) The device shall have high stability. Random fluctuations and general unsteadiness of the zero position shall be reduced to the lowest possible figure.

(d) The device shall be widely independent of external electrical disturbances. The specimen under test shall not be 'shielded'.

(e) Provision shall be made so that potential differences applied can be read off the instrument directly in microvolts or some multiple thereof.

(f) The sensitivity of the device shall be independent within wide limits of the resistance of the specimen under test. This condition is, of course, bound up with condition (a) above.

(g) The device shall be readily portable.

(h) Standard radio parts shall be used in its construction as far as possible, to keep the cost at a low figure."

The instrument employed in the present study conforms with the foregoing requirements in satisfactory degree. Specification (b) is fulfilled in part, maximum sensitivity being 45 microvolts/mm. Likewise specification (c) is not fulfilled in highest possible measure, but it is fulfilled to an extent which has been adequate for present needs. The instrument is, like that of Burr, Lane, and Nims ('36), essentially a "Wheatstone bridge" [1] network in which two resistance arms are vacuum tubes. [2] Unlike the instrument

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1. The term "bridge" will hereinafter frequently be employed, as synonymous with "voltmeter".
2. Type 89.

employed by Burr, Lane, and Nims ('36), however, both grid electrodes (rather than only one) are "floating" (i.e., at free grid potential). Furthermore, the "Wynn-Williams balance", for automatic compensation of filament battery fluctuations, as employed by Burr, Lane, and Nims ('36), has not been included in the set. The present network is figured in Fig. II, and the arrangement of controls, etc., is presented in Plate IV. Various aspects of the theory and employment of the foregoing network are dealt with in Appendix C.

Operating procedure employed with this bridge circuit can best be described in terms of Fig. II. Assuming that all switches are either "open" (S_2, S_7, S_9, S_{12}) or are in 0 position (S_1, S_3, S_4, S_5, S_6, S_8, S_{10}, S_{11}), the successive steps are as follows:

1. Adjust R_9 and R_{10} for low sensitivity. (This is a precautionary step, intended to protect galvanometer G against possible violent deflection.)
2. Throw S_8 to position 2.
3. Throw S_{10} to position 1.
4. Close S_7.
5. Wait 15 - 30 minutes. (Time for heaters of T_1 and T_2 to attain equilibrium.)
6. Close S_9.
7. Wait 5 - 10 minutes. (Time for the plate current to attain an equilibrium value.)
8. Throw S_{11} to position 1 or 2. (G is, in general, caused to deflect when S_{11} is closed, for the bridge circuit will not generally be "balanced".)
9. Balance the bridge (i.e., return G to zero) by adjusting, firstly R_4 and R_8, [1]

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1. The dial readings for the 2 resistors, R_4 & R_8, should be nearly the same.

and, secondly, R_5, R_6, R_7 (in succession). Increase the bridge output to G by adjustment of R_9 and R_{10}, and, if G deflects, continue balancing by adjustment of R_4 to R_7. (Continue this procedure until G shows no deflection when the entire bridge output is applied to G; the bridge is now balanced at highest sensitivity.)

10. Adjust R_9 and R_{10} for reduced sensitivity. (In order to protect G during the remainder of the procedure.)

11. Throw S_3 to position 2.

12. Throw S_1 to position 2. (G is, in general, again caused to deflect when both S_3 and S_1 have been thrown to position 2, because of the difference in the free grid potentials characteristic of T_1 and T_2.)

13. Throw S_6 to position 1 or 2.

14. Adjust R_1, R_2, and R_3, in succession, for zero deflection of G. (If adjustment is not obtainable, S_6 has been thrown to wrong position, and must be reversed.)

Steps 1 - 9 of the foregoing procedure provide a balance of the bridge circuit. Steps 10 - 13 compensate for difference in the free grid potentials of T_1 and T_2, thus making it possible to retain a bridge balance despite the fact that the two slightly dissimilar grids are connected to one another and are interposed between cathode and plate of T_1 and T_2.

The further procedure relevant to actual measurement of P.D.'s is given below (p. 44).

Since, in general, adjustment of the filament resistors R_{f_1} and R_{f_2} is required only infrequently, and constitutes no step in the usual measurement, the theory and adjustment of same is discussed in

Appendix C.

Calibration of the voltmeter may appropriately be outlined at this point. The procedure is as follows (see Fig. II):

1. Return S_1 to position 2. (It has been in position 1 during the course of a measurement) (p. 44).

2. Connect the selector switch S_4 with an appropriate [1] resistor, X.

3. Throw S_5 to position 1, and observe the deflection of G. (If desired, on grounds of accuracy, reverse B_3 by throwing S_5 to position 2, again observe deflection of G, and take an average of the two deflections.)

4. Throw S_3 to position 1.

5. Close S_{12}. (To "short out" grid compensating P.D.)

6. Read the P.D. applied to the potentiometer [P].

The P.D. applied to the potentiometer [P] is, of course, merely the P.D. across R_2; it is a P.D. applied to the two grids, and it is subject to variation in accordance with the resistor X selected by means of S_4. It is accurately measured by means of the potentiometer [P] (Step 6). However, this same P.D. has previously (Steps 1 - 3) been employed to cause an observed deflection of G. Hence, the deflection of G corresponding to a given value of P.D. applied between the two grids is known, i.e. a calibration of the voltmeter has been obtained, accurate to the accuracy of the potentiometer.

The adjustments of R_9 and R_{10} are, of course, all-important

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1. An appropriate resistor, X, is one such that, with the sensitivity employed during a current measurement, the deflection of G following upon closure of S_5 will be, say, 10 - 20 cm.

in determining the galvanometer deflection corresponding to a given P.D. input. Since, in the course of the great majority of experimental measurements, it was necessary to make some adjustments of these resistors in order to obtain a reading of desirable magnitude, [1] it became necessary to obtain a calibration reading subsequent to practically each experimental measurement. No particular difficulty is occasioned on this score, however, since the calibration readings are very quickly made.

Calibration showed the bridge to possess a maximum sensitivity — apparent when R_9 and R_{10} have been adjusted for maximum sensitivity — of approximately 45 microvolts/mm. deflection.

B. Assembly

The various apparatus described in the preceding section, i.e., the inverted microscope [1], micromanipulator [3,4], and voltmeter [5], must of course be combined into a single experimental unit. A detailed description of this assembly will now be given:

1. Combination of inverted microscope [1] with micromanipulator [3,4] ————:

The wooden block which constitutes the base [b] of the inverted microscope (see Plate II) is simply screwed down to the micromanipulator base [4], between the two control

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1. Say, 10 - 20 cms.

units. The microscope objective [16] is, of course, in line with the control units and approximately equidistant from both.

2. Combination of micro-electrodes [2] with micromanipulator [3,4] ———:

As aforementioned, two special brass plates [1] were fashioned in order to effect this combination. The plate is clamped into the control unit [3] in exactly the same manner as the pipette socket described by Taylor ('23-'25). The plate is drilled, and the screw projecting from the under side of the micro-electrode assembly [2] fits into this hole. A wing-nut contributes toward making the micro-electrode assembly readily demountable (Plate VI).

3. Combination of micro-electrodes [2] with voltmeter [5] ———:

As aforementioned, the conducting wire [C_w] is led from the micro-electrode [2] in a slender

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1. The micro-electrodes [2] could have been mounted directly on the control units [3], but this would have required drilling the latter.

(brass) tube which arises from the micro-electrode assembly. Leading out of the voltmeter case, now, are two brass tubes [13], [1](3/8 in. in diam.), each carrying a copper wire which is supported therein in Sulfur. At the distal end of the brass tube [15] there is a hollow chamber [14] into which the copper wire projects and which is drilled on its under side so as to receive the brass tube arising off the micro-electrode assembly. The chamber [14] is open to the front, so that the two wires, one [C_w] leading from the micro-electrode and the other toward the voltmeter [5], can be brought together and soldered. The brass tubes [15] leading out of the voltmeter [5] case are, of course, grounded.

Conductors supported in Sulfur in grounded metal shields, as just described, have given no leakage trouble whatsoever.

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1. See Plate V & Plate VI.

The junction [15] provided within the hollow chamber [14] allows adequate freedom of movement for the microelectrodes. [2]

4. Arrangement of galvanometer [6], galvanometer scale [7], and telescope [10]:

As already mentioned, a galvanometer serves as indicating instrument; this is a Leeds & Northrup Type P instrument, read by means of telescope and scale.[1] The instrument is mounted on the edge of a sturdy laboratory table, to which is also securely fastened the work table upon which the micromanipulator [3,4] and voltmeter [5] rest. The galvanometer [6] is suspended at such height that its telescope arm projects beneath the work table, the galvanometer scale [7] thus being located close beneath the top of the work table and near its front edge. An 18 in., 60 watt Mazda Lumiline bulb [9] mounted on the under side of the work table serves to illuminate the galvanometer scale [7] uniformly and

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1. Leeds & Northrup Catalog ED (1936); list #2239a.

brightly. The telescope [10] is removed from the telescope arm, and in its place there is a special mounting which supports a small 90° prism [8]. This prism [8] projects upward the reflection cast by the galvanometer mirror. Holes having been aligned thru the work table and the micromanipulator base [4] directly above the prism [8], it is now possible to observe the prism [8] with the galvanometer telescope [10] which is mounted on a special bracket just to the right of the microscope [1]. The arrangement thus effected is one of greatest desirability, on grounds of convenience as well as accuracy; for with the right eye it is possible to observe galvanometer deflections at the same time that the left eye observes events transpiring in the microscopic field.

