COMPARATIVE GROWTH OF BARLEY EMBRYOS IN VITRO AND IN VIVO

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Chong Won Chang

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COMPARATIVE GROWTH OF BARLEY EMBRYOS *IN VITRO* AND *IN VIVO*

by

Chong Won Chang

AN ABSTRACT

Submitted to the College of Science and Arts Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

In an attempt to obtain a more fundamental understanding of the growth of plant embryos in culture, barley embryo development *in vitro* was compared to development *in vivo* in the following ways: (1) by comparing their morphological development, (2) by comparing their relative sizes and rates of growth, and (3) by relating their growth to stages of embryonic development.

Most of the previous work dealing with embryo culture has been primarily concerned with attempts at growing "premature" embryos in any way possible irregardless of the growth patterns in culture, without determining accurately in what morphological stage of development embryos were at the time they were placed in culture, and without comparing their development *in vitro* with their normal growth *in vivo*. To date only a few satisfactory results have been obtained and most of these have been with embryos of dicotyledonous plants, e.g., Van Overbeek, *et al*. (1941, 1942) wherein they succeeded to grow two proembryos of *Datura*. More limited success has been obtained with immature monocotyledonous embryos: Kent and Brink (1947) achieving some success in the culture of immature barley embryos; Norstog (1955) reporting rates of growth and morphology in culture of young embryos of barley but for one week only. In neither case, however, was a comparison made between growth rates in culture and rate of development *in vivo*. The work most similar in nature to the present study

was that of Merry (1942) who compared growth rates of embryos in culture with those of the same aged embryos *in vivo*. The only embryos, however, with which Merry had any success in culture were in the mid-stages of differentiation at the time of placement in culture; therefore his results have only a very limited bearing on the present work.

In the present work, the barley variety. Hannchen (C. I. 531, a two-rowed variety), was used for the *in vivo* and *in vitro* study. Two kinds of media were used. The first was made according to White (1954), while the second was prepared by using one part of the above basic medium and nine parts of coconut milk. One hundred fifty embryos were cultured on the second type of medium. These embryos were retained on this medium without transfer even after a two-week period in order to see whether shoots or roots might be initiated. Eighty-eight embryos were cultured on the second type of medium. The fact that embryos cultured on the second type of medium only failed to undergo differentiation, would suggest that the addition of coconut milk to the basic medium was at least one of the critical factors for differentiation of young embryos. Orientation of the embryos on the agar medium had an effect in terms of the developmental morphology in culture since it was noted that embryos placed with scutellar surfaces in direct contact with the medium gave growth patterns more nearly approaching those of embryos *in vivo*.

It was found from the *in vivo* study that the lengths and lateral diameters of developing embryos are directly proportional to each other, while the lengths and widths of developing caryopses are not well correlated. When the sizes of caryopses and embryos are related to the stages of embryo development, the lengths of the caryopses show a rapid increase from the time in late proembryo development until stage 2, while the lateral diameters of the caryopses and the sizes of embryos *in vivo* undergo little change in measurements during this time. After stage 2, the increase in lengths of the caryopses gradually slow down until the middle of stage 6 is reached. Just prior to stage 5, the lateral diameters of the caryopses undergo rapid increases in size while the embryos increase in size at a somewhat lesser rate. By the middle of stage 6, the lengths of the caryopses become almost constant, while the lateral diameters are still increasing, but at a much diminished rate. The sizes of embryos within the caryopses, however, are at this time undergoing their most rapid growth. The fresh and dry weights of embryos show an ever-increasing relationship to embryo size. In addition to this, it was found that approximately 2/3 of the embryos' fresh weights was due to water. Of a total of 59 spikes from which embryos were excised for the *in vivo* study, 4 spikes contained embryos which averaged 0.55 x 0.30 mm. initially. Their final

average size at the end of two weeks was 3.15 x 2.38 mm. The ratio of the average final length to average final width *in vivo*, therefore, was 1.3 : 1.

