ON A CARBON DIOXIDE GRADIENT IN THE FUCALES EGG

Thesis by

Lionel F. Jaffe

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1954

ACKNOWLEDGMENTS

Thanks are due to my advisor, Dr. Albert Tyler, for his suggestions, criticisms, and assistance during the course of my work. It is a pleasure to express my appreciation to Dr. F. W. Went for his kindness, encouragement, and stimulating ideas. To Dr. A. J. Haagen-Smit and Dr. G. L. Keighley go sincere thanks for their generous assistance with chemical techniques. I am also indebted to Dr. W. R. Smythe for his liberal help with mathematical problems, to Dr. G. W. Beadle for calling my attention to the paper which suggested this investigation, to Messrs. W. P. Thomson, G. P. Keyes, and J. E. Cunningham of the staff of the Earhart Plant Research Laboratory, to Mrs. Burke H. Judd for her untiring patience in typing the manuscript, and to the Atomic Energy Commission for fellowship assistance.

ABSTRACT

Previous investigations suggest that a CO_2-pH gradient is involved in the development of polarity in the Fucales egg. To test this hypothesis, a method was developed to measure the relative CO_2 output from the two hemispheres of this egg. The eggs are photosynthetically tagged with C^{14} and embedded in a thin jelly membrane in which the eggs develop. One measures separately the respiratory $C^{14}O_2$ emitted from the two surfaces of the membrane during each of a series of intervals before, during, and after the eggs are unilaterally illuminated to orient their future axes. New methods are described for obtaining eggs, for controlling and measuring the thicknesses of egg-bearing jelly membranes, and for measuring $C^{14}O_2$. Because of a drop in the specific radioactivity of the emitted carbon dioxide, the sum of the rates of respiratory $C^{14}O_2$ emission from the two faces of a membrane decreases rapidly with time; but the $C^{14}O_2$-ratio, or the ratio of the rates of $C^{14}O_2$ emission from the two faces, changes very slightly with time. That face which had been lower while the membrane gelled nearly always emits $C^{14}O_2$ more rapidly than the other face. This is attributed partly to a settling of the eggs in the membrane while it gelled and partly to a higher respiratory rate somehow induced in the hemispheres of the eggs which point toward the formerly lower face. These "lower" hemispheres also tend to become the rhizoid poles. The changes of the $C^{14}O_2$-ratios with time are attributed primarily to changes in the relative specific radioactivities in the carbon dioxide emitted from the two hemispheres of the eggs, rather than to further changes in the relative respiratory rates of these hemispheres with time.

TABLE OF CONTENTS

INTRODUCTION

The visible differentiation of a germ is largely preceded by the establishment within it of a latent structure whose apparently undifferentiated parts bear a one-to-one correspondence to the structure of the developed organism. The transformation from the latent to the developed pattern is the preformationist or mosaic aspect of organic development. Development possesses an epigenetic or regulative aspect to the extent that the latent pattern develops afresh in each generation rather than being continuously maintained from one generation to the next. The presently available evidence - largely accumulated after 1891 when Driesch obtained a whole sea urchin larva from half an embryo - indicates that there is an important epigenetic aspect or phase in the development of all living organisms. Moreover, this evidence enables one to analyze the epigenetic phase into the following idealized sequence of stages:

1. Determination of the over-all character of the whole germ.

2. Polarization (primary, then secondary, and finally tertiary) of the whole germ.

3. Determination of the over-all character of each of various parts of the whole germ, called part germs.

4. Polarization (primary, then secondary, and finally tertiary) of each part germ.

5. Determination of the over-all character of still more circumscribed parts of each part germ . . . and so on until the fate

of each cell is determined.

Over-all determination of a whole or part germ establishes its development into a whole sea urchin, frog ear, leaf, etc. Primary polarization of a germ establishes one axis of differentiation. If the investigator cuts the singly polarized germ into parts, each part develops into a whole adult; but the primary polarity, or the direction of the first established latent morphological axis with respect to the substance of the germ, is the same in all the isolated parts. Furthermore, the established polarity of the whole germ or its isolated parts cannot be changed by chemical or physical influences. Secondary polarization determines a second latent morphological axis if the developed organism is asymmetrical with respect to a second axis etc.

To qualify this idealization properly or to review its origins is entirely beyond the scope of this paper (See Weiss, 1939, and Harrison, 1945). Suffice it to assert that this idealization is useful as a first step, but that the nature of epigenesis in physical and chemical terms remains a most challenging and almost total mystery.

Now, how can one attack this problem most effectively? A little consideration indicates that it should be technically easier to obtain physical and chemical information about epigenesis in whole germs than in part germs and about primary rather than secondary or tertiary polarization. With the limited exception of polymorphic organisms, the over-all character of whole germs is always preformed. Even in a highly regulative animal embryo like the hydroid, Amphisbestia, which may be divided into as many as sixteen parts, each developing into a normal larva, the primary polarity is irreversibly built into

the egg and develops unturned in the whole embryo and all its isolated parts (Teissier, 1931). In many plants, however, the primary polarity of the whole organism appears to arise epigenetically.

Thus, if the germs of the Equisetum gametophyte and of Fucus are unilaterally illuminated before their first mitosis, their rhizoids all develop on the sides of the germs that were away from the light (Stahl, 1885; Rosenvinge, 1888-9). The rhizoids mark one pole of the main morphological axis of these plants and the germs are glued down in a fixed position during the experiments. One could conclude that the primary polarity develops epigenetically were it not for the possibility that the whole protoplasm has a preformed polarity and merely rotates within the cell wall under the influence of the light, as in fact can occur in mature Bryopsis plants (Steinecke, 1925). In the Fucales, this latter possibility is rendered very unlikely by the frequent development of eggs with two oppositely situated rhizoid-bearing regions (See p. 9). Logically though the preformationist view could be partly rescued if external directing agents act by rotating the nucleus, or other self-duplicating asymmetrical inclusions, but an experimental test is as yet lacking.

In any event, in the Fucales the irreversible development of polarity can be studied in a single large undifferentiated cell, which is nevertheless the whole embryo of a highly differentiated adult. It is not surprising that the Fucales egg has been the most carefully studied object of researches upon polarization.

The bulk of the data on Fucales polarization concerns the sign and effectiveness of various factors as polarity directors. The

number of effective orienting agents known is large, but Whitaker (1940 c) suggested that some of them "can be resolved to a common action of hydrogen ions and CO_2." It occurred to me to ask whether the polarizing egg creates its own internal CO_2-pH gradient. To approach this question, the author attempted to determine directly whether the polarizing egg gives off more CO_2 at one pole than at the other.

How can one make such a measurement? The only similar one hitherto reported is that of Brachet (1939) on the frog egg held tightly within a capillary. The CO_2 emitted from each hemisphere of the egg traversed an air gap to enter a separate drop of bicarbonate solution containing phenol red where it was colorimetrically determined. However, since the smaller Pelvetia egg respires at only 1/600 the rate of the frog egg (Whitaker, 1931 a), a different technique was sought.

Following Whitaker and Berg (1944), this author embedded Pelvetia eggs in a thin jelly membrane supported only at its edges like a soap film in a loop. The eggs were polarized perpendicular to the film. Then during their subsequent development the respiratory CO_2 entering the two air gaps bordering the membrane was absorbed, thus effecting a partial separation of CO_2 from the basal and apical hemispheres of the eggs. The CO_2 was measured with the aid of $C^{14}O_2$.

LITERATURE REVIEW

Normal Development of Fucales*

The sexually mature fronds, or receptacles, discharge masses of haploid eggs, enclosed in öogonial capsules, into the sea where each egg is fertilized by a single minute sperm. The zygote is a relatively dense, radially symmetrical sphere of about .1 mm. in diameter. At first it lacks a vacuole or a cell wall but it is liberally supplied with small chromatophores and oil globules evenly dispersed in the hyaloplasm. Within a few hours the zygote escapes from the öogonial capsule and secretes a sticky wall rigid enough to allow plasmolysis. After a 12 to 24 hour diapause, during which the germ polarizes, a protuberance develops which marks the pole whence rhizoids and ultimately the adult holdfast develop. Cell division usually begins shortly after the protuberance first appears. Numerous cleavages and much growth create a turnip-shaped germling anchored by rhizoids at its narrow end.

The development of a tuft of hairs at the broad end initiates apical differentiation. The hairs soon degenerate but at the base of one of them the unicellular apical meristem appears at the bottom of a mucilage-filled crypt. In the Cystoseiraceae the symmetry remains

---

* This review will be restricted to the usual life cycle of the Fucaceae and the Cystoseiraceae since almost all experiments have been on species of these families. It is taken from secondary sources (Smith, 1938; Fritsch, 1945; Papenfuss, 1951).

axial; in the Fucaceae the thallus flattens and becomes biradially symmetrical. Much branching occurs, each branch terminating in a unicellular meristem which accounts for all increase in length. Large, colorless, longitudinally elongated cells suggesting conductive tissue differentiate internally and an epidermis forms externally. Eventually the branch ends differentiate into special fronds or receptacles which are dotted with nearly closed cavities called conceptacles which in turn bear the gametangia. Other prominent features of differentiation are hairs and gas bladders. Growth is indefinitely prolonged and individuals may reach 30 feet in length.

Studies of Polarization. (See Bloch, 1943; Whitaker, 1940c, for reviews.)

First Work. In 1888 the Danish investigator, Rosenvinge, began the study of the development of polarity in the Fucales. In his review of previous work he refers to at least three cases then known of plants whose main axes can be irrevocably determined by external gradients very early in development: polarization of the gametophytes of Marchantia and Equisetum by light (Mirbel, 1835; Stahl, 1885) and of pollen grains by nearby pollen grains (Kny, 1881). Examining the germination of five species of the Fucaceae in an analogous way, he found that the rhizoid forms away from unilateral light, toward nearby egg groups, and, under some conditions, toward a glass plate to which the eggs adhered. However, gravity and oögonial position*

---

* Studied with Pelvetia canaliculata in which germination occurs before the eggs leave the oögonial capsule.

had no effect, and normal germination occurred in the absence of obvious orienting influences, except that occasional bipolar forms developed. Moreover, the first cleavage plane bore no necessary relationship to the position of the rhizoid. Rosenvinge attributed some of these effects to the creation of an oxygen gradient.

Subsequent work will be reviewed by category rather than in a strictly historical order.

Conditions Compatible with Polarization and Germination. Some of the scanty and scattered data bearing on this question are listed here for reference. Cystoseira barbata eggs can polarize at both 1-2°C and 17-18°C (Knapp, 1931). Though direct reports of the effects of higher temperatures are lacking, various investigators generally seem to be careful to culture the Fucales at temperatures under 20°C. Moreover, Kniep (1907) reported that even transient exposure of F. serratus eggs to a temperature of 25°C greatly inhibited their germination.

F. furcatus eggs can polarize and germinate at pH's from 9.2 to 5.5 (Whitaker, 1937b). F. serratus eggs develop normally after centrifugation at 150,000g for one half hour (Beams, 1937). All F. serratus eggs can polarize but not germinate at 200% normal salinity and some F. serratus eggs can polarize and germinate in sea water with a salinity as low as 27% of normal (Kniep, 1907; also see Whitaker & Clancy, 1937).

Period of Sensitivity to Orienting Agents. There is almost no evidence as to the possibility of polarizing unfertilized eggs because the ordinary means of holding them in a fixed position would block

fertilization and therefore prevent the further development which makes the polarity visible. However, Knapp centrifuged C. barbata eggs before fertilizing them; an unstated fraction retained enough stratification for it to be observed that their rhizoids were all on the centrifugal ends of the eggs.

Between fertilization and germination there exists a short period showing a marked maximum in sensitivity to white light and to ultraviolet light (Winkler, 1900; Kniep, 1907; Knapp, 1931; Whitaker & Lowrance, 1936; Whitaker, 1941). Once the normal time of germination has passed, unilateral white light can no longer affect the polarity (Kniep, 1907; Whitaker & Lowrance, 1936), this holding even if morphogenesis is delayed for several days by hypertonic sea water (Kniep, 1907).

Nearly all that is known of other orienting agents in this regard is that they can be effective during some time between fertilization and germination.

Polarization in the Absence of Orienting Agents and by Opposing Agents. All investigators have confirmed Rosenvinge's observation that normal germination occurs in the apparent absence of orienting agents. However, in 1931, Knapp showed that the rhizoid of Cystoseira forms at the point of fertilization in the absence of other orienting agents. Probably this holds generally.

Since white light and centrifugation readily polarize Cystoseira eggs, these agents appear to reverse the fertilization-induced polarity. Furthermore, centrifugation can partly counteract the effect of white light which thus appears to be a second reversal

of polarity (Knapp, 1931). More striking is Whitaker's report of the apparent reversal of ultraviolet-induced polarity by white light in Fucus (1942). He unilaterally irradiated a group of eggs with ultraviolet light and then unilaterally illuminated a portion of these with white light from the opposite direction. The former eggs developed rhizoids away from the ultraviolet light; the latter, toward it. It is seen then that the effect of one agent may overcome the effect of an earlier one, but it is not known whether the polarity proper is reversed or whether some prior condition is counteracted.

Now various studies of other germs have demonstrated that if during polarization one agent is followed by an opposing but barely balancing one, then there develop some bipolar forms. For example, Harrison (1945) rotated otic placodes of Ambystoma 180° during their anterior-posterior polarization and obtained some enantiomorphically twinned ears. What do successive balanced orienting agents do to the Fucales egg?

Occasional cases of bipolar germination have been noted from the first, but their nature is obscure (Rosenvinge, 1888-9; Küster, 1906). Thus Whitaker (1931b) found that giant Fucus eggs, arising from the fusion of 2 to 8 normal eggs in the oogonial capsule, often germinated in a bipolar manner. However, Child (1941) reported, without data, that hourly reversals of light direction on Fucus eggs yielded many forms having a pair of opposite rhizoids or no rhizoids. Unfortunately no more information is available.

Direct Studies of the Character of Polarity. Reed and Whitaker's

investigation (1941) on polarized plasmolysis is virtually the only one in this group. They polarized Fucus eggs with various agents and then, before germination, they plasmolyzed them with sea water made hypertonic with added sucrose. More than 90% of the cells shrunk away from their walls only at the future rhizoid sides.

There is a broad maximum in sensitivity to white light polarization between 6 and 14 hours after fertilization; by 16 hours, half of the eggs form protuberances. When ultraviolet light, white light and centrifugation at pH 8 were the orienting agents (See Table 1), markedly polarized plasmolysis appeared at 8 1/2 hours or earlier; when nearby eggs at pH 6.0 were the agents, such plasmolysis first appeared at 13 hours.

Theoretically, this polarized plasmolysis might arise from the rhizoid pole of the cell proper being more permeable to water, less rigid, or less firmly attached to the cell wall; evidence on which to make a choice is not available.

Plasmolysis remained restricted to the future rhizoid pole even after protuberances had appeared. This is curious because in a very wide variety of other plant cells the protoplasm clings relatively tenaciously to newly forming portions of the cell wall (Weber, 1929; Reuter, 1953).

Another possibly germane observation is that of Child (1919, 1945). He reports that if Fucus eggs are subjected to sub-lethal concentrations of $KMnO_4$, then its brown reduction product, MnO_2, first appears within the rhizoid halves of the eggs. Also, KCN and "various other agents" kill the rhizoid regions more rapidly.