The essentials of the above arrangement are figured in Fig. III.

The entire assembly is seen in Plate V, and a close-up view of the micromanipulator units [3], inverted microscope [1], micro-electrodes [2], etc., is seen in Plate VI.

METHODS

A. Selection and preparation of Chaos chaos

Specimens of Chaos chaos showing normal vigorous activity were selected at random [1] for measurement. The criteria of "normal vigorous activity" are numerous, and one needs some experience with the organism in order to employ them. Briefly, one may cite 1) active streaming, 2) characteristic "shape", and 3) a "good color" (which depends upon the presence of not too many crystals in the endoplasm) as criteria for the estimation of a given specimen's state of well-being. In addition, care was taken to select specimens which clung tenaciously to the watch-glass, for experience indicated that such specimens were most likely to fasten themselves quickly and securely to the glass stage [S] upon which the micromanipulations were performed.

The organism selected for measurement was then put thru four successive washings of .001% NaCl. [2] Four thoroughly clean watch-glasses were prepared and .001% NaCl was poured into each. The chosen specimen was pipetted out of its culture medium and into the first watch-glass, from this it was pipetted into the second, and so on until it had passed thru four successive baths of .001% NaCl. (A different pipette was of course used for each transfer.) From the fourth watch-glass the cell was pipetted into a large drop of .001% NaCl on the stage [S]. The objective of the successive washings is, of course, to place the specimen onto the stage [S]

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1. Selection "at random" here means that the specimens were taken from various culture dishes.
2. C.P. NaCl, diluted with double-distilled water (second distillation out of a Pyrex still.)

in a known external medium, namely, .001% NaCl, free from unknowns present in the culture medium. That is, the washing process obviates transfer onto the stage [S] of unknowns in the culture medium, as would occur if the specimen were pipetted out of the culture vessel and directly onto the stage [S]. Of course, four washings may not completely obviate the transfer of such unknowns, but it is believed that so little is transferred onto the stage [S] as to be completely negligible. It is to be noted that the culture medium consists of 0.001% NaCl, and hence when the specimen is transferred into fresh 0.001% NaCl it suffers little alteration in the nature of its external medium; it is now in a medium, however, which is free of the metabolites which have accumulated in the culture medium.

For the ready establishment of contact by a micro-electrode tip [m], it is highly desirable that the specimen be attached to the stage [S], for, as mentioned earlier (p. 21), a floating ameba evades the micro-tip [m]. In this connection, it should then be mentioned that specimens frequently refuse to attach themselves to a glass surface which is not clean. Accordingly, the glass stage [S] was frequently cleaned by scrubbing with an abrasive powder and rinsing in double-distilled water. Such cleaning also, it should be noted, further insures purity of the external medium subsequently placed upon the stage. Amebas placed in a drop of .001% NaCl upon the stage [S] after such cleaning generally attach themselves quickly to the glass and are then in favorable condition for the establishment of contact by means of the micro-tip [m] of the internal electrode.

B. Establishment of electrical contact with the organism

Manipulation of micro-instruments by means of a Taylor micro-manipulator is adequately discussed by Taylor ('25-'25), and need not be enlarged upon here. It is sufficient to reiterate at this point that in the present arrangement, due to the use of the inverted microscope [1], the micro-tip [m] descends upon the organism from above. One micro-electrode tip [m] is brought into contact with the organism, while the second is submerged in the external medium, i.e., the .001% NaCl environing the organism. [1]

As previously explained (p. 29) the micro-electrode herein employed can also function as a micro-pipette. Because of this, it has been possible to suck protoplasm up into the micro-tip [m] and to observe intimate contact of same with the electrolyte [el] in the internal electrode. This is a technical step of considerable consequence since the area of contact of the protoplasm with the electrolyte is microscopically observable (Plate III, a); it is significant in that it allows unequivocal fulfillment of Requirement #3 (p. 13). The ectoplasm can be ruptured by suction of the electrolyte [el], whereupon the endoplasmic granules visibly commingle with the electrolyte [el].

Contact (with the organism) of the electrolyte [el] in the internal electrode has, for purposes of comparative study, been established in two ways, which are designated as "Type A Contact" and "Type B Contact":

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1. The position of the external electrode, with respect to the specimen, is of no importance. Thus, it can be moved about in the microscopic field and occasion no alteration in the value of the "cytoplasmic *P.D.*" being observed.

Type A Contact —— The micro-tip [m] of the internal electrode is simply lowered upon the cell, to a depth such that the tip [m] appears to indent the cell quite extensively.

Type B Contact —— The micro-tip [m] of the internal electrode is lowered upon the cell, again to a depth such that the tip appears to indent the cell extensively, and then protoplasm is gently sucked up into the micro-tip (p. 29) (Plate III, a). The length of the column of protoplasm drawn into the tip can be controlled by manipulation of the diaphragm [d] of the micro-electrode.

In the cases of all specimens of Chaos chaos studied, Type A Contact was first established, and then, as soon as the requisite data upon the P.D. provided by this type of contact had been observed (1 minute, or less), Type B Contact was established merely by manipulation of

the diaphragm [d] of the micro-electrode [z]

As has already been noted (p. 40), the external medium provided for the experimental specimen is 0.001% NaCl. But this same solution serves as electrolyte [el]. Hence, a liquid junction P.D. at the surface of contact of the external electrode with the external medium is avoided, and Requirement #2 (p.11) is fulfilled.

C. Procedure for reading a P.D.

The procedures required for balancing the voltmeter circuit [5], and for compensating for differences in free grid potentials, have already been given (p. 52). At this point, the procedure relating to actual measurement of the P.D. may appropriately be introduced.

Assume the voltmeter circuit [5] balanced, the free grid P.D. compensated, and the two micro-electrode tips [m] immersed in the same drop of .001% NaCl (on the stage [S]). If, now, S_1 is thrown to position 1 (see Fig. II), the two electrodes, shunted thru .001% NaCl, are connected to the grids of T_1 and T_2. There is generally a small "inherent P.D." between two such electrodes (Brown, '54), and since this P.D. is applied between the two grids, it unbalances the voltmeter circuit [5]. This electrode P.D. is now "balanced out" by further adjustment of R_1, R_2, and R_3,[1] so that the circuit balance is restored. Any P.D. now applied between the two micro-electrode tips [z], as by contact of the internal electrode with the specimen, again induces an unbalance of the bridge circuit. The degree of such unbalance, as indicated by the galvanometer [G],

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1. Rarely, S_6 must be reversed in order to effect this adjustment.

becomes the measure of the P.D. between the micro-electrode tips [m].

During the course of all the measurements reported in Table III (p. 51), S_3 was thrown from position 2 to position 0 a number of times (2 - 6), in order to make certain that the bridge balance had not altered during the course of the measurement. This procedure was hardly necessary, since, at the low sensitivity range in which the bridge was operated during these measurements, the "zero instability" of the instrument was small (around 4 - 5 millimeters); nevertheless, this procedure was adopted as a desirable precaution.

Furthermore, in order to make certain that the "inherent P.D." between the 2 electrodes had not altered during the course of the measurement, check was always made to see that the galvanometer deflection dropped to within the "zero instability" when, on completion of a "cytoplasmic P.D." measurement, the protoplasmic column was ejected from the micro-tip [m].

D. Recording of duration of the maintenance of the "cytoplasmic P.D."

The galvanometer deflections occurring as a consequence of the establishment of Type B Contact were observed continuously, and the total duration of the observation on each specimen was recorded.

E. Recording of temperature [1]

The temperature of the laboratory during the measurement on each specimen was recorded. An ordinary Hg thermometer [6] was used, its bulb being suspended in space at a point very close to the specimen

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1. The temperature data cannot be regarded as significant, except insofar as they indicate the constancy of this environmental factor within a small range.

being measured (Plate V).

OBSERVATIONS AND RESULTS

A. Qualitative Observations

1. General

Chaos chaos manifests an immediate reaction to contact of the micro-tip [m] of the internal electrode, as is to be expected. Locomotion is immediately affected, in that streaming is temporarily halted. Also, there occurs a quick contraction of the protoplasm, a contraction which is more intense in the immediate environment of the contacting micro-tip [m] than in parts of the cell body further removed. Within a short time (generally, less than 1 minute), the specimen resumes normal locomotion and appears normal in all respects. This quick return to normalcy obtains irrespective of whether the micro-tip [m] contacting the cell is maintaining Type A Contact or Type B Contact.

The specimens of Chaos chaos were observed quite generally to escape the contacting micro-tip [m], by virtue of their own locomotor activity, and this occurs irrespective of whether Type A Contact or Type B Contact is being maintained. In case the contact is of Type B, the ameba can be observed to sever itself free from the column of its protoplasm which has ascended into the micro-tip [m], the disjunction being effected at the mouth of the micro-tip [m]. Varying periods of time are required for the ameba to effect such release from the micro-tip [m], the shortest recorded instance being four minutes and the longest instance being ten minutes.

When the ameba frees itself from the contacting micro-tip [m], the P.D. which has theretofore been maintained is quickly dissipated,

the galvanometer deflection quickly being reduced to near [1] zero.