From the *in vitro* study, the average initial size of 38 embryos was 0.50 x 0.30 mm., while at the end of two weeks in culture they had attained a size of 1.20 x 0.90 mm. When the ratio of average final lengths to average final widths are compared, they are seen to be identical (1.3 : 1) in both *in vitro* and *in vivo* embryos. Embryos developing in culture, therefore, maintain length/width relationships which are identical with, and attain sizes which approach those of *in vivo* embryos. *In vitro* embryos differ, however, from those developing *in vivo* in that cultured embryos are larger at any given morphological stage, are slower in the rate at which they pass through the various stages, show a number of morphological deviations from normalembryogeny, and never attain the morphological development of stage 6.

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INTRODUCTION

Although the artificial culture of plant materials has received wide attention for a number of years, embryo culture should be distinguished from "tissue culture" in the broad sense. The latter aims at the growth *in vitro* of isolated tissues or of plant parts, while the former, embryo culture, theoretically at least, intends to induce normal embryogeny during embryo development (from the time of fertilization until embryo "maturity" as found in the seed) like that which is accomplished within the plant itself, that is, *in vivo*. The real aim, therefore, of embryo culture should be that of achieving continuous, normal embryonic development with the idea in mind of the ultimate production of seedlings which are morphologically and physiologically the same as those of embryos which develop *in vivo*. In order to approach this aim, then, it is most important to culture immature embryos as young as possible and to trace all stages of embryonic development until seedlings are formed. Thus, it is extremely hard to draw any positive conclusions as to the success that is being achieved in regard to embryonic development in culture, without first observing each stage of normal embryogenesis within the plant itself from fertilization time until the time when the embryo is "mature" (as found in the mature seed).

A survey of the work accomplished by researchers up to the present time may be grouped into two categories: one concerned with the production of plantlets; the other with the continuous embryonic growth of immature embryos. In the former case, the growth of immature embryos does not follow normal embryonic development, but rather gives rise to small plantlets which are the premature outgrowths of previously formed root and shoot primordia. The latter aims at inducing the same embryogenesis and ultimate formation of seedlings as would result from normal development *in vivo*. So far as the history of embryo culture is concerned, much more research has been done with this latter aspect in mind, yet to date few satisfactory results have been obtained. Among the more successful attempts has been the work of Van Overbeek, *et al.* (1941, 1942) using materials of dicotyledonous plants in which seven proembryos (0.14 mm. in diameter) of *Datura* were cultured, and, for the first time, apparently normal embryonic growth was induced in two of them.

In monocotyledonous plants Norstog (1955), culturing barley embryos, ranging from 0.3 to 0.8 mm. in length, reported the rate of growth and the morphology in culture during a one-week period only. He also succeeded in "growing" two "proembryos" which were only 0.16 to 0.2 mm. in length. In neither case, however, was a comparison made between growth rates of the cultured embryos and those developing *in vivo*.

So far as the writer knows, the research most similar to the present work is that of Merry (1942). He attempted to culture seven-day old (post-fertilization) barley embryos which measured approximately 0.3 mm. in length; nine-day old ones 0.5 - 0.6 mm. long; ten-day old one 0.7 mm. long; and eleven-day old embryos measuring 0.8 - 0.9 mm. in length. Although he did not succeed in growing any of these, he was able to produce plantlets from twelve-day old embryos and compared them morphologically with embryos growing *in vivo*.

Although the knowledge of plant embryo culture has accumulated over the years, especially research dealing with various aspects of nutritional requirements, thus far no one has succeeded in growing extremely immature excised monocot embryos in culture the way they normally develop *in vivo*. At this point it is quite important to compare the growth behaviors of embryos *in vitro* with those *in vivo* in order to more clearly evaluate their differences. Therefore, in the investigation undertaken here, particular attention has been directed toward a comparison of embryo development *in vitro* and *in vivo* in the following ways: (1) by comparing their morphological development; (2) by comparing their relative sizes and rates of growth; and (3) by relating their growth to stages of embryonic development.

HISTORICAL REVIEW

After a careful survey of the literature relating to embryo culture as a whole, it was decided to limit the discussion to the more pertinent work: that dealing primarily with the culture of immature embryos. Also, since the present work was designed to study in detail immature embryos, excised as young as possible, the culture of mature embryos has little relationship to the aspect that the writer intended to approach.