However, this pattern is not evident until a rhizoid cell has actually been cut off. Similarly, Kniep (1907) observed that after a rhizoid cell has been cut off, it may be killed by hypotonic sea water without injuring the rest of the embryo, which upon return to normal sea water continues to develop, soon regenerating rhizoids.

Direction and Effectiveness of Polarization by Various Agents

Most of the abundant data in this category are summarized in Table 1. The orienting agent is considered to be of threshold strength when 75% of the rhizoids originate in one hemisphere. (Of course, in the absence of orienting agents, 50% would do so.) The following twelve agents are known to be effective: unilateral short wave length visible light, unilateral ultraviolet light, a heat gradient, an electric current, centrifugal (or gravitational) force, shape of the egg, a diffusion barrier, gradients of CO_2-pH, dinitrophenol, and potassium indole acetate, nearby Fucales eggs or thalli, and fertilization. Four of these agents - centrifugal force, a diffusion barrier, a gradient of CO_2-pH, and nearby eggs - can polarize the eggs in opposite directions depending upon the pH. Hence, altogether, 16 positive effects are known. The only clearly negative result found is the failure of unilateral long wave length visible light to polarize.

Naturally, a negative result needs quantitative qualification. On general grounds it is very probable that the effective agent on each egg is a gradient of light rather than the direction of illumination. The author estimates that the Cystoseira eggs studied by Mosebach were unilaterally illuminated by 100 foot-candles of long wave length light

Table 1. Directors of Polarization (See Key to Species on p. 14)

Director	pH	Rhizoid Side	Threshold	Species and Investigators
Light White		Dark		Fbchi (Rosenvinge, 1888-9); Ca (Winkler, 1900; Knapp, 1931); Fbe (Kniep, 1907); Fa (Whitaker & Lowrance, 1936). (See also Farmer & Williams, 1898; Pierce & Randolf, 1905).
"		"	5 hrs. at .17 ft.- ca. = 5000 erg/mm^2	Ca (Mosebach, 1938)
>505 mμ (mainly >555 mμ)		No Effect		"
>485 mμ		Dark		"
366, 313 mμ		Dark	1000 ergs/mm^2	Fa (Whitaker, 1941)
280 mμ	8.0	Dark	1-10 ergs/mm^2	"
254 mμ	8.0	Dark	3 ergs/mm^2	Fa (Whitaker, 1941, 1942)
254 mμ	6.0	"	1.3 ergs/mm^2	"
265, 248, & 235 mμ	8.0	Dark	1-10 ergs/mm^2	Fa (Whitaker, 1941)
Heat	6.3-8.2	Hot	.4°C across egg	Fa (Lowrance, 1937)
Electric Current		Electropositive	25 millivolts across egg	Fd (Lund, 1923)
Gravity		No Effect		Fc (Rosenvinge, 1888-9)

(Cont.)

Table 1. Directors of Polarization (Cont.)

Director	pH	Rhizoid Side	Threshold	Species and Investigators
Centrifugation		Centrifugal	1-2 min. at 760g	Ca (Knapp, 1931)
	8.0	"		Fa (Whitaker, 1937, 1940b); Fg (Lowrance & Whitaker, 1940)
	6.3	No Effect		Fa (Whitaker, 1940b)
	6.0	Centripetal		"
Shape of Egg Made Prolate	7.8-8.2	Near End of Long Axis		Fa (Whitaker, 1940a)
Diffusion Barrier	6.4-7.6	Toward Barrier		Fa (Whitaker & Lowrance, 1937)
	8.0	No Effect		"
	8.1-8.6	Opposite Barrier		"
				(See also Rosenvinge, 1888-9)
CO_2 - pH Gradient				
Low pH: 5.9-6.4		Acid		Fa (Whitaker, 1938)
Low pH: 5.5-5.7		Basic		
Dinitrophenol	8.2	High DNP	2×10^{-5} M	Fa (Whitaker & Berg, 1944)
Potassium Indole Acetate		High Auxin	2×10^{-3} M	Fb (Olsen & Du Buy, 1937)
Nearby Fucales Egg or Thallus				
Fertilized Eggs of Same Species		Toward Neighbor		Fbcehi (Rosenvinge, 1888-9); Fd (Hurd, 1920); Fg (Lowrance & Whitaker, 1940)
"	6.0-7.5	"	One egg 2-3 egg diameters away	Fa (Whitaker, 1937b, Whitaker & Lowrance, 1940)
"	7.6	No Effect		
"	7.7-8.4	Opposite Neighbor	"	Fa (Whitaker & Lowrance, 1940)

(Cont.)

Table 1. Directors of Polarization (Cont.)

Director	pH	Rhizoid Side	Threshold	Species and Investigators
Unfertilized Eggs of Other Species		Toward Neighbor		Ff (Whitaker, 1931)
Thallus of Same or Other Species		Toward Thallus		Fe (Kniep, 1907)
Fertilization		Sperm Entry Point		Ca (Knapp, 1931)

Key to Species: F = Fucaceae; C = Cystoseiraceae; Fa = Fucus furcatus; Fb = F. vesiculosus; Fc = F. spiralis; Fd = F. inflatus; Fe = F. serratus; Ff = F. evanescens; Fg = Pelvetia fastigiata; Fh = P. canaliculata; Fi = Ascophyllum nodosum; Ca = Cystoseira barbata.

(>505 mμ) during a sensitive period of about five hours. This corresponds to 3,000,000 ergs/mm^2, which is more than 1,000 times the threshold energy required when using short wave length visible light. While measurements of visible light absorption by Fucales eggs are lacking, the simple fact that they are yellow-green in color indicates that light absorption in the blue-green and in the red are comparable. Hence, even if it exists, the threshold of red light polarization must be so much higher than that of shorter wave lengths as to indicate action by a different mechanism.

Previous to Mosebach's work Miss Hurd (1920) had studied the effects of colored light on the development of F. inflatus, obtaining similar results. However, she did not know the intensity of light used, as it came from unmeasured diffuse daylight passed through uncalibrated filters. Nienburg (1923) reported that two species of Fucus could be polarized by red light. However, Mosebach re-examined his red filter and found that it transmitted some "false light" in the violet.

While blue-green and violet light are directly known to polarize, the effectiveness of blue light alone can only be inferred. The fact that light of ultraviolet wave lengths, from 235 to 280 mμ, is more than 100 times as effective as the lines,313 mμ and 366 mμ, and a band in the blue-green suggests that the very short wave lengths act by means of a different mechanism.

One finds two reports that ultracentrifugation fails to polarize the Fucales egg, that of Nakazawa (1951) using two species of the Sargassaceae and that of Beams (1937) using F. serratus.

Interpretation is obscured since Nakazawa cultured the eggs in white light and neither investigator varied the pH of the medium.

Interpretations of the Data

Various investigators have emphasized the significance of oxygen gradients (Rosenvinge, 1888-9), electrical forces (Lund, 1923), auxin gradients (Olsen & Du Buy, 1937), and CO_2-pH gradients (Whitaker, 1940c).

The effect of an oxygen gradient has never been isolated, though four other effects - polarization by nearby eggs or thalli, diffusion barriers at low pH's, heat gradients, and short wave length visible light - might be explained as acting by creating a lower oxygen concentration at the future rhizoid pole. However, the failure of red light to polarize the phaeoplast-bearing eggs is difficult to explain on this basis.

The polarization threshold using potassium indole acetate is so high (2×10^{-3} M) and so nearly toxic that one doubts its specificity; Olsen & Du Buy did not test gradients of other organic acids, potassium compounds etc. Moreover, if IAA in the highest non-toxic concentrations is added at pH 6.3 or pH 8.0 to ultracentrifuged eggs or at pH 6.0 or pH 8.0 to ultraviolet-polarized eggs, then it has only a very slight effect on polarization (Whitaker, 1940, 1942). It is therefore improbable that IAA is involved in the polarization of the Fucales egg.

The CO_2-pH gradient theory is more attractive, since in addition to the four effects explicable by an oxygen gradient, three others - the actions of DNP, IAA, and of an external CO_2-pH gradient at higher pH's - are qualitatively explicable as acting by making the

rhizoid pole relatively acid. As before, however, the failure of red light to polarize is difficult to explain. One also notes that a pH gradient would create an electrical gradient with the positive pole at the acid end since hydrogen ions bind more strongly to immobile proteins than do their balancing anions. The facts that the rhizoid usually appears at the apparently acid pole and that it also appears at the electropositive pole might thus be correlated.

MATERIALS AND METHODS

Obtaining the Gametes

Almost all studies of Fucales development have utilized members of the Fucaceae or the Cystoseiraceae. Locally, one finds two members of the Fucaceae, the upper littoral Hesperophycus Harveyanus* and the mid-littoral Pelvetia fastigiata* plus three members of the Cystoseiraceae, the lower littoral or sublittoral Cystoseira osmundacea, C. Setchelli and Halidrys dioica (Setchell & Gardner, 1925; Papenfuss, 1951). P. fastigiata was selected since it is more easily collected than the lower littoral species and fruits during a longer portion of the year than Hesperophycus.

The author collected most of the material at Corona del Mar where P. fastigiata receptacles are mature from October 15 through June 1; during June and July of 1953 Dr. Lawrence Blinks mailed material that he collected at Pacific Grove where P. fastigiata fruits all year. Receptacles collected at Corona del Mar were cut free at their bases, brought indoors, and allowed to air-dry until liquid was no longer visible on them. Then they were placed inside a covered jar, which was in turn inside an ice-cooled thermos jug, and transported to Pasadena where they were stored in closed jars, still dry, in the dark, and at about 6°C. Even after more than two weeks of such storage, receptacles have yielded eggs showing more than 95% normal germination. Possibly because of overheating, the receptacles mailed from Pacific

---

* The author is indebted to Dr. Gilbert Smith for identifying specimens of these two species.

Grove did not always yield such healthy gametes.

Lowrance and Whitaker (1940) stimulated P. fastigiata to shed gametes simply by immersing the dry and cold-stored receptacles in sea water at 15°C. However, the author's efforts to stimulate shedding in this manner rarely yielded as many as 200 eggs per receptacle-hour (despite extensive variations in the time, temperature, and humidity of storage as well as in the temperature and illumination during immersal). Further experimentation required a new means of obtaining gametes. Thence it was discovered that receptacles shed gametes upon exposure to a brief period of darkness following a longer period of illumination. A detailed description of this phenomenon will appear elsewhere since it has been hitherto unreported in the algae and may resemble angiosperm photoperiodism. Here let it suffice to describe the routine method.

After two or more days of storage receptacles are transferred to sea water at 16°C and are illuminated by about 500 foot-candles of light from a daylight type fluorescent tube. After any period of such illumination longer than four hours, the receptacles are transferred to darkness. Five to ten minutes later rapid shedding ensues; within another fifteen minutes each receptacle yields up to 10,000 eggs and a sufficient number of sperm to fertilize them rapidly.

Labeling of Respiratory CO_2 with C^{14}

Twenty minutes after darkening a few receptacles the eggs thus yielded were allowed to settle in a graduated centrifuge tube for five to ten minutes, then the supernatant was drawn off down to approximately .1 cc., and the residual eggs and sea water were spread

out inside a paraffin barrier on a slide. In two early experiments this barrier enclosed 1 cm.^{2}, giving a pool .8 to 1.5 mm. deep; in the later ones it enclosed an area of 4 1/2 cm.^{2}, giving a pool .2 to .3 mm. deep. Thus covered shallowly, the eggs photosynthesized for 50 minutes under a total of approximately 500 foot-candles of white light coming from opposite directions in an air-filled 30 cc. chamber containing .1 to .2% of CO_2 labeled with .15 millicurie of C^{14} per mgm. of CO_2. Then after replacing the gas in the photosynthesis chamber with CO_2-free air, the eggs were thoroughly washed with fresh sea water and were now ready to be embedded in a film. Their subsequent respiratory CO_2 was always adequately labeled for measurement, though owing to readier diffusion of $C^{14}O_2$ through the shallower pool the respiratory CO_2 contained 2 1/4 times as high a concentration of C^{14} in the later experiments as in the earlier ones.

Embedding the Eggs in a Membrane

The method used was a modification of Whitaker and Berg's invention (1944). A description of preliminary experiments with a "polypectate" gel will precede that of the method utilizing agar which was finally adopted. Membranes of a "sodium polypectate"* gel containing normally developing eggs were formed as follows: Pelvetia eggs were washed with isotonic (1.1 M) glycerine and added to a 1%

---

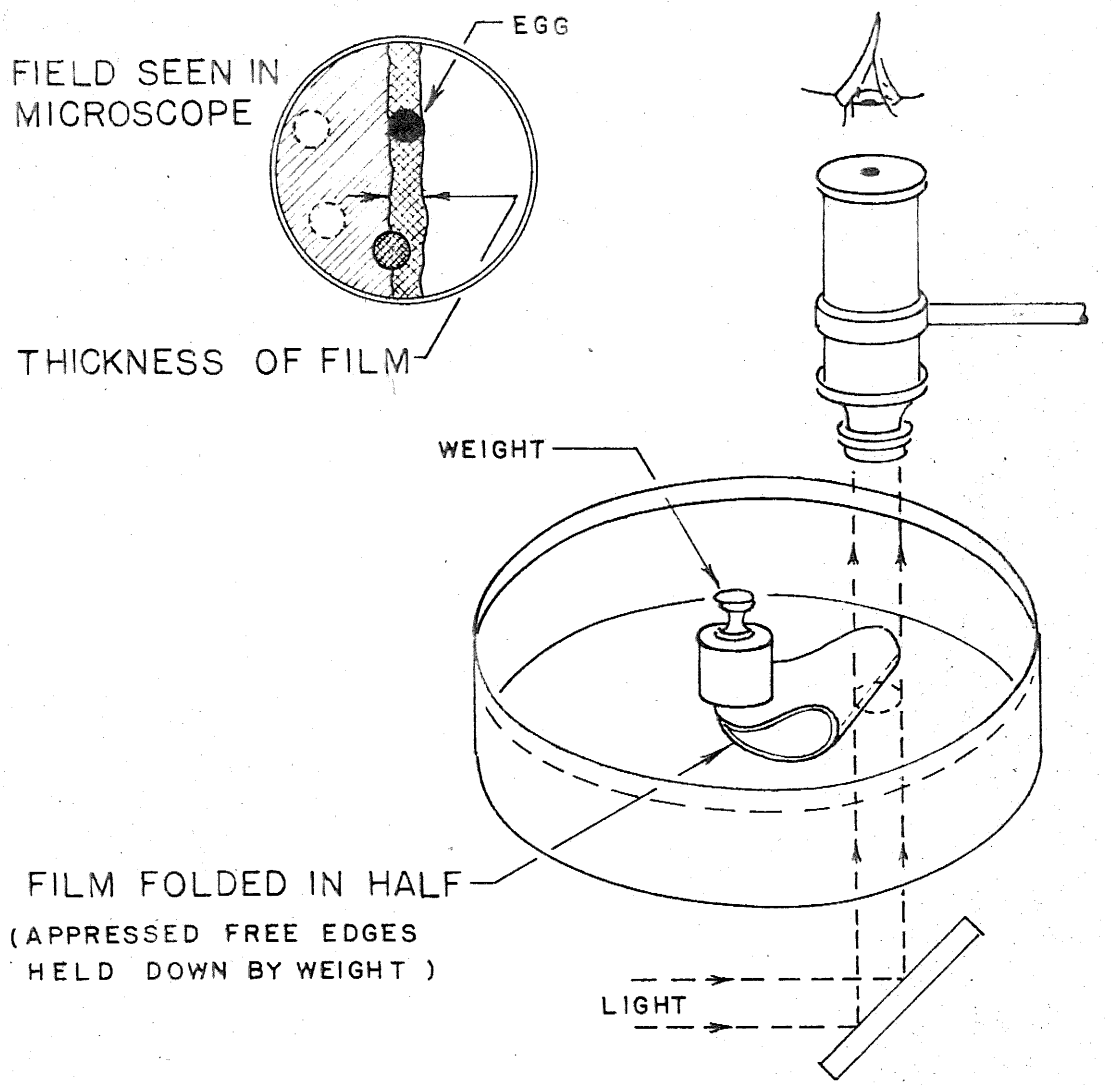
* "Sodium polypectate" is "product 24" of the California Fruit Growers Exchange (Corona, Calif.). It is described as a sodium salt of pectic acid of an average molecular weight of approximately 100,000. The author is indebted to Dr. Robert McColloch for a sample of, and information about, this material.

solution of sodium polypectate in isotonic sucrose*. As horizontally held rings, 1 or 2 cm. in diameter, were withdrawn from contact with this suspension they were spanned by liquid egg-bearing films. Turned to a vertical position, the rings were immediately dipped into sea water which instantly gelled the films. Since such a membrane gels while vertical, the eggs are probably embedded equidistant from its faces, but an agar membrane gels while horizontal and the eggs are found to set nearer its lower face (See p. 49). This shift greatly confuses interpretation of the CO_2-ratio experiments, but its existence was unsuspected when agar films were chosen.