The length of the column of protoplasm in the micro-tip [m] of the internal electrode does not influence the value of the "cytoplasmic P.D.", i.e., the magnitude of the "cytoplasmic P.D." observed with Type B Contact is independent of the amount of protoplasm drawn into the micro-tip [m]. Thus, during the course of an observation, it proved possible to shorten or to lengthen the protoplasmic column and yet to occasion no change in the P.D. value then being maintained.

Specimens upon which a measurement has been made have been kept under observation for hours subsequent to the measurement. They always appear normal as regards both morphological and physiological characteristics.

2. Relevant to Type A Contact

No special qualitative observations relative to Type A Contact have been made, other than the fact that the microscope does not reveal whether the electrolyte [el] in the micro-tip [m] of the internal electrode is in actual contact with the cytoplasm. The micro-tip [m] presses deeply into the cell, so that portions of the cell substance at a focus higher than the mouth of the micro-tip [m] tend to obscure this opening, and careful observation with a 16 mm. objective fails to reveal the nature of the contact at the mouth of the micro-tip [m];

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1. In a number of instances it was observed that if some of the protoplasm remained in the micro-tip [m] after the ameba had freed itself, and such was generally the case, then the galvanometer deflection might not return quite to zero, a small deflection (1 - 2 cm., generally) being maintained. This observation remains unexplained. Any protoplasm left in the micro-tip can, of course, be expelled at will.

the electrolyte [el] may make contact with the cytoplasm, or it may be separated from same by the ectoplasm (p. 13).

3. Relevant to Type B Contact

The column of protoplasm which has been sucked up is readily visible in the micro-tip [m] (Plate III, a). By manipulation of the diaphragm [d] of the micro-electrode [2], it is possible to cause rupture of the ectoplasmic layer covering this column of protoplasm, so that the cytoplasmic granules are seen actually to float free in the electrolyte [el]. Hence, there can be no doubt but that the electrolyte [el] is in intimate contact with the cytoplasm.

Protoplasmic streaming occurs within the column of protoplasm which is in the micro-tip [m]. The cytoplasmic granules flow in both directions in the protoplasmic column, so that there can be no question but that such movement of granules is due to protoplasmic activity; that is to say, it cannot be considered as mere passive movement due to suction of the electrolyte [el].

The column of protoplasm which has been drawn into the micro-tip [m], and which may have been contained therein over a period of a number of minutes, can be forced out of the micro-tip [m]. Such an ejected column (from which the ameba has not of course previously effected its separation), is rapidly filled with cytoplasm, so that it shortly (approx. 1/2 min.) cannot be distinguished from an ordinary pseudopod. Since the ectoplasm of such a protoplasmic column has suffered rupture (p. 49), this rupture must necessarily be healed. Detailed observations on this healing process were not undertaken, however.

B. Quantitative Observations

Quantitative observations on 32 specimens are recorded in Table III, below. Establishment of Type A Contact occasioned an immediate deflection of the galvanometer, the magnitude, polarity, and permanence of which are indicated (↓ indicates that the deflection quickly subsided to within the range of the "zero instability" [1] of the voltmeter; ↔ indicates that the deflection was maintained for approximately 1/2 - 1 minute.) The values recorded as "magnitude" for the deflection occasioned by the Type A Contact is the maximum value of the deflection shown by the galvanometer, either 1) before the deflection began to subside, or 2) during the course of the steady value maintained for 1/2 - 1 minute. Establishment of Type B Contact occasioned a deflection which was generally greater than that occasioned by the Type A Contact; the magnitude, polarity, and duration [2] of the observation on same are recorded. The deflections occasioned by Type B Contact are not, however, constant in magnitude. Rather, there is in general a relatively constant range [3] within which the deflection continuously varies at random, and from which it may deviate, in either a negative or positive sense, at random. The limits of the "Relatively Constant Range" are recorded, and also the upper and lower limits of the deviations from within this range which were observed [4] during the course of the measurement.

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1. See Appendix C.
2. In all cases, the deflection was maintained as long as Type B Contact was maintained.
3. Assignment of this range is, of course, somewhat arbitrary. It can be stated to be merely that range within which the deflection was maintained during the major part (about 4/5) of the duration of the observation.
4. Observation of the galvanometer deflections was, of course, continuous during any given measurement.

TABLE III

Specimen	Temp. (°C)	Deflection with Type A Contact: Permanence	Magnitude (Volts)	Polarity of Internal Electrode	Deflection with Type B Contact: Duration of Observation (Min.)	Relatively Constant Range (Volts)	Limits (Volts)	Polarity of Internal Electrode
1	27.5	↓	.035	–	6	.056 – .058	.048 & .063	–
2	26.0	↓	.016	–	5	.048 – .053	.043 & .056	–
3	24.5	X	.000	X	4	*	.040 & .070	–
4	25.0	↓	.004	–	5	.044 – .047	.039 & .049	–
5	24.0	↓	.025	–	8	.036 – .043	.035 & .057	–
6	24.5	↓	.031	–	3	.049 – .055	.040 & .063	–
7	25.0	↓	.006	–	9	.041 – .047	.036 & .050	–
8	25.0	↓	.024	–	6	.041 – .048	.028 & .059	–
9	25.0	↓	.018	–	7	.052 – .060	.043 & .066	–
10	24.5	↓	.010	–	9	.034 – .036	0	–
11	24.5	↓	.003	–	8	.051 – .051	0	–
12	24.5	↓	.039	–	4	.070 – .077	0	–
13	27.0	↓	.036	–	3	.029 – .031	.023 & .039	–
14	28.0	↓	.007	–	6	.025 – .029	.025 & .030	–
15	28.5	↔	.021	–	4	.056 – .063	.026 & .065	–
16	29.0	↓	.018	–	4	.082 – .085	.057 & .085	–
17	29.0	↓	.007	–	5	.043 – .050	.032 & .060	–
18	26.0	↔	.011	–	8	.065 – .070	.036 & .074	–
19	25.0	↓	.013	–	10	.070 – .073	.063 & .078	–
20	25.0	↓	.014	–	10	.050 – .058	.046 & .060	–
21	25.0	↓	.009	–	8	.043 – .046	.020 & .047	–
22	26.5	↓	.017	–	10	.079 – .110	0	–
23	24.0	↓	.022	–	10	.077 – .087	.091 & .045	–
24	27.5	↓	.009	–	11	.082 – .090	0	–
25	27.5	↔	.015	–	4	.021 – .023	.019 & .023	–
26	26.0	↓	.017	–	7	.052 – .058	.039 & .064	–
27	24.5	↓	.017	–	6	.056 – .065	.055 & .066	–
28	24.5	↓	.026	–	10	.090 – .095	.083 & .095	–
29	27.0	↓	.028	–	2	.053 – .061	0	–
30	27.5	↓	.035	–	4	.055 – .068	0	–
31	29.0	↓	.012	–	3	.054 – .080	0	–
32	28.5	↔	.043	–	5	*	.051 & .095	–

Symbols

↓ = Deflection quickly subsided to within the range of the "zero instability" of the voltmeter.

↔ = Deflection persisted for 1/2 – 1 minute.

X = No datum, because magnitude of deflection is zero.

* = No datum, as no Rel. Const. Range was manifested, deflection drifting steadily from a low value to a high value.

0 = No datum, as no (galvanometric) deflections from within the Rel. Const. Range occurred during the period of observation.

DISCUSSION

A. Concerning Original Observations

1. Qualitative Observations

Practically any type of biological experimentation is unavoidably subject to the criticism that injury is entailed by the experimental procedures employed, and, hence, that the results achieved do not obtain for the normal organism. From an experimental point of view, this criticism is not valid since, as just mentioned, there are few experimental procedures in biology against which it cannot be raised and, hence, practically no experimental investigation in biology could be carried on if investigators were to pause in the face of this objection. Nevertheless, it may appropriately be pointed out that the experimental procedures involved in the employment of Type B Contact seem to entail little or no injury to Chaos chaos. Three qualitative observations seem to the writer to constitute evidence in favor of this view. In the first place, the specimen resumes normal locomotor activity generally within a minute subsequent to the establishment of contact by the internal electrode. Secondly, protoplasmic streaming occurs in the protoplasmic column within the micro-tip [m], and protoplasmic streaming is a commonly accepted evidence of normalcy in those forms in which it normally occurs (Ettisch and Peterfi, '25; Gelfan '27; Gelfan '28). Lastly, there is the fact that a protoplasmic column [1] ejected from the micro-tip [m] will (provided, to be sure, that the ameba has not previously

---

1. The protoplasmic column can be varied in length (p. 29), and yet appear normal upon ejection. This indicates that the amount of protoplasm drawn into the micro-tip [m] does not influence the extent of any possible injury.

effected separation from same) rapidly fill with cytoplasm and take on the appearance of an ordinary pseudopod. These facts, taken together, strongly indicate that the protoplasm which is sucked into the micro-tip [m] is not injured thereby, and, furthermore, that the cell as a whole is not injured by the experimental manipulations involved in the use of Type B Contact.

2. Quantitative Observations

a. Type A Contact

The results on Type A Contact demonstrate that it/ causes galvanometric deflections which are generally transitory. Because of this feature alone, it seems that Type A Contact should not be employed in the study of "cytoplasmic P.D.'s", for certainly the possibility of investigating the durability of a "cytoplasmic P.D." should not be ruled out by the experimental technique itself.