For some unknown reason, it appears to be very hard to grow embryos of Gymnosperms *in vitro*. So far as the writer knows, no one yet has succeeded in culturing immature Gymnosperm embryos, although the culture of *Gingko* embryos was attempted by Radforth (1937), that of *Pinus* embryos by Loo and Wang (1943), and young embryos of *Larix* were investigated by Sterling (1949). The results obtained by these investigators were more or less similar in that they failed to induce normal embryonic growth, but did obtain undifferentiated masses of tissue.

In the culture of embryos of dicotyledonous plants, much greater success has been attained. Lofland (1950) was able to culture mature embryos of *Gossypium*, yet failed to grow the young, immature ones. Since certain varieties of sweet cherries produce no viable seeds (because prior to the

time of fruit ripening, the embryo and endosperm tissue cease development and abort) Tukey (1933) attempted to culture the immature aborting embryos. Although he was not able to induce normal embryonic growth, he did succeed in producing plantlets by using Knop's complete nutrient solution and Crone's nitrogen free solution. He also found that immature embryos of apple,and peach, among others, did not continue normal embryonic development in culture, but rather produced plantlets. Using a medium containing coconut milk, Van Overbeek, et al. (1941, 1942) cultured seven proembryos of Datura, 0.14 mm. in diameter, which were 14 days old. They, for the first time, succeeded in growing two immature embryos (of all those attempted) apparently normally, without a precocious differentiation into plantlets. Their success was apparently due to certain substances within the coconut milk which was referred to collectively as an "embryo factor."

As was mentioned above, it has been fairly well established that young immature embryos of monocotyledonous plants are much harder to grow than dicotyledonous plants. Many investigators have attempted to solve this difficult problem. In the years following the successful growth of proembryos of Datura with the addition of coconut milk, it has become generally accepted that young immature embryos in vitro require certain "embryo factors" for their normal embryonic growth. Therefore, a great deal of effort in recent years has been devoted to the determination of a more effective "embryo factor."

LaRue (1936) cultured young excised embryos of many plants to determine the minimum size of embryos which could be grown, and also to investigate the relationship of such culture with the developmental morphology of the embryos. He, particularly, succeeded in culturing embryos which were smaller than any previously cultured. Following LaRue's earlier work, LaRue and Avery (1938) compared the growth in culture of immature embryos of Zizania with those growing in vivo. By culturing, embryos, ranging from 0.2 - 0.35 mm. they found that the size could be doubled but were unable to develop further. In the culture of embryos 0.4 to 0.7 mm. in length, continuous cell divisions were found; any increase in embryo size was due to cell enlargement only. Embryos in which more cell divisions were obtained and in which a rudimentary leaf was induced, measured 0 mm. in length. Brink et al. (1944) grew a young hybrid embryo, which was the product of a cross between a wild species of barley and domestic rye, into a mature plant. Konzak et al. (1951) succeeded in obtaining seedlings by culturing young embryos of hybrids between common barley and wild perennial barley. In the culture of young embryos of Carex, Lee (1952) found that the seedlings from the cultured embryos were smaller and weaker than those grown from embryos developing in vivo. Interested in the factors responsible for normal embryonic development, Curtis (1947) cultured orchid embryos, using a medium to which barbiturates had been added, and obtained instead of plantlets

only an undifferentiated mass of tissue. Kent and Brink (1947) and Ziebur, et al. (1950) cultured immature barley embryos using, in addition to a basic mineral-sucrose mixture, various concentrations of casein hydrolysate, tomato juice, sodium nucleate from yeast, lactalbumin, and wheat gluten hydrolysate to test the effect of these substances as "embryo factors." Their "immature embryos" which were most successfully cultured were well differentiated embryos at the time of excision. Since the primary aim of these studies was to determine the effects of culture upon subsequent seedling growth, no comparisons were made with normal embryo development in vivo. Ziebur and Brink (1951) found that the addition of endosperm to the culture medium would also act as an "embryo factor." They reported limited success in culturing "proembryos," but again without any comparison with in vivo embryogeny. Haagen-Smit et al. (1945) tried to grow very young embryos of maize by the addition of coconut milk to a medium containing in addition to Van Overbeek's basic medium, sucrose, asparagine, and biotin, but failed to obtain significant results. The first successful attempt to culture immature monocot embryos by using an "embryo factor" source entirely different from any tried by previous workers was made by Pieczur (1952). He found that young maize embryos could be effectively grown if they were placed on a medium in which a mass of the maize endosperm tissue was already growing, yet would not grow if merely