In their procedure Whitaker and Berg mixed some Fucus eggs rapidly with a drop of a nearly congealed sol of agar in sea water. As they withdrew a horizontally held glass ring from contact with this mixture, it was spanned by a quickly gelling egg-bearing film. They measured the thicknesses of their films by examining "thin sections . . . cut with razor blades" and found each film to be fairly uniform except for a greatly thickened peripheral zone where it flared out in its attachment to the ring. To determine a film's thickness the present author detaches a film from its ring, immerses it in sea water, folds it in half and holds it down with a glass weight sitting on its appressed free edges. Microscopic observation of the fold shows a double line whose separation is considered the thickness of the film since eggs embedded in the folded region appear undistorted (See Fig. 1). Films made according to Whitaker and Berg's procedure, appear as they

---

* The nonelectrolytes were used because isotonic NaCl was found to gel 1% sodium polypectate.



METHOD OF DETERMINING FILM THICKNESS AT VARIOUS POINTS.

(DIAGRAMMATIC)

FIGURE 1

described. For example, film A, a section of which is represented in Figure 2, was made by withdrawing an ordinary glass ring having an inside diameter of 23 mm. from some nearly congealed 2% agar held in a watch glass. No eggs were included in this film.

Now in observing the effect of a gradient on cells, the thickened periphery does not interfere since attention can be restricted to the central cells; in measuring gradients produced by cells, however, metabolites liberated peripherally will mix with the central ones and obscure interpretation. It was therefore necessary to modify the technique so as to eliminate the peripheral thickening.

It would appear that if the ring were no thicker than the central region of the film, then the film would not flare out peripherally. The desired film thickness was approximately one Pelvetia egg-diameter or about 100μ. Therefore the ring should be less than or equal to 100μ in thickness. Such a ring might be constructed by cutting a hole in a sufficiently thin plate rendered hydrophobic except at the edge of the hole. This was achieved by cutting a roughly circular 10 mm. hole in a #0 glass cover slip with the aid of hydrofluoric acid guided by a paraffin barrier, siliconing* the entire resultant ring and finally removing the silicone from the edge of the hole with a fine paper-backed abrasive. The cover slip ring was supported and handled by means of a lucite ring attached to it with paraffin (See Fig. 3). When properly made, an agar gel membrane contacts such a ring only at the edge, the plane faces of the ring

---

* With "Desicote" made by Beckman Instrument Co., South Pasadena, Calif.

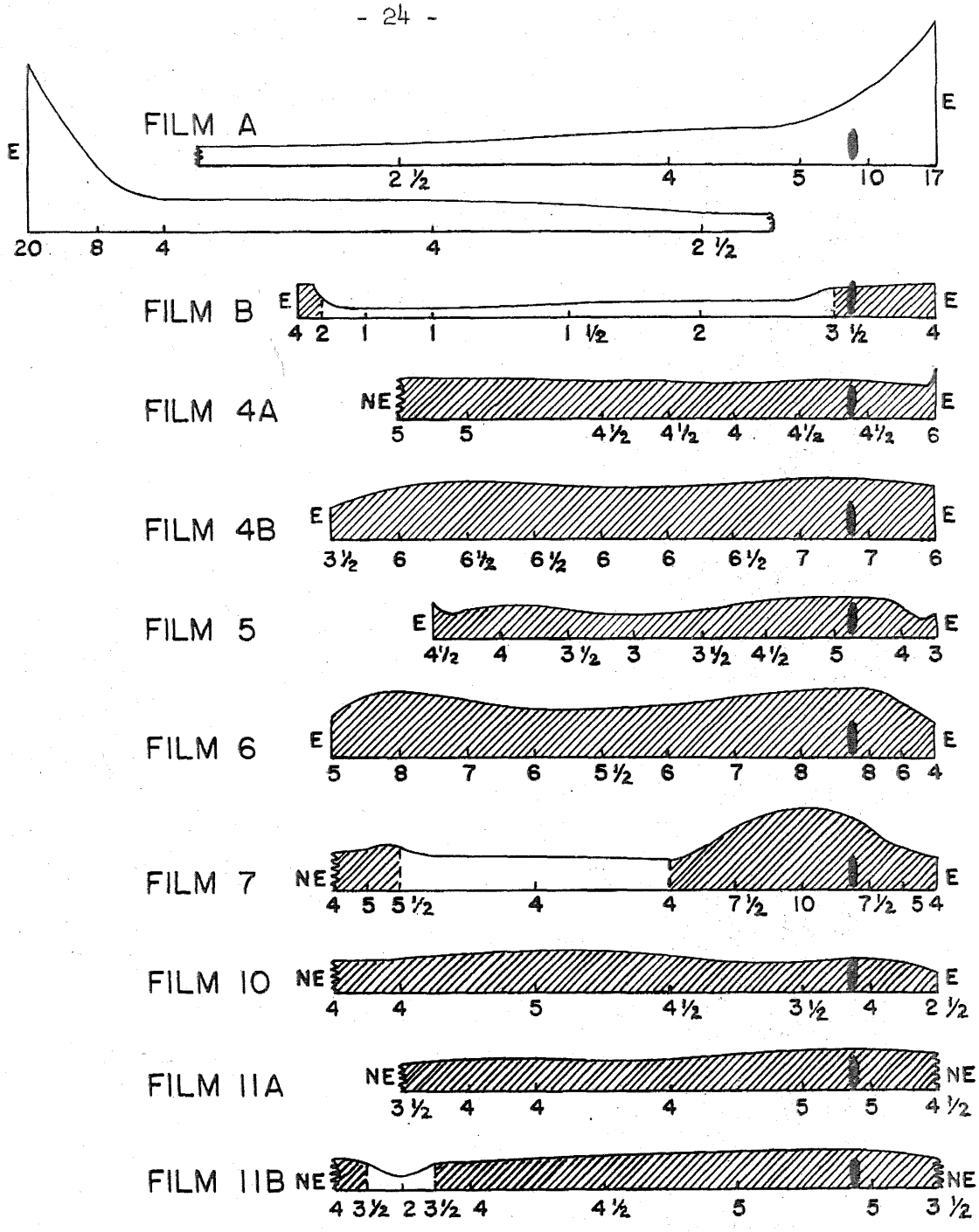
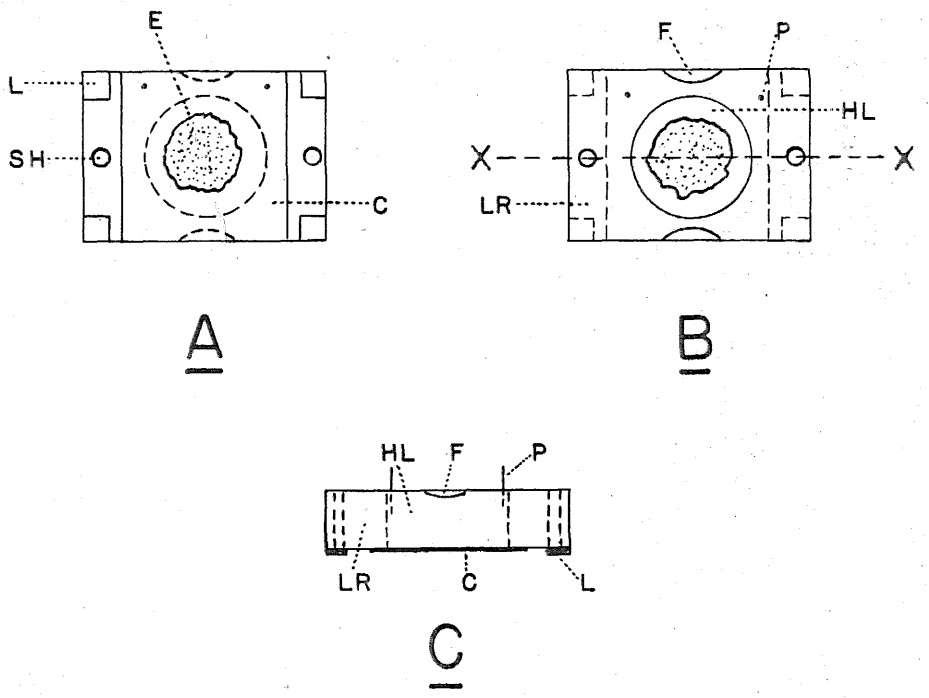


Figure 2. Sections of Films Reconstructed from Observations of Them Folded in Half (See Fig. 1). The indicated thicknesses are in ocular micrometer scale units of 25μ each. Film section A is about 2300μ long; the others are each about 1000μ long. The thicknesses have been magnified five times as much as the lengths. A spherical Pelvetia egg of 100μ in diameter is drawn to scale within each film. The shaded regions are the only ones that bore eggs. Film A was made according to Whitaker and Berg's method (See p. 23). The other films were made with cover glass rings. Film B was made with the aid of .008% sodium lauryl sulfate (NaLS); films 11A and 11B, with .004% NaLS; film 10, with .002% NaLS; films 4A, 4B, 5, 6 and 7, without NaLS. Films 4A, 4B, 5, 6, 7, 10, 11A and 11B were used in the principal experiments. E = edge of film; NE = near edge of film.



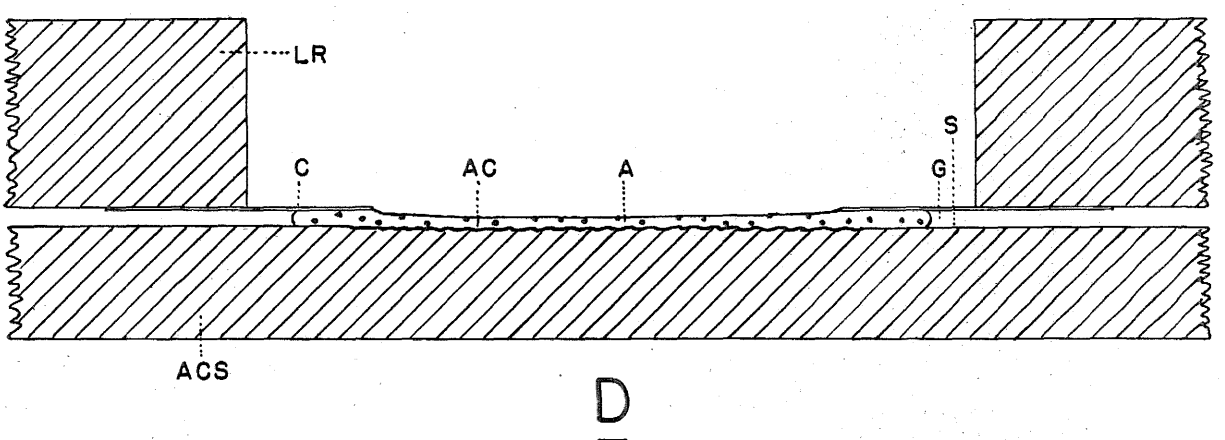


Figure 3. A - C, cover slip ring attached to a lucite ring and spanned by an egg-bearing film, x 1. D, section in the plane X--X of a cover slip ring sitting upon an abraded circle slide just before forming a film, x 6. (A, agar-sol-egg-mixture; AC, abraded circle; ACS, abraded circle slide; C, cover glass ring; E, egg-bearing film; F, finger groove which aids one in grasping CO_2 traps; G, gap maintained by legs; HL, hole in lucite ring; L, stainless steel legs 1/3 mm. high; LR, lucite ring; P, stainless steel pin which helps support CO_2 traps; S, smooth portion of abraded circle slide; SH, screw hole.)

being entirely free of agar. Since a #0 cover slip is about 100μ thick, it is not surprising that the edges of the membrane are found to be about this thick regardless of the thickness of the center. When the center is also about 100μ thick, a fairly uniform membrane of approximately one egg-diameter in thickness is obtained (See Fig. 2).

The author was unable to control the thickness of the main central areas of films made with cover slip rings and 1.0% agar in sea water*; they were usually too thick. Nevertheless, five of the eight principal experiments reported involved such films.

Later a reliable method was found for obtaining egg-bearing films with a fairly uniform thickness of one egg-diameter. This method consisted of adding .004% of the detergent, sodium lauryl sulfate (NaLS) to the filtered 1.0% agar solution before use. As is described in a later section, the membranes were always washed with pH 6.0 sea water a few minutes after formation, which presumably removed almost all of the NaLS. Nevertheless, one might well fear that even transient exposure to a powerful detergent would interfere with development.

Hence in two preliminary experiments, five films bearing unlabeled eggs were made with the addition of .004% NaLS, and then left to develop in pH 6.0 sea water. The eggs in these films exhibited 95 to 100% normal germination when observed 48 hours after fertilization. Another relevant experiment was performed with the intent of obtaining

---

* Higher temperatures, lower agar concentrations and slower withdrawal speeds all thin the films, but the first injures the eggs, the second yields excessively weak films, and the last gives films with small spots so thin as to exhibit interference colors.

$C^{14}O_2$-ratio data; some eggs were labeled, embedded in two films made by using .004% NaLS and then their respiratory CO_2 was trapped for eight hours. The $C^{14}O_2$ traps were defective and were discarded, but the film was then transferred to sea water of pH 6.0 for further observation. Twenty-eight hours after fertilization, 85% and 72% of the eggs in the respective films had germinated normally; about 85% of a batch of control eggs had likewise germinated normally by this time.

It would appear that the use of .004% NaLS had little or no effect upon germination. However, in one of the principal experiments reported (#11), involving two films made with .004% NaLS, germination and photosynthesis were markedly inhibited (See experimental results in the next chapter). The source of this discrepancy is obscure, though it may be noted that in this experiment, the egg-bearing films were kept in a CO_2-free atmosphere for the unusually long period of 15 1/2 hours.