It is to be observed, furthermore, that the deflections occasioned by Type A Contact are, with few exceptions, of considerably smaller magnitude than those occasioned by Type B Contact. This fact cannot constitute an indictment of the Type A Contact, but it does demonstrate that employment of the two types of contact (Type A and Type B) can but lead to exceedingly divergent results.

b. Type B Contact

Taking into account the construction of the micro-electrodes [2] employed (p. 25), it is obvious that employment of Type B Contact on Chaos chaos creates an electro-chemical chain which can be diagrammed as follows:

$$- \text{Ag, AgCl} \;\Big|_{a}\; \begin{matrix}\text{External}\\ \text{Medium}\\ (0.001\%\\ \text{NaCl})\end{matrix} \;\Big|_{b}\; \begin{matrix}\text{Cytoplasm}\\ \text{of living}\\ \underline{\text{Chaos chaos}}\end{matrix} \;\Big|_{c}\; \begin{matrix}\text{Ectoplasm}\\ \text{of living}\\ \underline{\text{Chaos chaos}}\end{matrix} \;\Big|_{d}\; \begin{matrix}\text{External}\\ \text{Medium}\\ (0.001\%\\ \text{NaCl})\end{matrix} \;\Big|_{a}\; \text{AgCl, Ag} + \quad [1]$$

This chain manifests what is herein designated as a "cytoplasmic P.D.", and which is possessed of the following three characteristics:

1. It is of the same polarity in all specimens of Chaos chaos;
2. It persists (in time) in all specimens of Chaos chaos;
3. It varies in magnitude (with time) in all specimens of Chaos chaos.

The two junctions designated "a" in the above diagram are equal in magnitude, and are opposed; hence, they cancel. The chain then resolves itself into three P.D.'s, indicated as "b", "c", and "d", all of which are unknown, and any one or two of which may be zero. The algebraic sum of these three P.D.'s maintains a constant polarity, and persists in time (in any individual specimen of Chaos chaos) as a quantity of varying magnitude.

Variation (with time) in the magnitude of the P.D. manifested by the above-diagrammed chain is apparently due solely to fluctuations in the values of any one or all of the three P.D.'s indicated as "b", "c", and "d". Such fluctuations are, further, undoubtedly attributable to vital activities. It is not possible at present to state with any degree of assurance precisely what may be the nature of such vital activities underlying fluctuations in the values of "b", "c", and "d". But the mere fact that such fluctuations do occur appears to be evidence for the extreme variability of the "protoplasmic system" which

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1. The writer is indebted to Dr. J. D. Stranathan, Physics Dep't., K.U., for pointing out the possible existence of the P.D. indicated by "c". Insofar as the writer is aware, the existence of this P.D. has not heretofore been postulated, though the existence of a P.D. between nucleus and cytoplasm has often been postulated.

we recognize as Chaos chaos.

B. Concerning Relationships to Results of Previous Investigators

Previous investigations on "cytoplasmic P.D.'s" in unicellular animals [1] have not involved employment of Type B Contact, nor, in the writer's view, of any other technique which can provide absolute assurance that the electrolyte in the internal electrode is in direct contact with the cytoplasm. Obviously, if the "cytoplasmic P.D." manifested by a given organism is to be studied, contact of the internal electrode with the cytoplasm itself is an inescapable requirement. Comparison, now, of Tables II and III (p. 18 and p. 51, respectively) brings to light the fact that the "cytoplasmic P.D.'s" manifested by Chaos chaos when Type B Contact is employed are of an order of magnitude which far exceeds those obtained by Telkes ('31), Kamada ('34), and Buchthal and Peterfi ('36-'37) in the various forms studied by these workers. On the other hand, Table III also shows that Type A Contact occasions P.D.'s which are generally transitory and generally smaller in magnitude than those provided by Type B Contact. It is not possible to conclude that the small values obtained by Telkes ('31), Kamada ('34), and Buchthal and Peterfi ('36-'37) are due to their having employed contact comparable to Type A, for the organism employed in the present study is not the same as that employed by any of the other workers; nevertheless, comparison of the data on Type A Contact and Type B Contact (presented in Table III)

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1. Osterhout ('24), working with the unicellular plant, Valonia macrophysa, has filled the tips of his electrodes with cell sap, the inner contents of the cell, and hence has employed a type of contact similar in principle to the Type B Contact described herein.

indicates this as a possible explanation for the small values recorded by the earlier workers.

Another evidence bearing on this same point occurs in the report of Buchthal and Peterfi ('36-'37, pp. 474-75). These writers state that when their internal electrode first contacts the cell (Ameba terricola), a temporary deflection ("einer momentanen, im Bruchteil einer Sekunde ablaufenden Ausschlag") occurs, and that their voltmeter thereupon subsequently indicates a steady value of 1 - 1 1/2 millivolts. This result is strikingly similar to those reported herein for Type A Contact, except that the deflections occasioned by the Type A Contact generally fall to within less than the 1 - 1 1/2 millivolt value.

The data herein reported for Chaos chaos demonstrate that the "cytoplasmic P.D." manifested by each individual when Type B Contact is employed persists in time (although varying somewhat in magnitude). In the writer's view, a time record on each measurement is extremely desirable, and it is regrettable that Ettisch ('28), Telkes ('31), Kamada ('34), and Buchthal and Peterfi ('36-'37) have not presented this datum. Buchthal and Peterfi ('36-'37) do state that the 1 - 1 1/2 millivolt value they obtain in Ameba terricola "über lange Zeit konstant ist", and Telkes ('31) presents scattered data on the time required for various salts added to the external medium to reduce the "cytoplasmic P.D." to zero; but there is no consistent record in the reports of Ettisch ('28), Telkes ('31), Kamada ('34) or Buchthal and Peterfi ('36-'37) on the total time during which the "cytoplasmic P.D." was observed in each specimen.

The "cytoplasmic P.D." manifested by Chaos chaos when Type B Contact is maintained has just (p. 53) been described as being due to the chain

- Ag, AgCl	External Medium (0.001% NaCl)	a	Cytoplasm of living Chaos chaos	b	Ectoplasm of living Chaos chaos	c	External Medium (0.001% NaCl)	d	AgCl, Ag +	a

It has further been pointed out (p. 54) that the P.D. measured is the algebraic sum of the three P.D.'s, "b", "c", and "d". This interpretation is unconventional in that the possible existence of a P.D. between cytoplasm and ectoplasm, designated as "c" in the above diagram, is herein postulated. Kamada ('34, p. 98) interprets his results as a manifestation of the chain

Non-polarizable electrode	External solution in Capillary	d	Protoplasm of Paramecium	a	External Solution		External solution in Capillary		Non-polarizable electrode	

and postulates (Kamada, '34, p. 99) that the P.D. marked "a", "which originates in the membrane of Paramecium", is greater than the liquid junction P.D. marked "d", "so that the measured potential is mainly the potential at 'a'". Throughout, Kamada ('34) refers to "d" as a "membrane potential", and points out the analogies as between the results he obtains with various salts as external medium and those obtained by Michaelis ('29) on collodion membranes. In the writer's view, it is advisable in discussing "cytoplasmic P.D.'s" to recognize the three P.D.'s indicated in the chain

Electrode	External Medium	a	Cytoplasm	b	Ectoplasm	c	External Medium	d	Electrode	a

as "b", "c", and "d", and to await further data before attempting to assay the contribution made by each to the total P.D. manifested by the chain.

CONCLUSIONS

1. The (electrochemical) chain

- Ag, AgCl	0.001% NaCl	a	Cytoplasm of living Chaos chaos	b	Ectoplasm of living Chaos chaos	c	0.001% NaCl	d	AgCl, Ag +	

manifests a P.D., the sign of which is indicated in the diagram.

2. The magnitude and permanence of the P.D. manifested by the above-diagrammed chain are importantly conditioned by the nature of the contact established at "b". Thus, if the contact at "b" is of Type A, the P.D. is, in general, transitory, and is almost invariably of smaller magnitude than that manifested by the chain when Type B Contact is established at "b".

3. The P.D.'s manifested by the above-diagrammed chain when Type B Contact is established at "b" are 1) constant in polarity, 2) persistent, and 3) varying (in magnitude) with time in any given specimen. The last-named characteristic of the P.D. (i.e., variation with time) cannot be correlated with locomotor activities of the organism.

4. The P.D.'s manifested by the above-diagrammed chain when Type B Contact is established at "b" are, with few exceptions, of greater magnitude than any values heretofore reported for "cytoplasmic P.D.'s" in a protozoan cell.

5. The P.D. manifested by the above-diagrammed chain when Type A

Contact is established at "b" is to be regarded, because of its usual transitoriness, as being of little value for studies directed toward analysis of the "cytoplasmic P.D.". On the other hand, the P.D. manifested when Type B Contact is employed is to be regarded, because of its permanence, as being of definite importance for further studies on the "cytoplasmic P.D."

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PLATE I

Culture vessel and contained

Chaos chaos

(Natural Size)

The organisms appear as white spots
in the watch glass which
serves as culture vessel.

PLATE I

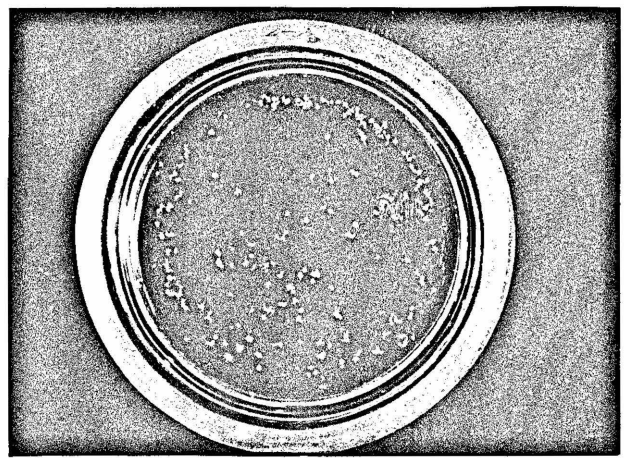


PLATE I

Culture vessel and contained

Chaos chaos

(Natural Size)

The organisms appear as white spots
in the watch glass which
serves as culture vessel.

PLATE I

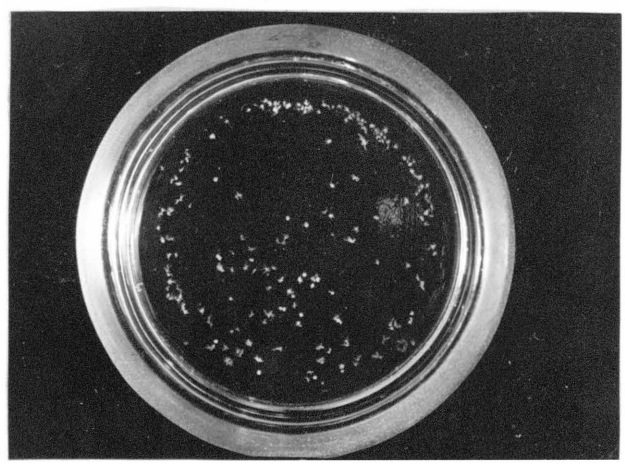


PLATE II

Inverted Microscope [1]

(Plate II, a, shows the 16mm. lens [16] aligned for use)
(" II, b, " " 32mm. " [32] " " ")

a — microscope arm

b — " base

il — illuminating objective

M — mirror

L — light source

S — stage

x — mounting (for [M] and [il])

p-c — prism-chamber

b-p — sliding (brass) plate

ms — mechanical stage

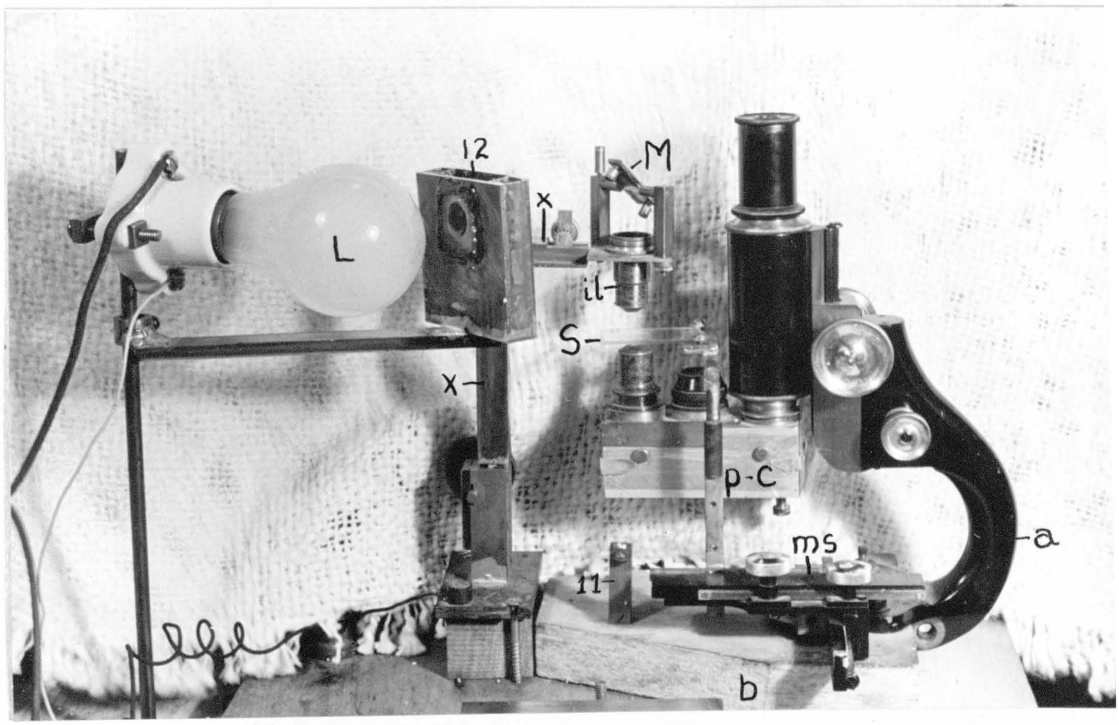
l1 — guide post

12 — water cell

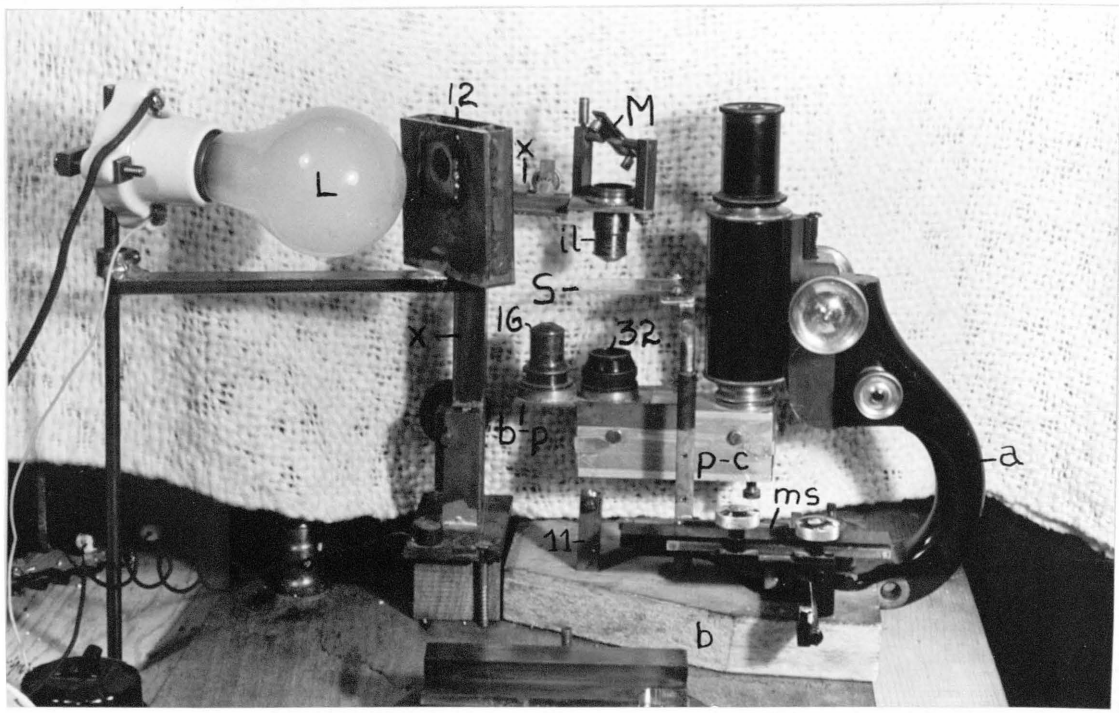
16 — 16 mm. lens

32 — 32 mm. lens

PLATE II



(a)



(b)

PLATE III

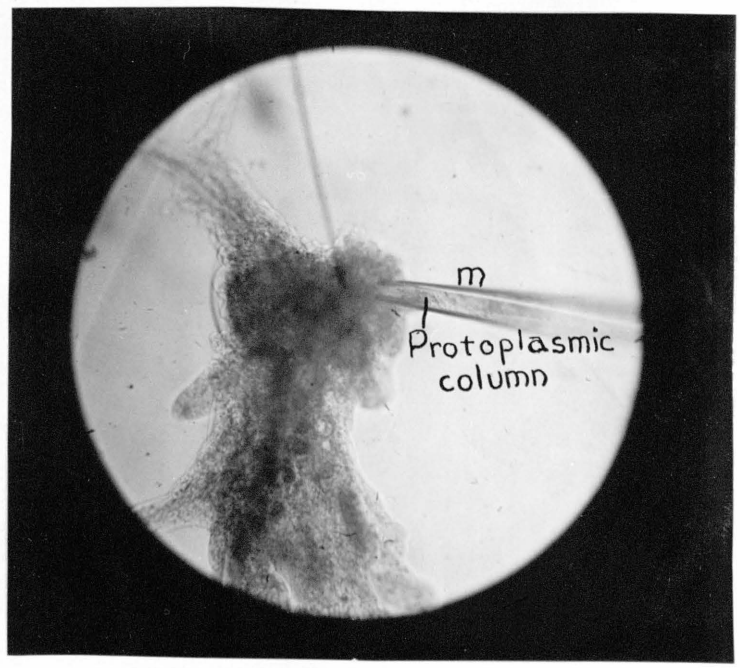
Chaos chaos, contacted by micro-electrode tip [m]

(Plate III, a, shows protoplasmic column in micro-electrode tip [m])

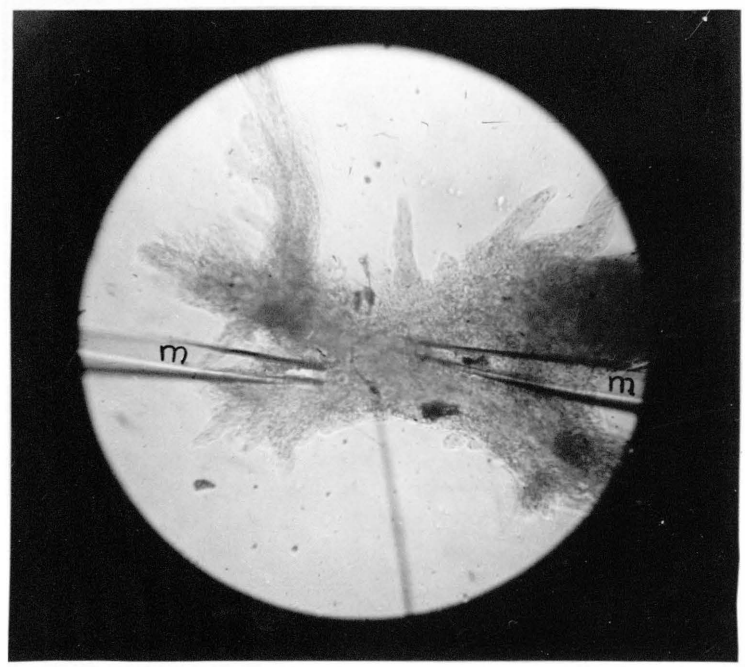
(Plate III, b, shows contact by two micro-electrode tips [m])

m — micro-electrode tip

PLATE III



(a)



(b)

PLATE IV

Panel of Voltmeter [5]

For symbols, see legend supplied
for Figure II.