Fearing the toxicity of NaLS and puzzled by the variable thicknesses of films made without the detergent, the author was impelled to wonder whether the sea water supernatant to gametes might have an effect upon film thickness. Hence, in a preliminary experiment some egg-bearing films were made using unwashed eggs and 1.0% agar without NaLS. These films were more frequently thin than films made with washed eggs, but their thicknesses were still not entirely under control.

Another difficulty arising from the use of cover slip rings is that the agar-sol-egg-mixture spreads out under the ring, consequently

gluing the ring down and crushing the eggs. This problem was solved by the joint use of an "abraded circle" mixing dish and legs attached to the ring to support it about 1/3 mm. above the dish (See Fig. 3). An abraded circle dish consists of a microslide entirely siliconed except for a roughened hydrophyllic 11 mm. circle. Such dishes are made by barely abrading an 11 mm. circle in a slide, siliconing the whole slide and then removing the silicone from the circle with alkali *. To keep the abraded circles hydrophyllic, it proved important to store the slides under 95% alcohol until shortly before use. It also appeared to be a worth-while precaution to swab clean the edge of the hole in a cover slip ring with 95% alcohol before use.

The procedure in forming a film is as follows: A cover slip ring is placed upon an abraded circle slide in a covered chamber immersed in a water bath at 39.6° ($\pm$ .8°) C. An aliquot of agar solution kept hot in a boiling water bath is poured into a hot corked 19 mm. shell vial which is then also immersed in the 39.6° bath. Three minutes later one or two drops of the cooled agar solution are placed in the abraded circle with a medicine dropper (previously warmed in a corked vial in the bath). While the agar has been cooling, about 1 $mm.^3$ of packed eggs has been drawn up into a siliconed medicine dropper**. These eggs are now added to the agar. The ring is rotated

---

* Measured with the aid of micrometer calipers, the abraded circles were found to be sunk from 5μ to 60μ below the surfaces of the slides.

** This is accomplished as follows: Some eggs are concentrated in the center of a dish by swirling it. A drop of egg suspension is drawn up in a siliconed dropper, the eggs allowed to settle in the dropper, and a droplet of settled eggs is forced out onto a siliconed plate. The supernatant is discarded and the egg droplet is then again drawn up in the dropper.

briefly to aid mixing, is then raised just enough to draw the spread out fluid back into the abraded circle during a brief pause, and finally is further raised to form a separated film. The entire process from egg addition to film formation required about two seconds. Finally after forming a film it is vigorously rotated for about five seconds while it is being raised into the cold room air and is gelling.

Since evaporation plasmolyzes the eggs during film formation, the films are washed with sea water (brought to pH 6.0 as described below). To avoid deformation of the films while washing them, they are allowed to set horizontally for two minutes after formation; to avoid detaching the films from their rings they are then dipped slowly into the sea water in a vertical position, held there quietly for two minutes, and withdrawn as they were dipped.

Absorption and Measurement of Respiratory CO_2

Figure 5 shows the general design of the apparatus (See also Fig. 3 and Fig. 4). The eggs developed in a buffered membrane on each side of which was an air gap separating it from a CO_2 trap in the form of a drop of isotonic alkali. After exposure to respiratory CO_2 for a desired period, each pair of CO_2 traps was replaced by a fresh pair and later dried and measured with a Geiger counter. Details follow in sections:

Membrane Buffering

It was feared that if the films were too alkaline that some respiratory $C^{14}O_2$ would first accumulate as $HC^{14}O_3^-$ before passing into the air gaps as $C^{14}O_2$, thus introducing an error since such $C^{14}O_2$ would always reach the two traps at equal rates. Hence the membranes

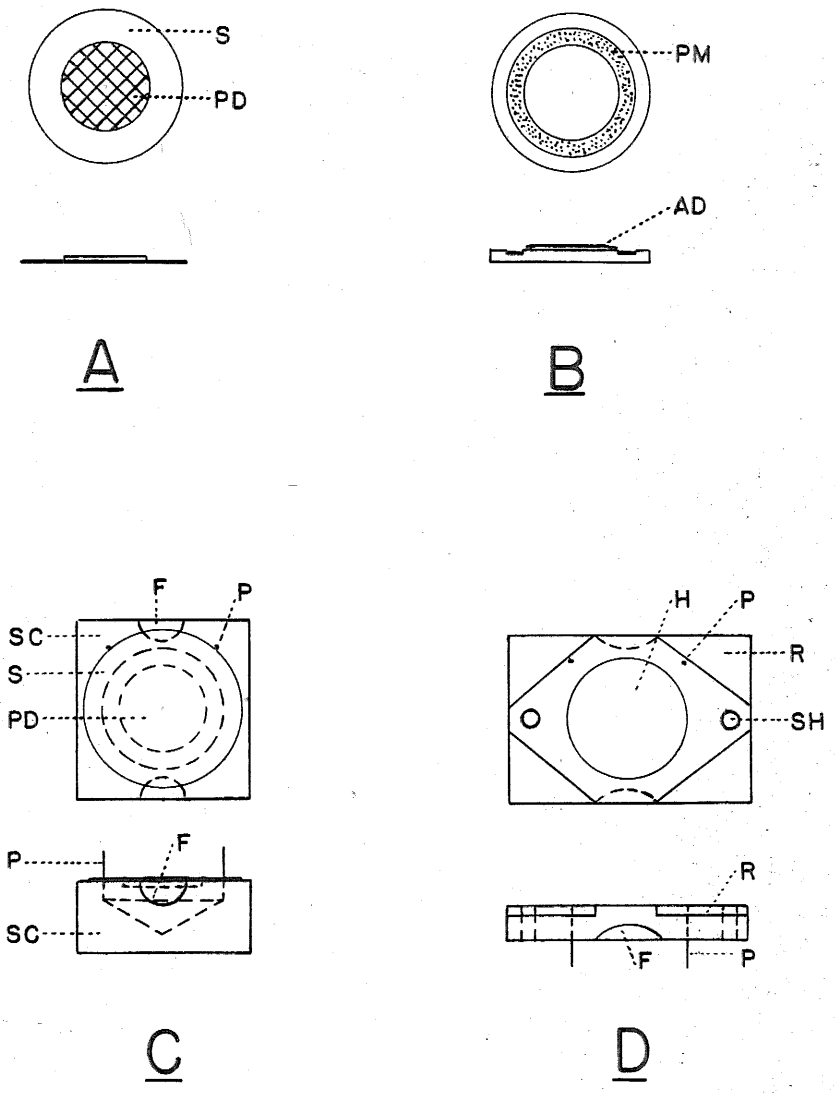
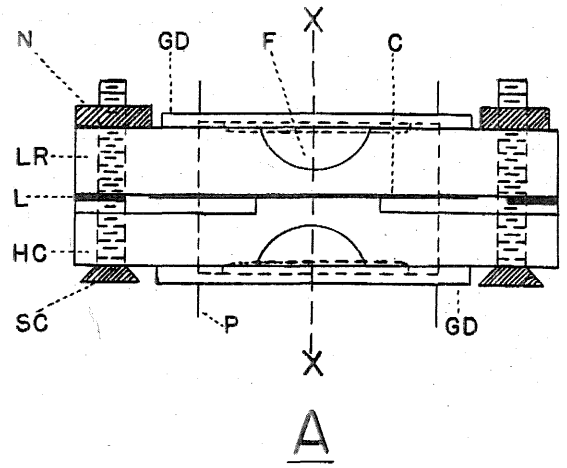
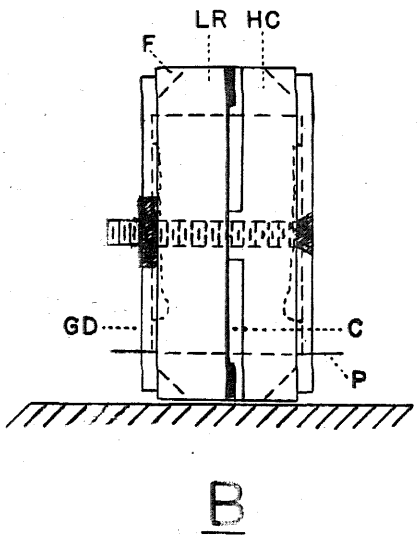


Figure 4. A, steel CO_2 trap, x 1. B, glass CO_2 trap, x 1. C, steel CO_2 trap sealed with grease on a lucite storage cup. D, lucite half cell which is a part of the device for measuring $C^{14}O_2$ emission ratios, x 1. (AD, alkali droplet adhering to glass disc; F, finger groove; H, main hole in half cell; P, stainless steel pin; PD, lens paper disc holding an alkali droplet in place; PM, paraffin-filled moat; R, recess in the half cell to accommodate legs on cover slip ring; S, stainless steel disc; SC, lucite storage cup; SH, screw hole.)





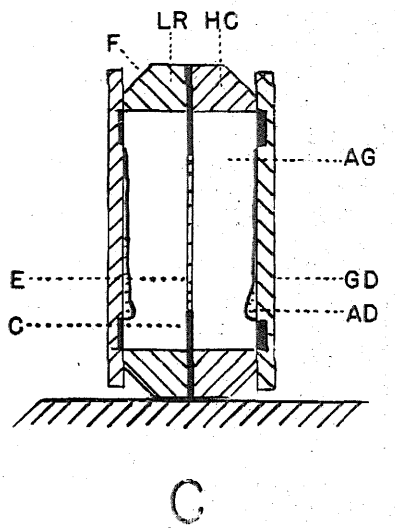


Figure 5. Assembled Device for Measuring the Ratio of $C^{14}O_2$ Emitted from the Two Faces of an Egg-bearing Film (glass CO_2 traps shown). A, top view, x 2. B, side view, x 2. C, view in the plane X--X, x 2. (AD, alkali droplet adhering to glass disc; AG, air gap; C, cover slip ring sealed with grease onto half cell; E, egg-bearing film; F, finger groove; GD, glass disc; HC, half cell; L, leg; LR, lucite ring attached with paraffin to cover slip ring; N, nut; P, pin; SC, screw.)

were buffered at pH 6.0 by dipping them for more than one minute into sea water brought to pH 6.0 with .0026 M phosphate buffer. Unfortunately it was not until after completing the experiments that mathematical analysis revealed that the alkali reserve error would have been less than 1% even at pH 8.0 (See Appendix I). Moreover, the low pH probably interfered markedly with the orientation of the eggs since it greatly intensifies the group effect (Whitaker & Lowrance, 1937).

Design of CO_2 Trap

A .02 cc. droplet of isotonic lithium hydroxide in water was chosen to absorb the $C^{14}O_2$. It has the advantages that neither LiOH, $LiOH.H_2O$, $LiHCO_3$, nor Li_2CO_3 are hygroscopic, that it is soluble enough to form an isotonic solution, and that it introduces minimal self-absorption. For measurements made in the dark, each alkali droplet was placed on a 20 1/2 mm. stainless steel disc and an 11 mm. disc of Harshaw lens paper was then carefully dropped on top. This was done in the open air. Then the CO_2 traps were immediately sealed with silicone stopcock grease onto lucite cups (See Fig. 4). As an additional precaution against atmospheric CO_2 the lucite cups with traps upon them were stored in a sealed chamber containing .55 M KOH. To prevent the alkali droplets from spreading too rapidly on the steel discs before the paper discs were added, it proved helpful to rub them first with a finger, but upon drying traps so prepared, the paper tended to curl off.

Measurements of $C^{14}O_2$ emitted under illumination were made with the aid of glass traps. The alkali droplet was spread out on a 12 mm. glass disc bounded by a paraffin-filled moat (Fig. 4). These methods of supporting a radioactive sample are modified from Calvin et al. (1949).

Drying and Counting Samples

The steel traps were first dried under a nitrogen stream and then in vacuo (.05 mm.), which curled some of the paper discs partly off their steel supports. Hence one drop of 1/10 normal strength collodion* was then placed on every disc. While they then dried in the open air, each curled disc was held down flat with the point of a needle, and then the drying of all discs was completed in vacuo (.05 mm.)**. The glass traps were either dried under a nitrogen stream and then in vacuo or in an aspirated desiccator containing P_2O_5 and KOH.

Soon after being dried, the samples were measured either with a thin window G. M. counter or, when available, with a windowless flow type G. M. counter***, the latter being preferable since it registered 3.7 times as many counts.

---

* 1 volume collodion: 2 1/4 ethanol: 6 3/4 ether.

** Occasionally this collodion treatment had to be repeated as many as seven times to flatten a stubbornly curling disc, but checks showed no effects on measured radioactivity.

*** Nuclear Instrument and Chemical Co. Q-gas counter D46-A.

As previously indicated, a series of pairs of CO_2 traps were exposed to respiratory CO_2 from the two faces of the membrane during successive intervals. Upon measuring the radioactivities in these pairs of samples, one obtained pairs of counting rates above background, b: A_1-b and B_1-b, A_2-b and B_2-b, etc. Then for successive intervals $C^{14}O_2$-ratios were obtained: $\frac{A_1-b}{B_1-b}$, $\frac{A_2-b}{B_2-b}$, etc. The most important final information desired were the changes in these ratios with time, which were expressed by dividing one ratio by another ratio so as to get the function, $F = \frac{(A_2-b)\ (B_1-b)}{(B_2-b)\ (A_1-b)}$.

The standard counter error in F, σ_F, was calculated using long known formulas set forth in many secondary sources (e.g. Calvin et al., 1949).

Let σ = a fractional standard error

R = a counting rate

t = a time taken for counting

Then: $$\sigma_{(x-b)} = \frac{\sqrt{\frac{R_x}{t_x} + \frac{R_b}{t_b}}}{R_x - R_b} \qquad \text{where } x = A_1,\ B_1,\ A_2, \text{ or } B_2 \qquad (1)$$

$$\sigma_F = F\sqrt{\sigma^2_{(A_1-b)} + \sigma^2_{(B_1-b)} + \sigma^2_{(A_2-b)} + \sigma^2_{(B_2-b)}} \qquad (2)$$

Formula (2) is only an approximation but the author was advised by two mathematicians that it would be very laborious to calculate

how exact it is. The experimental results (q. v.) suggest that this formula overestimates σ_F. This can be partially explained by noting that Formula (2) is derived by assuming that $\sigma_{(A_1-b)}$, $\sigma_{(B_1-b)}$ etc. are independent, but insofar as (A_1-b), (B_1-b), etc. all involve the same b, their error would tend to have the same sign; this situation tends to reduce σ_F below the values given by Formula (2).

Orientation of Eggs in Films

The egg-bearing films were unilaterally illuminated with light originating in a 75 watt tungsten bulb within an Argus slide projector. The light then passed through 1 1/2 inches of a 2% $CuCl_2$ solution to remove the infrared and then through a C-47 Wratten filter to restrict the light mainly to the blue*, and then through a glass CO_2 trap before finally reaching the eggs. Essentially the same set-up was used in all the principal experiments, and hence the eggs must have received approximately the same intensity of light. Just what this intensity was can only be estimated because the glass CO_2 traps reflected and refracted the light to an unknown degree. The intensity striking the trap was measured with a Weston (Model 75b) photoelectric light meter and found to be 40 foot-candles. This meter had been adjusted with white light; a small error arises from the fact that the light was blue.

---

* This Wratten filter is reported by the manufacturer to transmit virtually no light of wave lengths longer than 530 mμ.

PROCEDURES AND RESULTS OF PRELIMINARY EXPERIMENTS

Some Observations upon Hesperophycus harveyanus

At Corona del Mar, the author found healthy, mature Hesperophycus receptacles from June through September but not during the other eight months. Unlike Pelvetia, mature Hesperophycus receptacles shed copiously when dried and rewetted, but they do not respond at all to the light-to-dark shift effective for Pelvetia.

This plant is monoecious and fertilization normally occurs within fifteen minutes after shedding (Walker, 1931). However it was found possible to obtain healthy unfertilized eggs by taking advantage of the observation that acidification of the sea water delays the escape of sperm from an antheridial capsule more than that of an egg from an oögonial capsule. The eggs are dense enough to be easily concentrated in the center of a dish by swirling it in the usual embryological way, but neither the oögonia nor the antheridia can be aggregated by this means. Now then, if some receptacles are caused to shed gametangia directly into sea water buffered at pH 6.0, the eggs are freed an hour later, but the sperm, though moving vigorously, remain trapped within the intact antheridia. Then unfertilized eggs free of antheridia can be easily obtained by repeating the process of concentrating eggs in the center of one dish and transferring them to another. If the gametes are allowed to remain together in the pH 6.0 sea water for an indefinite period, however, up to 50% fertilization and germination eventually occur.

In ordinary filtered sea water in the dark at 21°C, half the eggs first become pear-shaped after about 20 hours; at 24°C and 13°C, this stage is reached after about 30 and 40 hours respectively; at 27°C, the eggs never germinate. Dwarf eggs of one half of the usual diameter often germinate normally, and even polar bodies of 1/3 of the usual diameter sometimes become partitioned by a cell wall and develop a rhizoid.

In all cases, the rhizoids tend to develop away from unilateral white light.

The Effect of Transient High Temperatures upon the Germination of Pelvetia Eggs.

In the process of embedding eggs in a thin agar membrane they must be transiently exposed to a temperature of at least 35°C, for even at 35°C sea water containing 1% agar gels in a few minutes. Therefore in a preliminary study portions of a batch of Pelvetia eggs were immersed for short periods (starting 45 to 75 minutes after being shed) in heated sea water and then returned to sea water at 16°C to develop in the dark. Germination was observed during the next four days and the results are summarized in Table 2.

Table 2

High T	Duration	Percentage Germinating by 18-20 hours	24-5 hours	42 hours	96 hours
Untreated Controls		80	98	98	
26-27°C	6 min.	89	98		
"	36 min.	77	91	99	
37°C	10 sec.	80	99	100	
"	1 min.	19	85	99	
34-37°C	6 min.	0	11	94	98
42°C	10 sec.	0	0	83	96
"	1 min.	0	0	0	0

It is apparent that there is a rapid increase in the rate of injury as the temperature increased from 37°C to 42°C: Ten seconds of exposure to 42°C is much more injurious than 1 minute of exposure to 37°C.

Development of Pelvetia Eggs in Sea Water at pH 6.0 and Containing Only a Trace of CO_2

Since, in the principal experiments, the Pelvetia eggs are subjected to sea water at pH 6.0 and containing only a trace of CO_2, a preliminary test was made of the effects of these conditions on the eggs. Two cc. of sea water buffered at pH 6.0 with .008 M Na_2HPO_4 and .0021 M citric acid was placed in a 5 cm. petri dish. To this was added .5 cc. of a suspension in sea water of recently spawned eggs. This culture developed in the dark within a sealed chamber containing .55 M NaOH. Similar control cultures at pH 6.0 in a normal atmosphere and at pH 8

in either type of atmosphere were run at the same time.

Examination of all the cultures three days later showed that germination had proceeded normally in all the dishes. However, the bottom of the experimental dish happened to be so convex that the sea water only barely wetted its central portion thus stranding some eggs there. It was recorded that these eggs showed a definite tendency to develop their rhizoids upward, away from the glass and into the CO_2 deficient air. Unfortunately, this observation was forgotten and not repeated. It may have represented the same kind of orientation that the eggs were much later observed to exhibit in agar films (See next section).

Polarization of Pelvetia Eggs by Embedding Them in a Film

It was assumed, until the principal experiments were completed and time and material had nearly run out, that merely embedding eggs in films would not affect their orientation. However, three last minute experiments indicate, at least in films made using NaLS, that the rhizoid poles tend to develop toward those faces that were lower while the films gelled.

Experiment 1 In the usual way some Pelvetia eggs were allowed to photosynthesize in an atmosphere containing $C^{14}O_2$; they were then embedded in a film made with the aid of .004% NaLS. The film was dipped for two minutes in pH 6.0 sea water, a half-cell and two steel CO_2 traps attached and the film left to develop in darkness in a vertical position for eight hours. Then the film was detached and transferred to pH 6.0 sea water in a lucite box in the dark.

Forty-eight hours after fertilization the film was examined:

It was found to be roughly divisible into regions about 10% thicker than one egg-diameter and regions about 10% thinner. In the thick phase, 90 ± 1.4% of the eggs (188 of 210 observed) had germinated and 80 ± 2.8% of these (72.5 of 91) had rhizoids pointing toward the originally lower face of the film; in the thin phase, 76 ± 3.0% of the eggs (69 of 91) had germinated and 68 ± 2.6% (97 of 143) of these had rhizoids pointing toward the originally lower face*.

In this experiment, and even more so in the others, germination was incomplete. Hence the observed preferential orientation of the rhizoids toward the originally lower face might be interpreted as having arisen from a relative inhibition of germination toward the originally upper face, instead of or as well as from true polarization of the eggs with the rhizoid poles toward the originally lower face. However, in Experiment 1 , as least, high germination and downward orientation were correlated; hence if a differential inhibition of germination existed at all, then "downward" germination was more suppressed; this would make the apparent degree of downward polarization less than the true value.

Experiment 2 Some Pelvetia eggs (which had not photosynthesized under $C^{14}O_2$) were embedded in two films made with the aid of .004% NaLS. The two films were made one and two hours respectively after fertilization. Each film was then washed with pH 6.0 sea water and allowed to develop in a vertical position in a moist chamber so arranged that each face of both films was one cm. from blotting paper soaked in .55 M KOH. Ten hours later both films were removed from the CO_2-free chamber, again washed with pH 6.0

---

* The probable errors arising from the sizes of the samples are given. Each egg with a rhizoid in the plane of the film was counted as one half egg germinating toward each face.

sea water and then placed vertically in a dark moist chamber filled with ordinary air. Two days later, both films were detached and immersed in pH 6.0 sea water for further development. Four days after fertilization the films were examined.

In the film made first, 24% of the eggs had germinated and 71 $\pm$ 4.7% (30.5 of 43) of the rhizoids pointed "downwards"; in the second film 33% of the eggs had germinated and 72 $\pm$ 3.0% (74.5 of 103) of the rhizoids pointed "downwards". Both films were of fairly homogeneous thicknesses averaging about 1/6 thicker than one egg-diameter. Observation of development in control eggs not embedded in films showed that the batch used for film formation was seriously subnormal: Only 68% and 90% of groups of control eggs left in ordinary sea water and pH 6.0 sea water respectively had germinated.

Experiment 3 Two hours after fertilization, some Pelvetia eggs (which had not photosynthesized under $C^{14}O_2$) were embedded in a film made without the use of NaLS. The film was washed with pH 6.0 sea water and then placed in a dark, CO_2-free moist chamber as in Experiment 2 . Nine hours later the ring was transferred to a dark, moist chamber containing ordinary air and three days later the film was detached and immersed in pH 6.0 sea water. Prominent fungal hyphae were seen in the film at this time.

Cleavage proceeded normally in most eggs but the development of rhizoids was extremely delayed. After 3 days, only 10 of a total of about 400 eggs in the film or 2 1/2% had germinated; 8 of these 10 had developed rhizoids downward. After 8 days, 17 eggs or 4% of the total had germinated; 11 1/2 of these or 70% had developed downward. After 14 days, 60 of 324 eggs

observed or 19% had germinated; 33 of these or 55% had developed downward. Apparently, those few eggs that germinated after only a relatively small delay tended to develop downward as in Experiments 1 and 2 but the subsequently germinating eggs developed with random orientations.

Observation of development in control eggs left in plain sea water showed them to be somewhat subnormal; 47 hours after fertilization, only 89% of them had germinated. However, the extreme inhibition observed can probably be accounted for principally by the fungus noted above.

Polarization of Pelvetia Eggs by Short Exposures to Unilateral Light

In a preliminary experiment portions from four batches of Pelvetia eggs were allowed to develop in the dark except for exposure to one hour of unilateral illumination beginning at about one, two, etc. hours after fertilization. They developed in filtered sea water within 5 cm. petri dishes at a temperature which varied from 16°C to 18°C. The white light included wave lengths from about 350 mμ to 800 mμ; the blue light from about 350 mμ to 530 mμ.

The results are listed in Table 3. The experiment was not repeated, the sampling errors were large, and different batches of eggs were used in different parts of this experiment. Nevertheless, it is notable that the period of maximum sensitivity advances to times later in development as the intensity of the unilateral light decreases. An interpretation of this observation is complicated by the fact that during the first few hours after fertilization the eggs are not attached very securely to the glass dishes; consequently the light may act not only by polarizing the eggs but by affecting their adhesion to the substrate and

hence the extent to which rolling of the eggs brings about randomization.

Table 3

Hours A. F.	White Light in Foot-candles					Blue (foot-candles)	
	2000	400	100	25	6	120	30
1	93±4	60±4	54±3	42±5	43±4	56±3	60±3
2	73±5	91±3	84±2	66±4	67±4	75±3	85±2
3	79±4	86±3	86±2	84±3	75±4	77±2	82±2
4	76±6	87±3	79±3	70±4	71±5		
5	60±6						
6	50±7						
7	53±8						

Polarization of Pelvetia Eggs by One Hour of Unilateral White or Blue Light Applied at Various Times after Fertilization (A. F.). In the body of the table are listed the percentages of eggs counted which developed rhizoids away from the light and the probable errors of these percentages resulting from the sizes of the samples.

In other experiments, Pelvetia eggs were exposed to unilateral white light while developing in sea water brought to pH 6.0 with a phosphate buffer. The rhizoids developed away from the light as usual.

The Rate of $C^{14}O_2$ Emission by Labeled Pelvetia Eggs Developing in Dishes

Two preliminary experiments belong under this heading. In the first of these some eggs were shed and about 30 minutes later were allowed to photosynthesize for 90 minutes in the presence of $C^{14}O_2$. After being thoroughly washed, about 1000 labeled eggs were placed in each of two straight-wall concavity slides bearing depressions 16 mm. in diameter

and 3 mm. deep. The eggs were barely covered with pH 6.0 sea water and allowed to develop either in darkness or under approximately 500 foot-candles of light from a daylight type fluorescent lamp. CO_2 traps were placed over the egg-bearing concavities and changed at intervals. The trapped $C^{14}O_2$ was later measured with a thin-window G. M. counter. Dish A was in darkness (D) during the first two measurement intervals and illuminated (L) during the third while dish B was treated oppositely. The measured activities in counts per minute above background per hour of egg development per thousand eggs are listed in the body of Table 4. The intervals of measurement are in minutes after the end of photosynthesis (A.P.):

Table 4 (See Text above)

	Interval A.P.		
	54-118	123-420	422-544
Dish A	460 (D)	168 (D)	14 (L)
Dish B	129 (L)	17 (L)	98 (D)

Control measurements of the $C^{14}O_2$ emitted by pH 6.0 sea water that had been supernatant to the eggs right after washing them gave no counts significantly above background.

A second preliminary experiment using eggs cultured in one concavity slide gave essentially similar results. Since a film bears about 1000 eggs, it was apparent that by means of photosynthesis the respiratory CO_2 of the eggs could be easily gotten "hot" enough for the author's purposes. However, it was seen that the rate of $C^{14}O_2$ emission decays fairly rapidly after photosynthesis ceases. In Figure 6 on p. 56, this

preliminary data is graphed together with similar data on the decay of the rate of $C^{14}O_2$ emission taken from the principal experiments upon eggs in films.

PROCEDURES AND RESULTS OF THE PRINCIPAL EXPERIMENTS

In the eight principal experiments, recently shed Pelvetia eggs labeled with C^{14} were embedded in a thin jelly membrane where they developed. Then during each of a series of increasingly longer intervals, the $C^{14}O_2$ escaping from each side of the membrane was separately absorbed to be measured later. During the first one or two intervals of $C^{14}O_2$ absorption, the membrane was in darkness; during the next one or two intervals it was unilaterally illuminated with about 30 foot-candles of blue light which partially polarized the eggs; during the last one, two or three intervals, it was again in darkness. CO_2 absorption was stopped before the eggs germinated. Subsequent development occurred either with the film in a moist chamber bearing air over sea water or with the film immersed directly in sea water*. In the course of the experiments room temperature varied between 14.5°C and 17.5°C.

$C^{14}O_2$ Ratios

The principal purpose of these experiments was to determine the ratio of $C^{14}O_2$ emitted from the two faces of egg-bearing films before, during, and after unilateral illumination. This information

---

* Film 5 was left in moist air until 59 hours after fertilization. Then it was transferred to sea water where it remained for another 24 hours before the final observations upon it were made.

Table 5

The Relative $C^{14}O_2$ Output from the Two Faces of Egg-bearing Membranes and Related Data. The moment of making the film and the various intervals are in hours after stimulating shedding. The standard errors listed are the theoretical counter errors, i.e., those due only to the randomness of radioactive disintegration. The film thicknesses are in average egg diameters. Bottom and top refer to the upper and lower faces of the film while it gelled.

Experiment	4A	4B	5	6
Time Film Made	2.2	1.8	2.0	1.8
% NaLS	0	0	0	0
Number of Eggs	1,300		1,500	1,300
Light From	Bottom	Top	Top	Top
Develop In	Moist Air	Moist Air	See Text	Sea Water
"Photosynthesis"				39%
% Germination	100	100	54	90
% Oriented Toward Dark	71		71	72
Thickness Mean	1.1	1.5	.95	1.7
" Range	1.0-1.2	.9-1.7	.6-1.2	1.0-2.0
Pre-interval 1	None	None	None	2.00-2.25
$C^{14}O_2$: Bottom/Top				1.56±.04
Pre-interval 2	2.8-3.8	2.5-3.5	2.5-3.5	2.3-3.0
$C^{14}O_2$: Bottom/Top	1.38±.02	1.71±.07	1.27±.03	1.64±.03
Light Interval 1	3.9-5.9	3.6-5.6	3.5-6.0	3.1-4.1
$C^{14}O_2$: Bottom/Top				1.66±.05
% Change in Ratio				+1±4
Light Interval 2				4.1-5.2
$C^{14}O_2$: Bottom/Top				1.52±.05
% Change in Ratio				-8 ±4
Post-interval 1	5.9-7.9	5.6-7.6	6.0-9.0	5.2-7.2
$C^{14}O_2$:Bottom/Top	1.29±.05	1.68±.07	1.12±.03	1.63±.03
% Change in Ratio	-7±4	-2±6	-13±5	0±3
Post-interval 2	8.0-12.1		9.0-13.0	
$C^{14}O_2$: Bottom/Top	1.50±.08		1.11±.03	
% Change in Ratio	+9±6		-15±4	

Table 5 (cont.)