PLATE IV

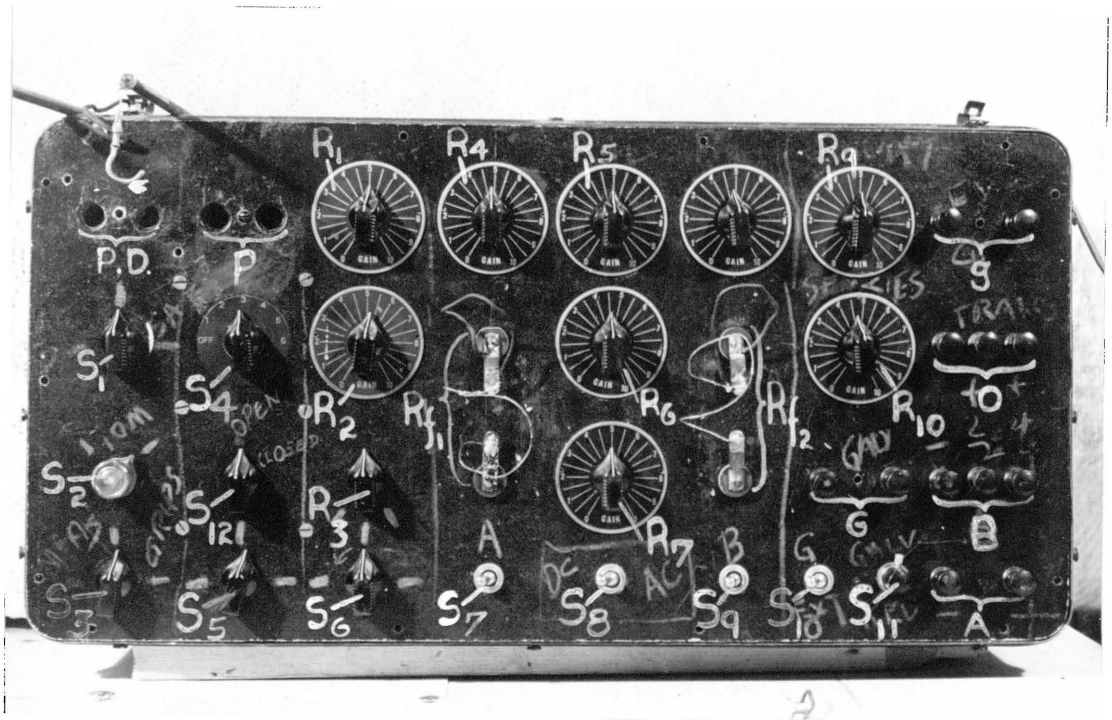

P.D.
P
R1
R4
R5
R9
R2
R3
R6
R7
R10
R11
R12
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
GAIN
OFF
OPEN
CLOSED
A
B
G
GND
TRAILS
10

PLATE V

(View of Assembly)

1 — inverted microscope

2 — micro-electrode

3 — micromanipulator control unit

4 — " base

5 — voltmeter

6 — thermometer

10 — telescope

13 — input lead

14 — hollow chamber on input lead

PLATE V

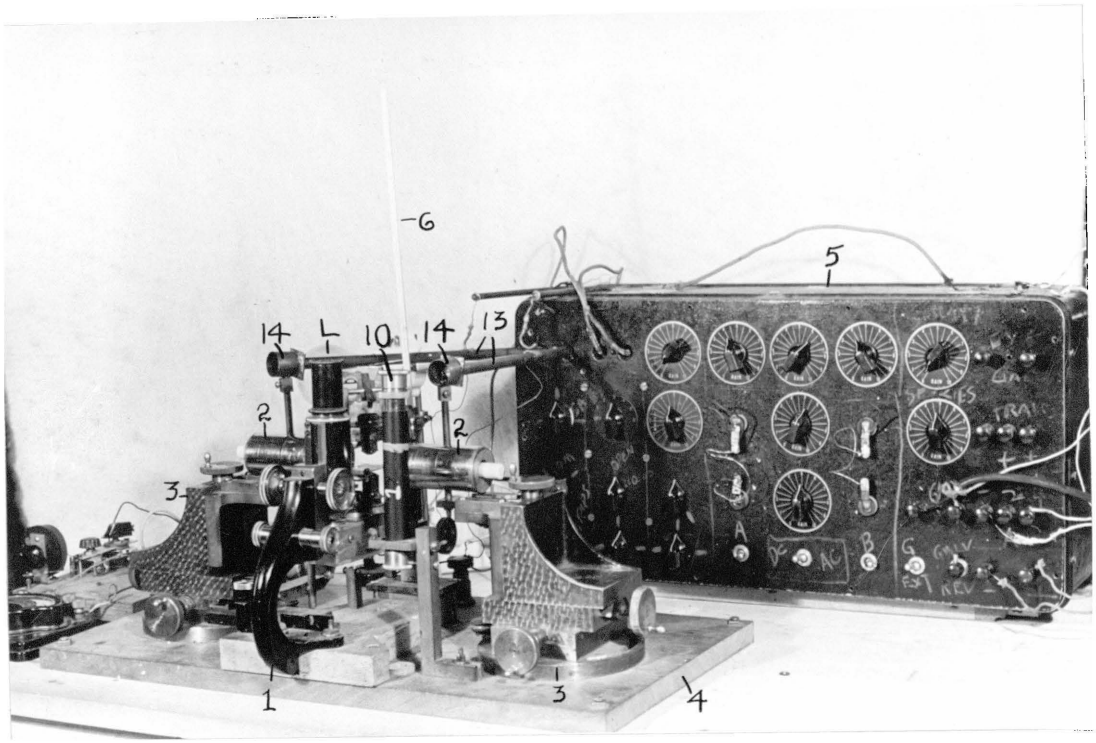


PLATE VI

(Close-up view of micromanipulator control units, inverted microscope, micro-electrodes, etc.)

1 — inverted microscope

2 — micro-electrode

3 — micromanipulator control unit

4 — micromanipulator base

6 — thermometer

10 — telescope

11 — guide post

12 — water cell

13 — input lead

14 — hollow chamber on input lead

15 — junction of [C_g] (Fig. I) and wire in input lead

il — illuminating objective

ms — mechanical stage

p-c — prism chamber

m — micro-electrode tip

b — inverted microscope base

x — mounting (for [M] and [il])