Experiment	7	10	11A	11B
Time Film Made	2.4	2.2	2.2	1.9
% NaLS	0	.002	.004	.004
Number of Eggs	1,200		1,700	2,600
Light From	Bottom	Bottom	Top	Bottom
Develop in	Sea Water	Sea Water	Sea Water	Sea Water
"Photosynthesis"	62%	27%	2%	5%
% Germination	100	100	14	10
% Oriented Toward Dark	87	74	78	78
Thickness: Mean	1.6	.95	1.1	1.05
" Range	1.0-2.4	0-1.2	.9-1.2	.5-1.2
Pre-interval 1	2.5-2.8	2.4-2.8	2.35-2.60	2.0-2.5
$C^{14}O_2$: Bottom/Top				$1.11 \pm .01$
Pre-interval 2	2.8-3.5	2.8-3.6	2.7-3.4	2.6-3.3
$C^{14}O_2$: Bottom/Top	$1.32 \pm .04$	$1.01 \pm .02$	$1.31 \pm .02$	$1.08 \pm .01$
Light Interval 1	3.6-5.6	3.8-5.8	3.4-5.4	3.4-5.4
$C^{14}O_2$: Bottom/Top	$1.18 \pm .04$	$.95 \pm .02$	$1.24 \pm .01$	$1.04 \pm .01$
% Change in Ratio	-11 ± 5	-6 ± 2.5	-6 ± 2	-4 ± 2
Post-interval 1	5.6-7.6	5.8-7.8	5.5-7.4	5.4-7.4
$C^{14}O_2$: Bottom/Top	$1.29 \pm .03$	$.98 \pm .02$	$1.19 \pm .02$	$1.01 \pm .01$
% Change in Ratio	-2 ± 4	-3 ± 3	-10 ± 2	-7 ± 2
Post-interval 2			7.5-11.5	7.4-11.4
$C^{14}O_2$: Bottom/Top			$1.20 \pm .02$	
% Change in Ratio			-9 ± 2	
Post-interval 3			11.5-15.5	11.4-15.4
$C^{14}O_2$: Bottom/Top			$1.16 \pm .02$	$1.10 \pm .01$
% Change in Ratio			-13 ± 2	$+1 \pm 2$

is summarized in Table 5. Each film listed contained a portion of labeled eggs from a different batch except that films 4A and 4B both contained eggs from one batch, and films 11A and 11B likewise both contained eggs from a single batch.

The percentage of the eggs in each film which germinated served as one criterion of their normality; the intensity with which the eggs photosynthesized, under the unilateral light that oriented them (Tabulated as "Photosynthesis"), served as another criterion of normality. The tabulated percentages of germination were obtained two or three days after fertilization; before this time protuberances that have developed perpendicular to the film are very difficult to see. The intensity of photosynthesis under the unilateral light was measured by the percentage decrease in the total rate of $C^{14}O_2$ emission from both faces of the illuminated film as compared with the expected total rate during the interval of illumination had the eggs been in the dark during this period. This expected total rate was obtained by extrapolating to the light interval, with the aid of the graph in Figure 6, from a measurement made during a dark interval, in the same experiment (See p. 55). No measure of the intensity of photosynthesis under unilateral illumination is available for experiments 4A, 4B, and 5 because adequate traps for CO_2 during illumination had not yet been developed when they were performed.

Applying the above criteria it will be seen that the relative normality of the eggs in Experiments 7, 4A, and 4B was the same, while the relative normality was successively lower in Experiments 10, 6, 5, 11A, and 11B.

In Experiments 7, 4A, and 4B, 100% of the eggs germinated; moreover, the highest intensity of photosynthesis, 62%, was found for Experiment 7. In Experiments 10 and 6, 100% and 90% of the eggs germinated while the intensities of photosynthesis were respectively 27% and 39%. In Experiment 5, 54% of the eggs germinated. In Experiments 11A and 11B, only 14% and 10% of the eggs germinated respectively while the calculated intensities of photosynthesis, 2% and 5% respectively, are not definitely different from zero. The 14% and 10% germination percentages tabulated for Experiments 11A and 11B were recorded 72 hours after fertilization but development was followed for an additional 7 days. In Experiment 11A, at 5 days after fertilization, 100% of the eggs were observed to be finely cleaved into cells but only the original 14% had yet developed rhizoids. At 6 days, an additional 1% of the eggs had begun to develop very thin rhizoids; at 7 days, 3%; at 8 days, 9%; at 9 days, 23%; at 10 days, 39%. In Experiment 11B at 10 days after fertilization, one observed in addition to the 10% of the eggs originally germinating, which now bore thick rhizoids, an additional 28% with thin, tardily developed rhizoids; most of the remaining eggs were finely cleaved into cells. The cause of the extreme inhibition observed in Experiments 11A and 11B is obscure (See pp.26-7).

Now returning to the $C^{14}O_2$-ratio data, let us first consider the base ratio or the ratio observed in the second and main dark interval before illumination. Note first that in all cases except Experiment 10 that the base ratio is markedly greater than 1, that is, the formerly lower surface or "bottom" of each film emitted $C^{14}O_2$ at a significantly greater rate than the formerly upper surface or "top" of each film.

This fact is interpreted as being caused primarily by the eggs having set nearer to the formerly lower surface of each film (by reason of having settled while the film gelled). Comparison of the observed base ratios of the film with the theoretical expectation based primarily upon the observed film thicknesses offers further support for this interpretation.

First consider what effect the thickness of the film would theoretically have on the position that the eggs became fixed in (or set in) and hence on the base ratio. The principal forces determining the position of an egg in the film at mechanical equilibrium are gravity and surface tension. An egg entirely inside of a film will be affected only by the downward pull of gravity. An egg which bulges out of the surface of a film will also be subjected to surface tension forces acting upon the line of contact between the exposed segment of the egg and the surface of the film in directions tangent to the egg's surface so as to pull the egg back into the film with a force which increases as the line of contact lengthens and hence as the egg bulges out farther. If the film is thicker than one egg-diameter, mechanical equilibrium will be attained when the egg bulges out of the lower surface of the film far enough to create an upward surface tension pull which balances the downward pull of gravity. A simple calculation shows that the segment exposed at equilibrium is less than 100 angstroms in height. Hence for the present purposes an egg at equilibrium in a film thicker than one egg-diameter is considered to be tangent to the lower surface. Similarly, in a portion of a film which is thinner than one egg-diameter, the center of an egg at equilibrium will be shifted below the mid-plane

of the membrane by the negligible distance of less than 100 angstroms.

That the eggs really are shifted toward the lower surface of a thick film was confirmed by direct examination of a folded one. Film 4B was folded with the formerly lower surface of the film on the outside (See Fig. 1 on p. 22). Of 65 eggs seen in the edge, that is, bounded by the apparent double line, only one was apparently separated from the formerly upper surface by as little as one scale unit, while 49 were apparently separated from the upper surface by two scale units or more. Note that no eggs would appear farther from the upper surface and some* would appear closer to it than they really were. Then consider the section of Film 4B (Fig. 2, p. 24) in conjunction with the foregoing and it will be clear that in this relatively thick film that the majority of eggs were fixed nearer to the lower surface. When thinner films were observed in a folded condition, no evidence that the average position of the eggs was off-center could be obtained; however, the method is too crude to attach significance to this negative result.

In Appendix II, a theoretical upper limit to the effect of the off-center position of the eggs on the base ratio is derived from a consideration of the diffusion of CO_2 molecules in the film. Let D be the mean excess of the film thickness over one egg-diameter (regions less than one egg-diameter thick are considered in the averaging process to be just one egg-diameter thick). Let R be the base ratio.

Then, $R < 1 + 2D$

Now let us compare the theoretically expected base ratios

---

* Those out of the plane which bisects the fold.

with the observed facts. The relevant data are arranged in Table 6.

Table 6

Correlation Between Base Ratio and Film Thickness

Experiment	10	11B	5	11A	4A	4B	7	6
D	.03	.1	.05	.1	.1	.5	.6	.7
1 + 2D	1.06	1.2	1.1	1.2	1.2	2.0	2.2	2.4
Egg-free Area	Yes	Yes	Yes	No	No	No	Yes	No
Base Ratio Observed	1.01	1.08	1.27	1.31	1.38	1.71	1.32	1.64

The listed values of D are taken from the egg-bearing regions of the film "sections" shown in Figure 2 (See p. 24)*. Four of the films showed regions which were thinner than average and which were free of eggs. The presence of these thin egg-free regions is considered as evidence that in these films the eggs became fixed in a position far from that of mechanical equilibrium**.

---

* Likewise, the mean film thicknesses listed in Table 5 are taken only from the egg-bearing regions as shown in Figure 2.

** As Figure 2 shows, the folds from which sections were taken went through these egg-free regions in films 7 and 11B. The egg-free regions were relatively small in films 5 and 10 and the folds did not go through them.

It is apparent that the base ratios tend to have higher values in the films with higher D's and in the films without egg-free areas, both correlations being expected from the eccentric position theory presented. However, the base ratios in Films 5, 11A, and particularly 4A are so much larger than the theoretical upper limit, even taking the crudeness of the measurements of D into account, as to suggest the existence of a second cause of the high base ratios in addition to the eccentric position of the eggs.

Now let us consider the changes in the $C^{14}O_2$-ratio during the period of unilateral illumination. Disregarding the unreliable Experiment 11, data from the most reliable Experiment 7, as well as from 10 and 6 are available. In Experiment 6, two measurements were made during the light interval. Lumping these so as to make 6 comparable with 7 and 10, the "bottom/top" ratio during the light was found to be $1.59 \pm .04$, a drop of $3 \pm 3\%$ from the base ratio of 1.64. Noting the direction of illumination then, the relative changes in $C^{14}O_2$ output from the lighted face are as follows: film 7, $-11 \pm 5\%$; film 6, $+3 \pm 3\%$; film 10, $-6 \pm 2\ 1/2\%$. While statistical analysis cannot be intelligently applied here, the author judges this data to offer limited evidence that the normal eggs tended to emit less $C^{14}O_2$ from their illuminated sides.

Consider next the changes in the $C^{14}O_2$-ratio after the light was extinguished. These data fall into two distinct internally consistent groups: Films 10, 4A, 7, 4B, 6, and 11B on the one hand and Films 5 and 11A on the other hand. The cause of this dichotomy is uncertain; here let it suffice to present and organize the facts. Consider the large group first. Since some films were lighted from the "bottom" and some

from the "top", the changes in the ratios may be considered either as between the sides formerly toward and away from the light or between the former bottom and top sides. Consider the data from the former viewpoint. The relative changes in the formerly lighted side were $-7\pm2\%$, $-7\pm4\%$, $-3\pm3\%$, $-2\pm4\%$, $0\pm3\%$, $+1\pm2\%$, $+2\pm6\%$, and $+9\pm6\%$. The mean change in this larger group is $-.9\pm1.8\%$, where $\pm1.8\%$ is the empirical standard deviation of the mean. It is largely accounted for by the 1.4% expected standard deviation of the mean resulting from the counter errors alone. If the larger group is considered from the bottom versus top viewpoint, the bottom is found to decrease by $-1.4\pm1.8\%$. Obviously the ratios in the larger group as a whole did not change to a measurable degree. From either point of view the conclusion that they did not change by more than 5% is certainly trustworthy. However, it may be noted that in Experiments 4A and 11B, in which a second post-illumination measurement was made, these latter appear to have indicated more $C^{14}O_2$ from the bottom or lighted side than the measurements during the first post-illumination intervals. The first post-interval mean change from the bottom/top viewpoint is $-3\pm1.2\%$; the second one, $+5\pm4\%$. The two means differ by $8\pm4.2\%$. It is hardly permissible to draw the conclusion that the ratio increased between the first and second interval.

Turning now to the second group of films, 5 and 11A, an entirely different picture emerges. Both these films were illuminated from the top. The faces formerly at the bottom and formerly away from light underwent relative changes in the post-illumination intervals of $-9\pm2\%$, $-10\pm2\%$, $-13\pm5\%$, $-13\pm2\%$, and $-15\pm4\%$. The mean change in this smaller group was $-12\pm1.1\%$. This empirical standard deviation of the mean of

1.1% is even less than the 1.5% expected standard deviation of the mean resulting from counter errors alone. One finds no significant changes in the $C^{14}O_2$-ratio between the first, second, and third post-illumination intervals. Preparatory to a consideration of why the two groups of films behaved so differently after illumination, one notes two correlations with double conditions. Films 5 and 11A were the only films illuminated from the formerly upper side which also exhibited relatively high base ratios for their thicknesses. They were likewise the only "top"-lighted films bearing relatively abnormal eggs.

Total $C^{14}O_2$ Output

As a by-product of the $C^{14}O_2$-ratios, the principal experiments upon $C^{14}O_2$ emission by eggs embedded in films also yielded information about the decay of the rate of total $C^{14}O_2$ emission. These data were obtained for each interval by adding the measurements of $C^{14}O_2$ emitted from the two faces of each film rather than by dividing them. Figure 6 shows a graph of all the measurements of total $C^{14}O_2$ emission in the dark taken from the development of eggs in the eight films studied as well as from the preliminary cultures in depression slides. The data from which the graph was plotted are carried in Table 7.

Since the rate of $C^{14}O_2$ emission varied with the conditions of photosynthetic labeling, which in turn varied from one egg batch to another, normalized values are actually plotted. Each measurement, s, of total $C^{14}O_2$ emitted per minute of respiration per 1000 eggs during some interval, was divided by s_r, the total measured during a reference

The Decay of the Total Rate of $C^{14}O_2$ Emission in the Dark by Pelvetia Eggs after Photosynthesis under $C^{14}O_2$ Has Ceased

The time when photosynthesis stops is taken as zero hours. The data from ten experiments involving eight batches of labeled eggs are graphed; to make the course of decay in different experiments comparable, each measurement of a $C^{14}O_2$ emission rate was divided by a reference value taken from the same experiment or one using the same batch of eggs. Table 7 carries the data graphed here.