M — mirror

S — stage

L — light source

PLATE VI

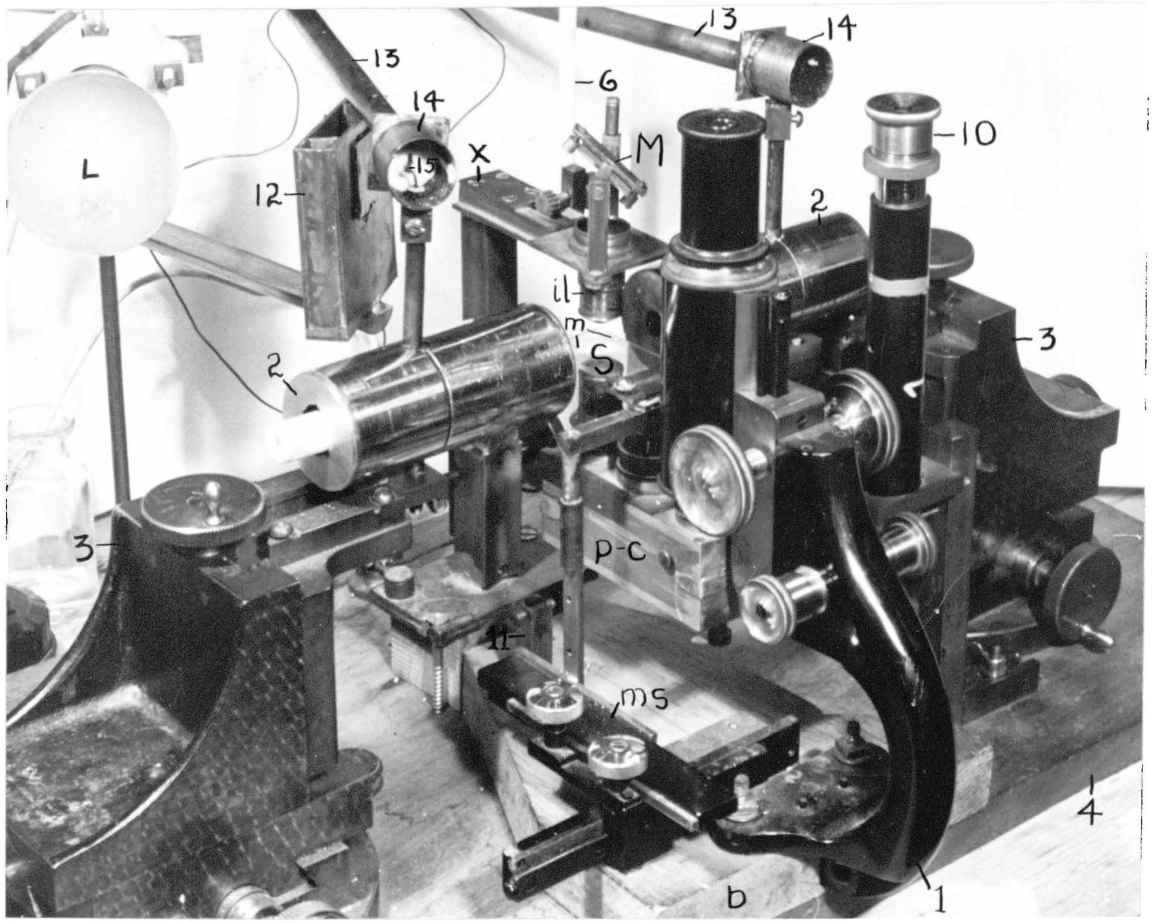


FIGURE I

Cross-section of Combined Micro-electrode

and Micro-pipette Assembly [2]

(Natural Size)

S_f = Shield, front section

S_r = " , rear "

p = Pipette body

p' = " shank

h = Brass head

h' = Neck on brass head

H = Sulfur handle

E = Reversible electrode

R = Ring

C_w = Conducting wire

m = Micro-electrode tip

r = Rubber stopper

d = Diaphragm

s = Screw (32 threads/in.; 6 gauge)

t = Side-tube

el = Electrolyte (.001% NaCl)

⁘ = Sulfur (insulation)

\\\\ = de Khotinsky Cement

≡ = Solder

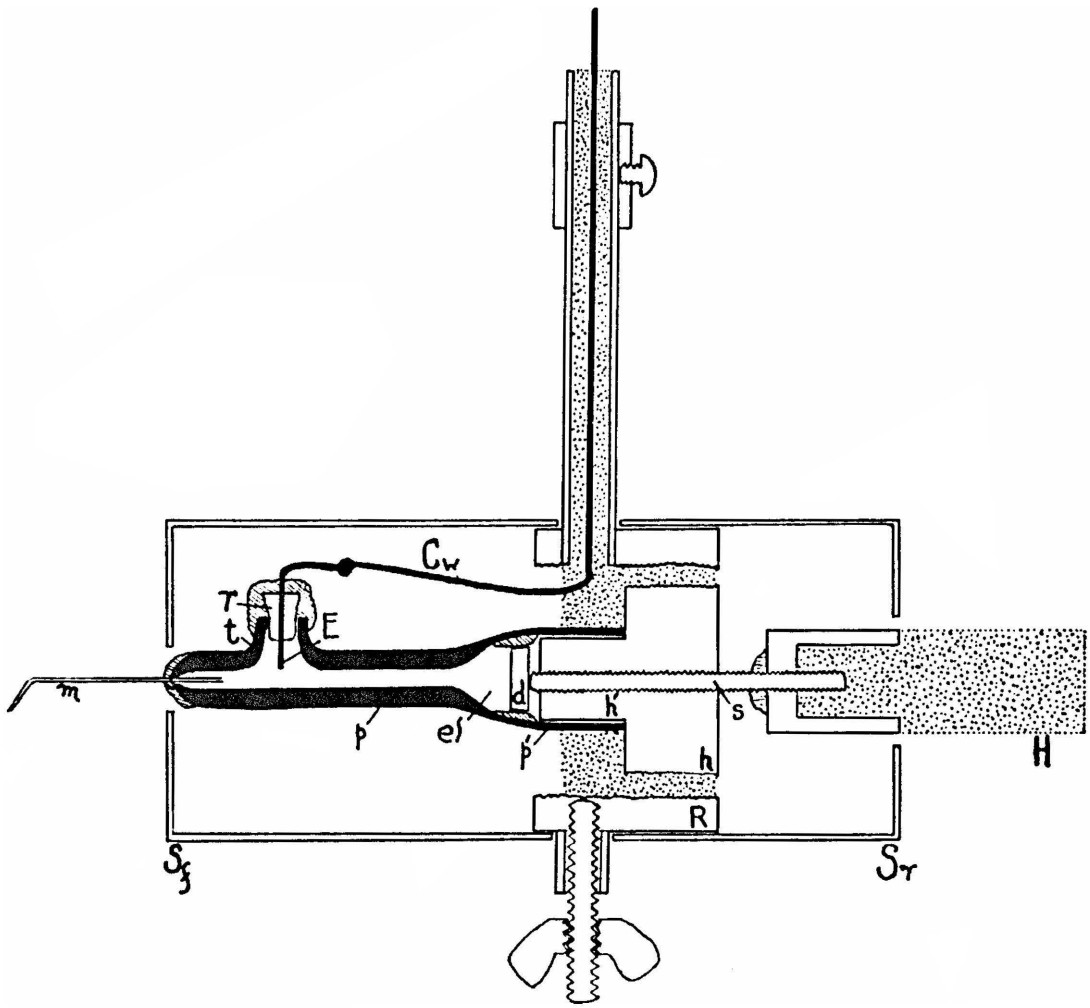

Cw
T
t
E
m
d
h
s
H
p
es
p'
h
R
Sf
Sr

FIGURE II

Circuit Diagram of Voltmeter [5]

Fixed Resistors	Variable Resistors	Miscellaneous
R_a — 10 ohms	R_1 — 2×10^4 ohms	P.D. — Input P.D. (measured)
R_b — 10^5 "	R_2 — 1×10^4 "	G — Galvanometer
R_c — 10^4 "	R_3 — 1×10^3 "	T_1, T_2 — Type 89, Vacuum Tube
R_d — 10^4 "	R_4 — 5×10^3 "	A — 6 Volt Storage Battery
R_e — 10^3 "	R_5 — 1×10^3 "	B — 45 " Dry "
R_f — 10^3 "	R_6 — 1×10^4 "	B_3, B_4 — 1 1/2 " " "
R_g — 10^2 "	R_7 — 1×10^5 "	S_2, S_7, S_9, S_{12} — Single pole, single throw switches
R_h — 10^2 "	R_8 — 5×10^5 "	
R_i — 10^7 "	R_9 — 1×10^4 "	S_1, S_3, S_8, S_{10} — Double pole, double throw switches
X_1 — 10^3 "	R_{10} — 2×10^4 "	
X_2 — 3×10^3 ohms	R_{f_1}, R_{f_2}	S_5, S_6, S_{11} — Double pole, double throw reversing switches
X_3 ——— 10^4 "		
X_4 ——— 10^6 "		S_4 — Selector switch
		P — Connections to potentiometer
		O — Connections to oscillograph (not used in present study)
		g — Direct connections for galvanometer. (not used in present study)