- o Eggs in Films
- ▲ Eggs in Dishes
- · Reference Values for Normalization

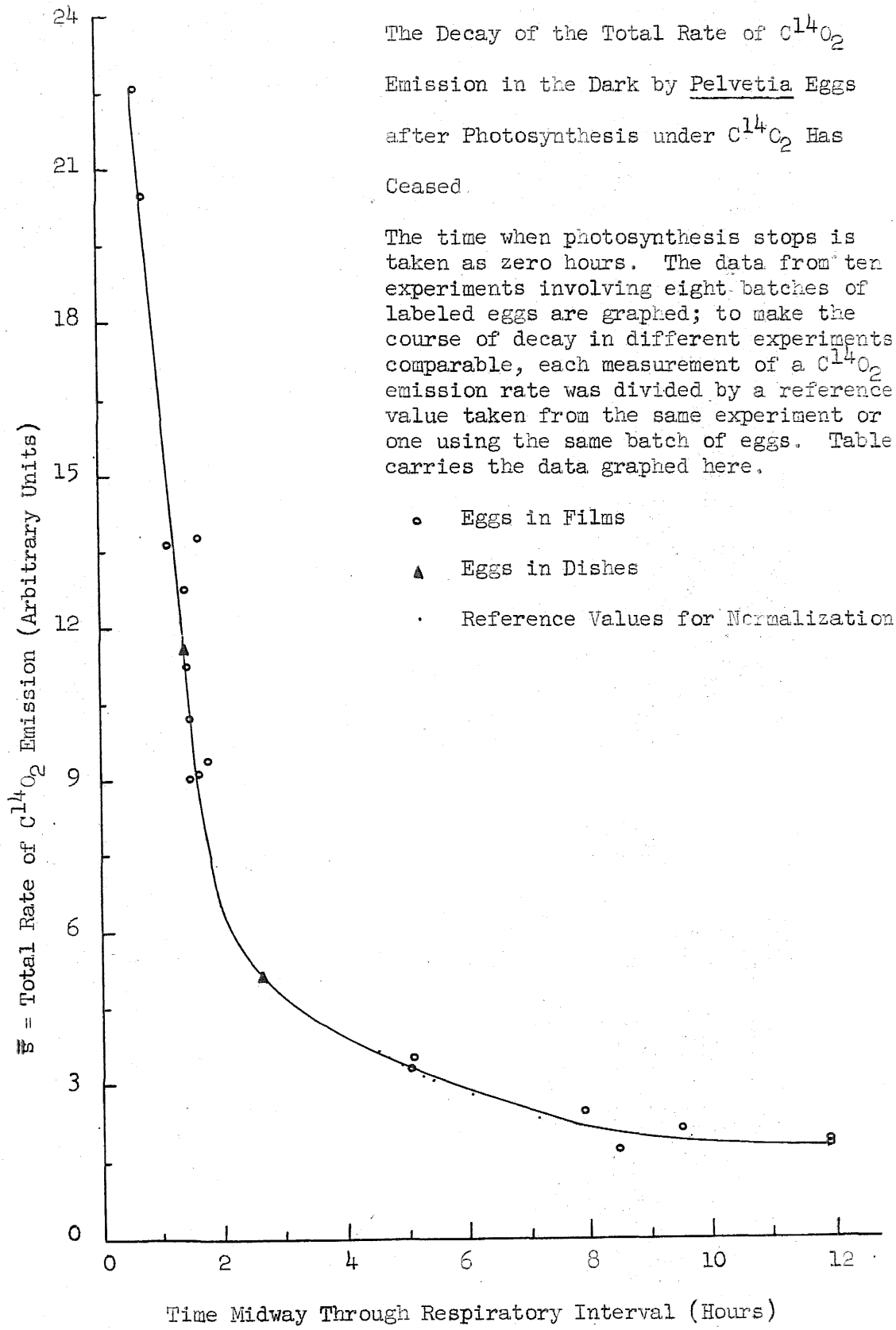


Figure 6

Table 7

The Decay of the Total Rate of $C^{14}O_2$ Emission in the Dark by Pelvetia Eggs after Photosynthesis under $C^{14}O_2$ Has Ceased (Plotted in Figure 6)

s = Total counts per minute in the $C^{14}O_2$ emitted (per minute of respiration per thousand eggs).
t = Time midway through respiratory interval (in minutes).
s_r = s during reference interval; t_r = t during reference interval.
$\bar{s}$ = $1000 \cdot s/s_r t_r$ = normalized s.
s' = $1{,}080/t + 12$.

		Dish A	Dish C	Film 4A	Film 4B	Film 5	Film 6	Film 7	Film 10	Film 11A	Film 11B
Reference interval (post-interval 1)	t_r	272	431	325		363	282	302	315	295	
	s_r	.250	.0151	.0355		.0453	.128	.134	.174	.260	
	$s_r t_r$	68.0	6.51	11.53	From	16.44	36.1	40.5	54.8	76.7	From 11A
	$\bar{s}_r/s'$			.92	4A	.93	.92	.92	.92	.92	
Pre-interval 1	t						38				45
	$\bar{s}$						22.6				20.5
	$\bar{s}/s'$						1.05				1.08
Pre-interval 2	t	86	157	108	87	88	70	97	99	90	85
	$\bar{s}$	11.6	5.58	9.37	11.3	9.06	13.7	9.14	13.8	11.2	12.8
	$\bar{s}/s'$	1.05	.87	1.04	1.04	.84	1.04	.92	1.42	1.06	1.01
Post-interval 1	t				307						305
	$\bar{s}$				3.52						3.36
	$\bar{s}/s'$				1.00						.99
Post-interval 2	t			510		571				476	
	$\bar{s}$			1.73		2.16				2.50	
	$\bar{s}/s'$			.84		1.17				1.13	
Post-interval 3	t									717	715
	$\bar{s}$									1.80	1.88
	$\bar{s}/s'$									1.21	1.24

interval of emission by the same batch of eggs and also divided by t_r, the interval between the end of photosynthesis and the time midway through this reference period:

$$\text{Normalized } s = \bar{s} = s/s_r t_r$$

The quantity s is divided by $s_r t_r$ instead of by s_r because s was seen to be approximately proportional to 1/t within each experiment. As far as possible the different reference intervals were chosen to be at equal times after photosynthesis ceased. The time coordinate of each value is the arithmetical mean of the corresponding interval, $1/2\ (t_2-t_1)$*. The smooth curve shown was drawn by eye so as to give the best fit. Consideration of all the data shows that the points are fitted nearly as closely by the hyperbola, $s' = 1{,}080/t + 12\ (\text{min.}^{-1})$, as by the smooth curve drawn. To make this point clear, the ratio of the normalized experimental values, $\bar{s}$, to the hyperbolic values, s', has been calculated and listed in Table 7.

---

* Since $s \propto 1/t$, it may be easily shown that the proper time coordinates to plot are the geometrical means, $\sqrt{t_1 t_2}$, but the latter differ too slightly from the arithmetical means to justify recalculation.

DISCUSSION AND CONCLUSIONS

$C^{14}O_2$ Ratios

Thus far it has been tacitly assumed in this thesis that $C^{14}O_2$ and CO_2 are emitted at the same relative rates from the two faces of an egg-bearing film, but this assumption proves seriously incorrect when the hemispheres of the eggs that point toward one face of a film respire more rapidly than the hemispheres pointing toward the other face. Consider the observed fact that the total rate of $C^{14}O_2$ emission by Pelvetia eggs decreases rapidly with time. The total rate of carbon dioxide emission can be assumed to remain constant or even increase with time. Hence, the specific activity of the total emitted carbon dioxide must decrease rapidly with time. Therefore, if one hemisphere of an egg respires more rapidly than the other, then the specific activity of the carbon dioxide emitted from this hemisphere must fall more rapidly than that from the other.

In Appendix IV, this matter is quantitatively considered. Suffice it here to state the results. Let one hemisphere of an egg respire more rapidly and hence emit carbon dioxide at $(1 + f)$ times the rate that the other hemisphere does. Let zero time be .2 of an hour before photosynthesis ceases. Let the differential respiration begin at the time t_1 and continue to the time t_2 when the relative specific activities in the emitted carbon dioxide are desired. Let R_{sa} be the ratio of specific activity from the more rapidly respiring hemisphere to that of the other.

Then,
$$R_{sa} = 1 - f \frac{(t_2 - t_1)}{t_2}$$

Clearly, R_{sa} decreases steadily from 1, when $t_2 = t_1$, to $1 - 1/2f$ when $t_2 = 2t_1$, to $1 - 2/3f$ when $t_2 = 3t_1$, and so on to the limiting value of $(1 - f)$ when $t_2 = \infty$.

This means that the ratio, R_{ce}, of the rate of $C^{14}O_2$ emission by the more rapidly respiring hemisphere to that of the other would be $(1 + f)$ at first but would fall with time according to the formula:

$$R_{ce} = (1 + f)\left[1 - f\left(\frac{t_2 - t_1}{t_2}\right)\right] \cong 1 + f(t_1/t_2)$$

Clearly, R_{ce} decreases steadily to $1 + 1/2\ f$ when $t_2 = 2t_1$, to $1 + 1/3\ f$ when $t_2 = 3t_1$, and so on to the limiting value of 1 when $t_2 = \infty$.

Now let us apply this analysis to the experimental data. As has been discussed in the previous chapter, an analysis of the base ratios, or the $C^{14}O_2$-ratios measured during the first long dark interval, indicates that in some cases they are so high as to be only partly explicable by the eccentric position of the eggs in the films and must be partly attributed to a higher rate of $C^{14}O_2$ emission by the "bottom" hemispheres of the eggs, that is, those that are pointed toward the "bottoms" of the films. This increased rate of emission from the "bottom" hemispheres might arise either from an increased rate of respiration in the "bottom" hemispheres or an increased permeability to carbon dioxide of the cell membrane in the "bottom" hemisphere. Differential permeability would not affect the relative specific activities of emitted carbon dioxide, but as discussed above, differential respiration would decrease the specific activity emitted from the "bottom"*

---

* This consideration may serve as a valuable tool for distinguishing a differential CO_2 emission by different regions of a cell, produced by differential respiration, from one caused by differential permeability.

and would therefore result in a falling $C^{14}O_2$-ratio (from the "bottom/top" viewpoint).

Whatever it is that induces the increased "bottom" emission must begin to be effective shortly after the eggs are embedded in the films or at $t_1 \cong 1$ hour. The base ratio interval lies between $t_2 \cong 1.2$ hours and $t_2 \cong 2$ hours; the first post-illumination dark interval lies between $t_2 \cong 4$ hours and $t_2 \cong 6$ hours. Thus if the relative increase in "bottom" emission were attributed to differential respiration, the $C^{14}O_2$-ratio should have fallen by about 1/2 f between the base interval and the first post-illumination interval.

In a similar manner, it can be argued that the unilateral illumination should result in a marked relative decrease in specific activity in the carbon dioxide emitted from the hemispheres away from the light both during and after illumination.

Finally then, where the illumination came from the "top" direction, photosynthesis and the "bottom" respiration should have reinforced each other's action in decreasing the specific activity of the carbon dioxide emitted from the "bottom" and thus in decreasing the $C^{14}O_2$-ratios in the post-illumination intervals (from the bottom/top viewpoint); where the illumination came from the "bottom" direction, photosynthesis and the "bottom" respiration should have had opposing effects upon these $C^{14}O_2$-ratios.

Now recall that two of the eight films studied, numbers 5 and 11A, were sharply distinguished from the other six by exhibiting a marked decrease in their $C^{14}O_2$ ratios, from the bottom/top viewpoint, in the post-illumination intervals. Moreover, films 5 and 11A alone can be characterized by the double condition of being "top"-lighted and of exhibiting a relatively high

base ratio for their thicknesses.

These are exactly the conditions which are expected to result in a decreased specific activity in the $C^{14}O_2$ emitted from the bottom and hence in a decreased $C^{14}O_2$-ratio.

One concludes then that none of the films exhibited any measurable change in the relative rates of carbon dioxide output from their two faces after unilateral illumination; the apparent change exhibited by Films 5 and 11A only reflects a change in the relative specific radioactivities. Moreover, this successful prediction rests in part upon the previous conclusion that the high base ratios partially reflect a higher respiration in the hemispheres of the eggs pointed toward the formerly lower faces. Hence this latter conclusion is reinforced.

Both these conclusions are furnished some further support by the fact that Films 4A and 11B, the only "bottom"-lighted films on which more than one post-illumination measurement was made, showed a tendency for their $C^{14}O_2$-ratio to change in one direction and then in the other. This checks with the prediction that two opposing effects upon the relative specific activities are present. Moreover, for Film 6, the only film on which two measurements were made during the light interval, the second measurement, as compared to the first, showed a significant increase in the relative $C^{14}O_2$ emission from the lighted side, which again checks qualitatively with the expected change in specific activities.

The conclusion, in some of the films at least, that the hemispheres of the eggs which were pointed toward the "bottom" respire more rapidly can be correlated with the observation that in the dark the rhizoids tended to develop toward the formerly lower face. There is no direct evi-

dence available to determine whether these two conditions are causally related or whether they are merely consequences of a third condition. However, it may be recalled that a variety of agents which polarize the Fucales egg may be considered as acting by creating a CO_2-pH gradient with the high CO_2 concentration at the rhizoid pole (See p. 16). This is at least not inconsistent with a causal relationship between higher respiration and the development of the rhizoid pole.

Total $C^{14}O_2$ Emission

As has been stated previously, the decay of the total rate of $C^{14}O_2$ emission must reflect a decay in the specific activity of the carbon dioxide rather than a decrease in carbon dioxide emission. Evidently the tagged photosynthate is oxidized more rapidly than other substrates of respiration. Various other studies have indicated that the carbon atoms of photosynthetically assimilated CO_2 are distributed so that a very wide variety of cellular compounds soon become labeled but with markedly different specific activities (e.g. Clendenning, and Gorham, 1952). In view of these studies, the decay of $C^{14}O_2$ emission is not very surprising, but the hyperbolic shape of the decay curve is probably of a complex origin. In the simplest imaginable situation, respiration would proceed at a constant rate at the expense of a single substrate which contains all the tagged photosynthate but which is steadily replenished from a pool of unlabeled materials. This would yield an exponential decay curve, which again suggests that the hyperbolic decay curve has a complex origin.

LITERATURE CITED

Beams, H. W. 1937. The Air Turbine Ultracentrifuge, Together with Some Results upon Ultracentrifuging the Eggs of Fucus serratus. J. Mar. Biol. Assoc. 21:571-88.

Bloch, R. 1943. Polarity in Plants. Bot. Ref. 9:266-9.

Brachet, J. 1939. Etude du Métabolisme de l'Oeuf de Grenouille (Rana Fusca) au cours du développement. V Le métabolisme protéique et hydrocarboné de l'oeuf en relation avec le problème de l'organisateur. Arch. Biol. 50:233-67.

Calvin, M. (and others) 1949. Isotopic Carbon, Techniques in its Measurement and Chemical Manipulation. New York. J. Wiley.

Child, C. M. 1941. Patterns and Problems of Development. U. of Chicago Press, Chicago.

Clendenning, K. A. and Gorham, P. R. 1952. Intracellular Distribution of Assimilated C^{14} in Briefly Exposed Aquatic Plants. Arch. Biochem. and Biophys. 37:56-71.

Driesch, H. 1891. Entwicklungsmechanische Studien I. Der Werth der Beiden ersten Furchungszellen in der Echinodermenentwicklung. Experimentelle Erzeugung von Theil- und Doppelbildungen. Zeitschrift f. Wiss. Zool. 53:160-84.

Farmer, J. B. and Williams, J. L. 1898. X. Contributions to our Knowledge of the Fucaceae: Their Life History and Cytology. Phil Trans. Royal Soc. 190:623-45.

Fritsch, F. E. 1945. The Structure and Reproduction of the Algae: Vol. II. Cambridge U. Press.

Harrison, R. G. 1945. Relations of Symmetry in the Developing Embryo. Trans. Conn. Acad. Arts Sci. 36:277-330.

Harvey, H. W. 1945. Recent Advances in the Chemistry & Biology of Sea Water. Cambridge U. Press, Cambridge.

Hurd, A. M. 1920. Effect of Unilateral Monochromatic Light and Group Orientation on the Polarity of Germinating Fucus Spores. Bot. Gaz. 70:25-50.

International Critical Tables, V, 1929. McGraw-Hill Co., New York.

Knapp, E. 1931. Entwicklungsphysiologische Untersuchungen an Fucaceen-Eiern. I. Zur Kenntnis der Polarität der Eier von Cystosira barbata. Planta 14:731-51.

Kniep, A. 1907. Beiträge zur Keimungs-Physiologie und Biologie von Fucus. Jahrb. f. wiss. Bot. 44:635.

Kny, M. 1881 Verhandlungen d. bot. Vereins d. Prov. Brandenburg. 23:7.

Küster, E. 1906. Normale und abnorme Keimungen bei Fucus. Ber. Deutch. Bot. Ges. 24:522-28.

Lowrance, E. W. 1937. Effect of Temperature Gradients upon Polarity in Eggs of Fucus furcatus. J.C.C.P. 10:321-37.