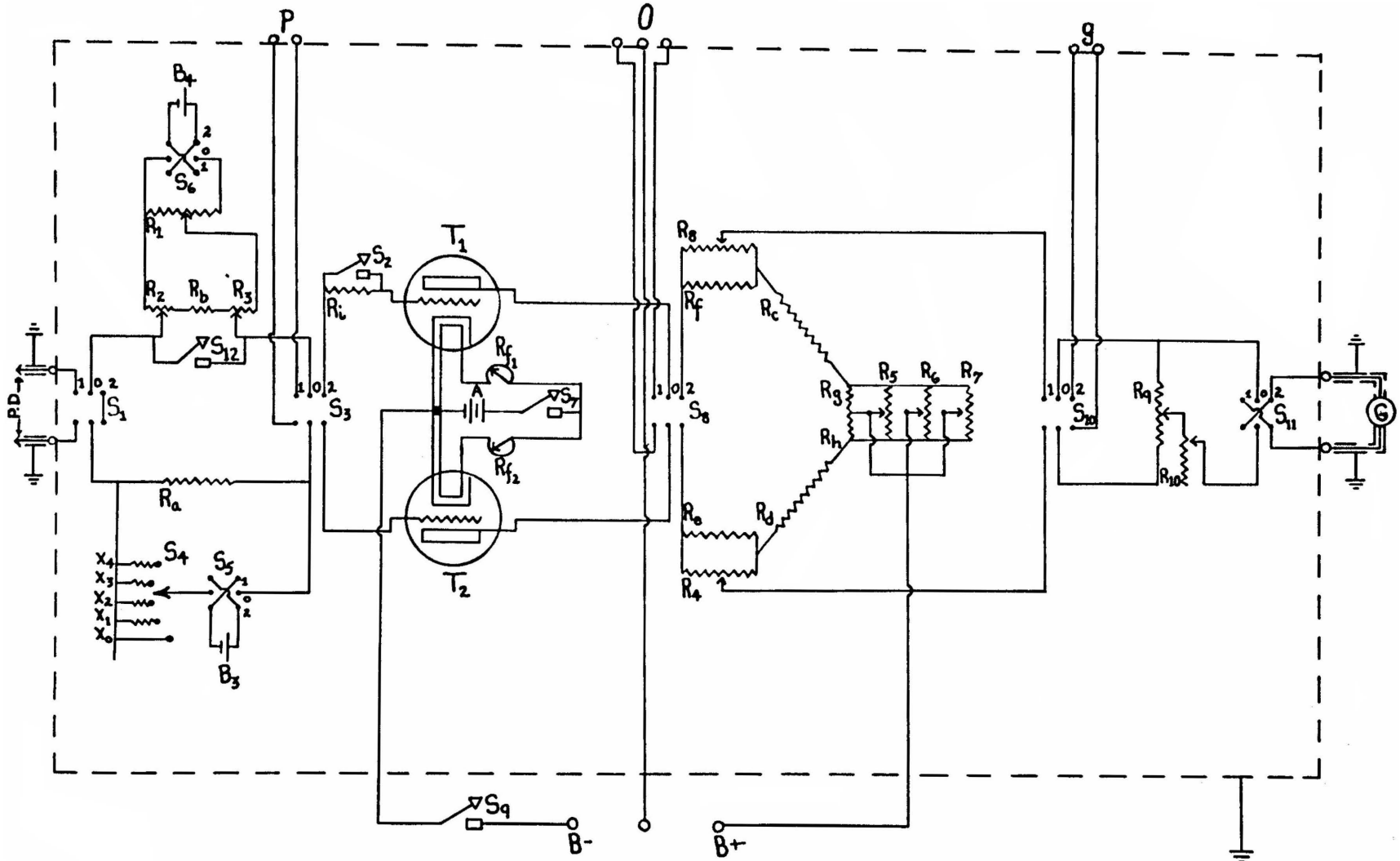

P
O
g
B+
S6
R1
R2
Rb
R3
S12
P.D
S1
S2
Ri
T1
Rf1
A
S7
Rf2
T2
S3
Ra
S4
X4
X3
X2
X1
X0
S5
B3
S9
B-
R8
Rf
Rc
R5
R6
R7
Rg
Rh
S8
Re
Rd
R4
S10
R9
R10
S11
G

FIGURE III

Diagram of Apparatus Assembly, as seen from side

1 — Inverted microscope

3 — Control unit of micromanipulator

4 — Base of micromanipulator

5 — Voltmeter

7 — Galvanometer scale

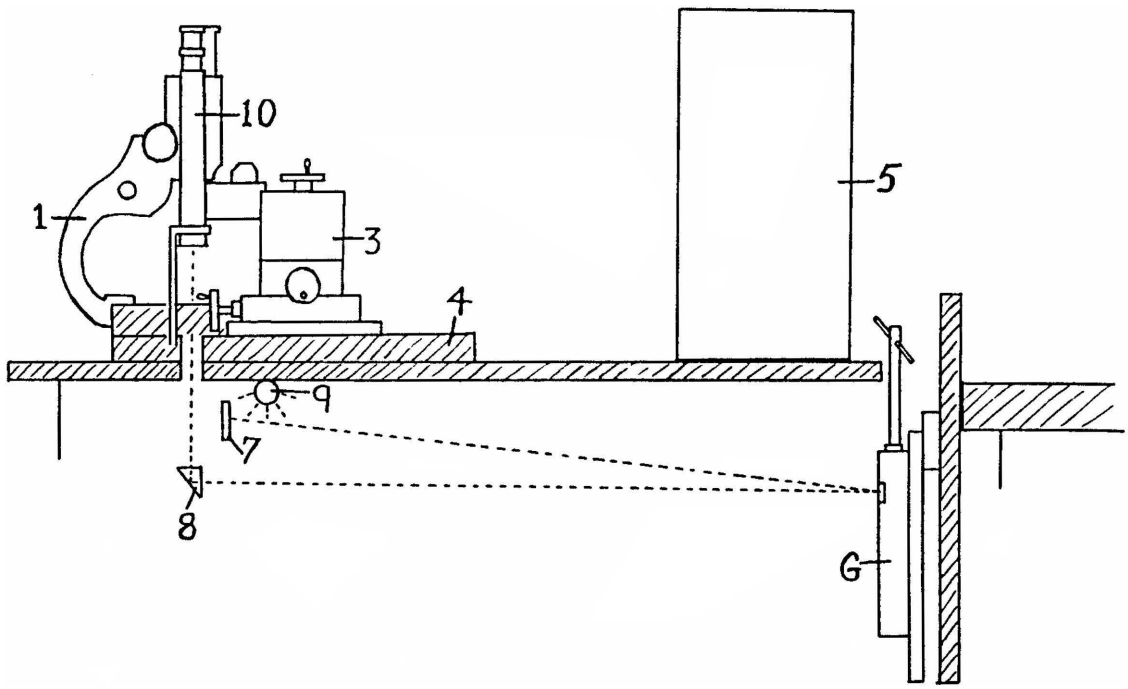
8 — Prism

9 — Lumiline bulb (18 in., 60 watt)

10 — Telescope

G — Galvanometer

Note: This figure indicates arrangement of galvanometer [G] with respect to microscope [1] and micromanipulator [3,4], etc. A number of items which would be apparent in such a side view are omitted; for these, see Plates V & VI.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APPENDIX A

Production of micro-electrode tips [m].

For a recent discussion of methods for the production of micro-needles and micro-pipettes, Chambers and Kopac ('57) should be consulted.

In the present work, the micro-electrode tips [m], which are really micro-pipettes, have been produced in the following way:

> 1/4" Pyrex tubing is thoroughly cleaned in cleaning solution and rinsed. In an oxygen flame, it is drawn down to about 1 mm. diameter. A 2" section of this 1 mm. tubing is drawn very thin in the middle, by warming in a micro-flame and pulling gently and carefully with the fingers. This 2" section, thinned at its mid-region, is now mounted in a special device which applies heat (radiated from a hot Nichrome wire) at the thinned mid-region, at the same time that it applies tension at the two ends of the capillary. The capillary is thus severed at its thinned mid-region, fine tips being produced at the point of disjunction. Quite often, these tips are closed, in which case they may be opened by being rubbed against a glass surface; this must be done carefully, of course, preferably under microscopic observation.

APPENDIX B

Further discussion of micro-electrode [2].

The micro-electrode assembly [2] is faulty in two respects. In the first place, control of the column of liquid electrolyte [el], effected by means of the action of a screw [s] against the rubber diaphragm [d], is not sufficiently delicate for work with small cells (as, for example, *Paramecium caudatum*). Since, however, the organism employed in the present study (*Chaos chaos*) is quite large, and since, furthermore, mouths of the micro-electrode tips were quite large (10 - 40 microns), precision control of the electrolyte column proved to be unnecessary. Secondly, the fact that the electrolyte column [el] contacts rubber (i.e., the rubber of diaphragm [d]) and the de Khotinsky cement which holds this rubber in place, is viewed as an objectionable feature. To be sure, both the rubber and the de Khotinsky cement are inert substances, and, hence, it is probable that they do not effect the reversible electrode [E]; nevertheless, it would be best if all possibility of such an effect were eliminated. In the present study, error resulting from possible action of the rubber and cement constituents on the reversible electrode [E] has been guarded against by frequent change of the electrolyte [el] and of the reversible electrode [E].

APPENDIX C

Aspects of theory and use of the voltmeter [5].

The network of the voltmeter [5] employed in the present study is diagrammed in Fig. II. It is a Wheatstone bridge network, in which two arms of the bridge are Type 89 vacuum tubes, and is similar in design to the instrument described by Burr, Lane, and Nims ('36).

The three grids of each tube are in electrical contact, and may be considered as one. The grids of both tubes operate at "free grid potential", any difference in these "free grid potentials" being compensated by a P.D. across R_b, R_2, and R_3. The input P.D. (measured) is applied to the two "free" grids, causing a change in the cathode-anode resistance of the tubes T_1 and T_2, and consequent unbalance of the bridge balance. The degree of such unbalance is the measure of the input P.D.

The two vacuum tubes (T_1 and T_2) constitute a "matched set". Matching contributes to bridge stability, for battery voltage fluctuations effect both tubes of a "matched set" similarly, and hence the effects of such fluctuations tend to become minimized.

R_{f_1} and R_{f_2} are short lengths of Nichrome wire, brought out onto the control panel (see Plate IV). Adjustment of these filament-heater resistors causes the bridge balance to drift in one direction or the other, so that proper adjustment of both R_{f_1} and R_{f_2} practically eliminates all drift due to A battery decay.

Drift of the bridge balance due to B battery decay is similarly compensated by adjustment of R_4 and R_3, wire-wound variable resistors paralleled with wire-wound fixed resistors.

Procedure for use of the instrument, as employed in the present study, is described on pp. 32–33, 44–45.

The input impedance of the circuit is AT LEAST 5×10^8 OHMS.

The stability of the network is not as great as is needed for work in the region of the instrument's maximum sensitivity. The "zero instability" (i.e., random fluctuations of the galvanometer) is too great to allow successful employment of the bridge when adjusted for maximum sensitivity. However, in the sensitivity range employed in the present study, the "zero instability" was not sufficiently great to prevent successful operation, being approximately 5 mm.