Lowrance, E. W. and Whitaker, D. M. 1940. Determination of Polarity in Pelvetia Eggs by Centrifuging. Growth. 4:73-6.

Lund, E. J. 1923. Electrical Control of Organic Polarity in the Egg of Fucus. Bot. Gaz. 76:288-301.

Mirbel, M. 1835. Recherches anat. et physiol. sur le Marchantia polymorpha. Annales du Muséum, 3e série, Ier, p. 107.

Mosebach, G. 1938. Über den Einfluss des Lichtes auf die Polariesierung des befruchteten Eies von Cystosira barbata Ag. Ber. Deutchen Bot. Ges. 56:210-25.

Nakazawa, S. 1951. Sc. Rep. of the Tohoku U., 4th series. 19:73-8.

Nienburg, W. 1923. Die Polarization der Fucus-Eier durch das Licht. Wiss. Meer. N. F. Abt. Helgoland 15.

Olsen, R. A. and Du Buy, H. G. 1937. The Role of Growth Substance in the Polarity and Morphogenesis of Fucus. Am. J. Bot. 24:611-15.

Papenfuss, G. F. 1951. Chap. 7 in Manual of Phycology, ed. by G. Smith. Chronica Botanica Co., Waltham. Mass., U. S. A.

Pierce, G. J. and Randolph, F. A. 1905. Studies of Irritability in Algae. Bot. Gaz. 40:321-50.

Reed, E. A. and Whitaker, O. M. 1941. Polarized Plasmolysis of Fucus Eggs with Particular Reference to Ultraviolet Light. J.C.C.P. 18: 329-38.

Rosenvinge, M. L. 1888-9. Influence des agents exterieurs sur l'organisation polaire et dorsiventrale des plantes. Rev. Gen. Bot. 1:53-62, 123-27. (Partly Published in Danish in 1888.)

Roughton, F. J. W. 1941. The Kinetics and Rapid Thermochemistry of Carbonic Acid. J.A.C.S. 63:2930-34.

Setchell, W. A. and Gardner, N. L. 1925. The Marine Algae of the Pacific Coast of North America. Part III: Melanophyceae. U. Cal. Pub. in Bot. 8, part 3:662-711.

Smith, Gilbert. 1938. Cryptogamic Botany. Vol. I. McGraw-Hill, New York.

Stahl, M. 1885. Einfluss der Beleuchtungsrichtung auf die Theilung der Equisetum-sporen. Ber. d. deutsch. bot. Gesellsch. 3:334.

Steinecke, F. 1925. Über Polarität von Bryopsis. Bot. Arch. 12:97-118.

Teissier, G. 1931. Étude Expérimentale du Developpement de Quelques Hydraires. Ann. Sci. Nat., Zool. 14:5-60.

Weiss, P. 1939. Principles of Development. Holt & Co., New York.

Whitaker, D. M. 1931a. On the Rate of Oxygen Consumption by Fertilized and Unfertilized Eggs. I. Fucus vesiculosus. J. Gen. Physiol. 15:167-82.

Whitaker, D. M. 1931b. Some Observations on the Eggs of Fucus and upon their Mutual Influence in the Determination of the Developmental Axis. Biol. Bull. 61:303-7.

Whitaker, D. M. 1937a. Determination of Polarity by Centrifuging Eggs of Fucus furcatus. Biol. Bull. 73:249-60.

Whitaker, D. M. 1937b. The Effect of Hydrogen Ion Concentration Upon the Induction of Polarity in Fucus Eggs. I. Increased Hydrogen Ion Concentration and the Intensity of Mutual Induction by Neighboring Eggs of Fucus furcatus. J. Gen. Physiol. 20:491-500.

Whitaker, D. M. 1938. Ibid. II. Gradients of Hydrogen Ion Concentration. J. Gen. Physiol. 21:833-45.

Whitaker, D. M. 1940a. The Effect of Shape on the Developmental Axis of the Fucus Egg. Biol. Bull. 78:111-16.

Whitaker, D. M. 1940b. The Effect of Ultracentrifuging and of pH on the Development of Fucus Eggs. J.C.C.P. 15:173-87.

Whitaker, D. M. 1940c. Physical Factors of Growth. Second Growth Symposium (in Growth 4:75-90).

Whitaker, D. M. 1941. The Effect of Unilateral Ultraviolet Light on the Development of the Fucus Egg. J. Gen. Physiol. 24:263-78.

Whitaker, D. M. 1942. Ultraviolet Light and the Development of Fucus Eggs as Affected by Auxin and pH. Biol. Bull. 82:127-37.

Whitaker, D. M. and Berg, W. E. 1944. The Development of Fucus Eggs in Concentration Gradients: A New Method for Establishing Steep Gradients Across Living Cells. Biol. Bull. 86:125-29.

Whitaker, D. M. and Clancy, C. W. 1937. The Effect of Salinity upon the Growth of Eggs of Fucus furcatus. Biol. Bull. 73:552-56.

Whitaker, D. M. and Lowrance, E. W. 1936. On the Period of Susceptibility in the Egg of Fucus furcatus when Polarity is Induced by Brief Exposure to Directed White Light. J.C.C.P. 7:417-24.

Whitaker, D. M. and Lowrance, E. W. 1937. The Effect of Hydrogen Ion Concentration upon the Induction of Polarity in Fucus Eggs. II. The Effect of Diffusion Gradients Brought About by Eggs in Capillary Tubes. J. Gen. Physiol. 21:57-70.

Whitaker, D. M. and Lowrance, E. W. 1940. The Effect of Alkalinity upon Mutual Influences Determining the Developmental Axis in Fucus Eggs. Biol. Bull. 78:407-11.

Winkler, H. 1900. Ueber den Einfluss äusserer Factoren auf die Theilung der Eier von Cystosira barbata. Ber. d. Deutsche. Bot. Ges. 18:297-305.

APPENDIX I. A DEMONSTRATION THAT THE "ALKALI RESERVE ERROR", EVEN AT pH 8, IS NEGLIGIBLE

The "alkali reserve error", or the error in the measured $C^{14}O_2$-ratios arising from $C^{14}O_2$ emitted indirectly by way of $H_2C^{14}O_3$ and $HC^{14}O_3^-$ in the film, will be shown to be less than 1% even at pH 8.0. Before presenting the principal argument in section D, some necessary preliminary information and analyses are given in sections A, B, and C.

Table 8

A. Physical and Chemical Constants of Carbon Dioxide (all at 16°C).

Symbol	Constant of	Value	Source
D_w	Diffusion in Water	1.64×10^{-5} cm.^{2}/sec.	I.C.T., 1929
D_a	Diffusion in Air	1.55×10^{-1} cm.^{2}/sec.	"
$\bar{K}_H$	Hydration Rate of CO_2 in Sea Water*	.018 sec.$^{-1}$	Roughton, 1941
$\bar{K}_D$	Dehydration Rate of H_2CO_3 in Sea Water*	14. sec.$^{-1}$	"
K_o	Equilibrium of $(CO_2)/(H_2CO_3)$ in Water	760	"
K_1	Equilibrium of $(H^+)(HCO_3^-)/(CO_2)$ in Sea Water**	8.8×10^{-7} moles/liter	Harvey, 1945
K_1'	$(H^+)(HCO_3^-)/H_2CO_3 = K_1K_o$	6.7×10^{-4} moles/liter	Derived

* Corrected for small catalytic effect of chloride in sea water.

** Chlorinity of 19.00 o/oo.

B. The Relative Rates of CO_2 Diffusion Out of a Film and CO_2 Hydration

It is a good first approximation to analyze a steady-state model in which the respiratory CO_2 is all liberated uniformly from a plane bisecting the film. This is a simple one-dimensional diffusion problem. Consider a unit area of the film.

Let K_p = rate of CO_2 production

Let K_H = rate of CO_2 hydration in moles/sec.

Let L = rate that CO_2 leaves film in moles/sec.

Let C_c = concentration of CO_2 at film's center

Let C_s = " " " " " surface

Let T = total quantity of CO_2 in film

Let 2r = thickness of film

Let g = length of air gap

Let $R = L/K_H$

Then, $$1/2\ K_p = \frac{D_w(C_c - C_s)}{r} = \frac{D_a C_s}{g}$$

and $$L = K_p$$

$\therefore$ $$C_s = \frac{g\ K_p}{2\ D_w}$$

$$C_c = \frac{r\ K_p}{2\ D_w} + \frac{g\ K_p}{2\ D_a} = 1/2\ K_p \left[\frac{r}{D_w} + \frac{g}{D_a}\right]$$

$$T = r\ (C_s + C_c) = 1/2\ r\ K_p \left[\frac{2g}{D_a} + \frac{r}{D_w}\right] = \frac{K_p\ r^2}{2\ D_w}$$

Now, $$K_H = \bar{K}_H\ T = \frac{K_p\ \bar{K}_H\ r^2}{2\ D_w}$$

$\therefore$ $$R = L/K_H = \frac{2\ D_w}{\bar{K}_H\ r^2}$$

$r \cong$ one egg-radius $= 5 \times 10^{-3}$ cm.

$D_w = 1.64 \times 10^{-5}$ cm.^{2}/sec.; $\bar{K}_H = .018$ sec.$^{-1}$

$\therefore \quad R = 70$

Thus, CO_2 diffuses out of a film seventy times as fast as it is hydrated. Furthermore, the rate with which CO_2 is turned over by diffusing out the film is simply R x $\bar{K}_H$ or 1.3 sec.$^{-1}$

C. The Half Life of Bicarbonate in a Film

Let X = the turnover rate of HCO_3^-, that is, the velocity of the reaction, $HCO_3^- + H^+ \longrightarrow (H_2CO_3) \longrightarrow CO_2 + H_2O$, per mole HCO_3^- per liter.

$$X = \bar{K}_D \cdot \frac{(H_2CO_3)}{(HCO_3^-)} = \frac{\bar{K}_D \cdot (H^+)}{K_1'} = 1.3 \times 10^6 \, (H^+) \text{ moles/liter x min.}$$

At pH 8.0, $(H^+) = 10^{-8}$ moles/liter, and therefore, X = .013 min.$^{-1}$. In section B it was shown that the rate with which CO_2 is turned over by diffusing out of the film is 1.3 sec.$^{-1}$. Evidently then, the rate, X, with which bicarbonate is converted to carbon dioxide, rather than diffusion, limits its escape from the film. Therefore, at pH 8.0, the half-life of bicarbonate in a film is 1/2X = 40 minutes.

D. The "Alkali Reserve" Error

At pH 8.0, the half-life of bicarbonate in a film is 40 minutes (See C). The total rate of liberation of $C^{14}O_2$, s, is inversely proportional to time and after first forming a film, s falls in half in no less than 40 minutes. Thus without attempting rigor, it is seen that C^{14} would never leave the alkali reserve more than twice as fast as it enters the

reserve. On the other hand, at 80 minutes after forming a film, when the first important interval of measurement is less than half completed, $C^{14}O_2$ would always leave the reserve faster than it enters. Hence samples of trapped carbon dioxide will not differ in their proportion of $C^{14}O_2$ which has come from the reserve by more than the relative rate that $C^{14}O_2$ enters the reserve. This is 1/70 the rate of direct liberation (See B). The largest $C^{14}O_2$-ratio measured was 1.71. An addition of 1/70 indirect $C^{14}O_2$ would change this ratio by only .8%.

APPENDIX III. THE HIGHEST $C^{14}O_2$-RATIO WHICH CAN BE ATTRIBUTED TO THE POSITION OF THE EGGS IN A FILM

A. The Film is Thicker Than One Egg-Diameter

The most eccentric position that an egg will become fixed in, on the average, is the position of equilibrium, internally tangent to the "bottom" surface of the film (See Appendix II). An egg not at equilibrium will tend, on the average, to be nearer to the upper surface than at equilibrium. One wishes to know the relative rate, R, with which, in the steady state, CO_2 emitted from the egg escapes through the "bottom" surface as compared to the "top" surface. The author was advised by Dr. William Smythe that an exact mathematical analysis of this diffusion problem would be a matter of such great difficulty that it would be easier to solve it by building a "fluid-mapper" analogue. However, the following argument will demonstrate that a simplification of this problem to one dimension can be made which yields the upper limit of $(1 + 2D)$ to the desired ratio, R. (D is the excess, in egg-diameters, of the film's thickness over one egg-diameter.)

The cell membrane is probably impermeable enough to CO_2 that in the steady state there is a large drop in CO_2 concentration across the membrane from inside to outside. The surface of the sphere can then be considered a uniform one-way diffuse source, that is, a uniform source at the surface of a spherical barrier.

Consider what happens when the spherical barrier is removed but the spherical source remains. The barrier is such as to cause CO_2 originating

from the upper hemisphere to tend to escape more rapidly from the upper surface and vice versa. If the barrier were a complete partition between the upper and lower hemispheres, a ratio of 1 would be obtained. Even the spherical barrier, then, which is an incomplete partition between the hemispheres, will tend to create a lower ratio. Hence, removal of the spherical barrier yields a new field in which R is higher than in the original one.

Once the barrier is removed, the problem is readily reduced to a simple one-dimensional field. It is well known that the field outside of such a spherically symmetrical source is unchanged when it is replaced by a point source at its center. Let this be done then. Furthermore, in the absence of the barrier, the diffusion of a molecule parallel to the surfaces of the film does not affect its diffusion perpendicular to the surfaces of the film. Hence the field can be finally reduced to diffusion along a finite line bounded by sinks with a point source at a distance a from one sink and (a + d) from the other, (where a is the radius of the egg and d is the excess of the film's thickness over one egg-diameter).

Hence by simple calculus,

$$R \quad \frac{a + d}{a} = 1 + 2D$$

Q.E.D.

Note that 1 + 2D exceeds R for two different reasons. First, the eggs may not be at mechanical equilibrium. Secondly, a mathematical approximation was introduced which results in too large a solution.

APPENDIX IV. THE GRADIENT OF SPECIFIC RADIOACTIVITIES CREATED BY A RESPIRATORY GRADIENT

Let one hemisphere of an egg respire at $(1 + f)$ times the rate that the other one does, starting at time t_1. Let us examine the ratio, R_{sa}, of specific activities between the hemispheres at time t_2.

Let zero time be at .2 hours before photosynthesis ceases.

The total rate of $C^{14}O_2$ production is observed to be inversely proportional to time, where zero time is defined as .2 hours before photosynthesis ceases (See p. 58). Let us assume that if the respiratory rate of a region of the egg increases or decreases, that the course of the decay of the specific radioactivity of this region remains unchanged except for a corresponding compression or expansion of the time scale. Then,

$$R_{sa} = \frac{\dfrac{K}{t_1 + (t_2-t_1)(1+f/2)}}{\dfrac{K}{t_1 + (t_2-t_1)(1-f/2)}} = \frac{1 - \dfrac{(t_2-t_1)f}{2t_2}}{1 + \dfrac{(t_2-t_1)f}{2t_2}} \cong 1 - \frac{f\,(t_2-t_1)}{t_2}$$

Now, $(1 + f)$ is the ratio of respiratory rates between the hemispheres. The measured ratio, of the rates of emission of $C^{14}O_2$, will really be less than $(1 + f)$ because of diffusional equilibration. However, if the respiratory ratio is taken from the emission ratio without a correction for diffusional equilibration, then the presently calculated ratio of specific radioactivities between the hemispheres can, in turn, be applied to the ratio of emitted radioactivities without correction.