excised endosperm (from the grain) was placed on the medium at the same time that the embryos were started in culture. In a recent investigation concerned with the culture of excised embryos of oats, barley, rye and wheat, Norstog (1955) reported the successful embryonic growth of immature barley embryos by culturing with a modified White's nutrient medium to which 90 percent coconut milk had been added. Of the four smallest embryos cultured (ranging from 0.16 - 0.20 mm. in length) two produced leaves and roots. While Norstog described the morphology of his cultured embryos in some detail, he did not use in vivo controls. In an earlier experiment, using a different culture medium, Merry (1942) compared barley embryos in culture with those in vivo in an effort to determine their morphological relationships. He failed, however, to induce embryonic development and to produce seedlings when culturing embryos younger than eleven days post-fertilization. The youngest age at which he could produce plantlets was from twelve-day old embryos.

After a survey of the brief history dealing with immature monocot embryo culture up to the present time, it appears to be true that no one has yet succeeded in taking extremely immature embryos and producing normal embryonic growth with subsequent plant seedlings which are morphologically and physiologically the same as those which develop in vivo. When previous workers have referred to "successful" culture, they apparently have meant the ability to keep immature monocot

embryos alive or growing for a short period of time irregardless of their nature of development, and without making a critical comparison between embryo growth *in vitro* and *in vivo*.

MATERIALS AND METHODS

The barley variety, Hannchen (C. I. 531, a two-rowed variety) was used for the *in vivo* and the *in vitro* study. This variety was chosen because it has been used extensively for radiation research here during the past four years and is a uniformly growing plant of a long inbred line well adapted to the Michigan climate. Plants were grown in the greenhouse where temperatures were kept at 75° F during the day and 65° F at night. Diurnal optimum temperature differentials for this strain of barley should be 10° - 15° F. It is particularly important to maintain the lower night temperature to avoid sterility problems often encountered with higher temperatures. Since barley is a long day plant, the day length was increased to 20 hours by the use of artificial light in order to hasten flowering, thus permitting extra "crops" to be grown during a given period.

In Vivo Study

In order to determine the growth rates of the caryopses and embryos *in vivo*, samples were taken at two-day intervals and the lengths and lateral diameters were measured. Measurement of embryos and caryopses were carried out under a calibrated dissecting microscope. There was little difficulty in

determining the exact lengths and lateral diameters of the embryos (after excision from the fruits) and the lateral diameters of the caryopses. The lengths of the caryopses, however, were much more difficult to obtain since the fusion of the two stigmas (Fig. 1) at the apical end made an exact delimitation of that end hard to determine. This difficulty was also encountered by Harlan (1920). In the present study, this difficulty was resolved by carefully determining the fusion point of the stigmas with a dissecting needle before measurements were made.

Formalin-acetic acid-alcohol, FAA (Johansen, 1940), was used for killing and fixing caryopses sampled for the histological study. Materials were dehydrated, embedded in paraffin by standard procedures (Johansen, 1940) and serially sectioned at 12-15 microns. Staining was carried out by using saffanin.

Selection of caryopses of the same age was made possible by the fact that the caryopses in the middle of the spike are pollinated on the same day, while in the terminal four and the basal four, pollination occurs slightly later. Therefore, if the caryopses of the four terminal and four basal nodes are discarded, the remaining caryopses are, for all practical purposes, identical. Sampling of the caryopses and embryos was done in order, from the top of the spike toward the base.

Fig. 1. Caryopses at different stages of development.

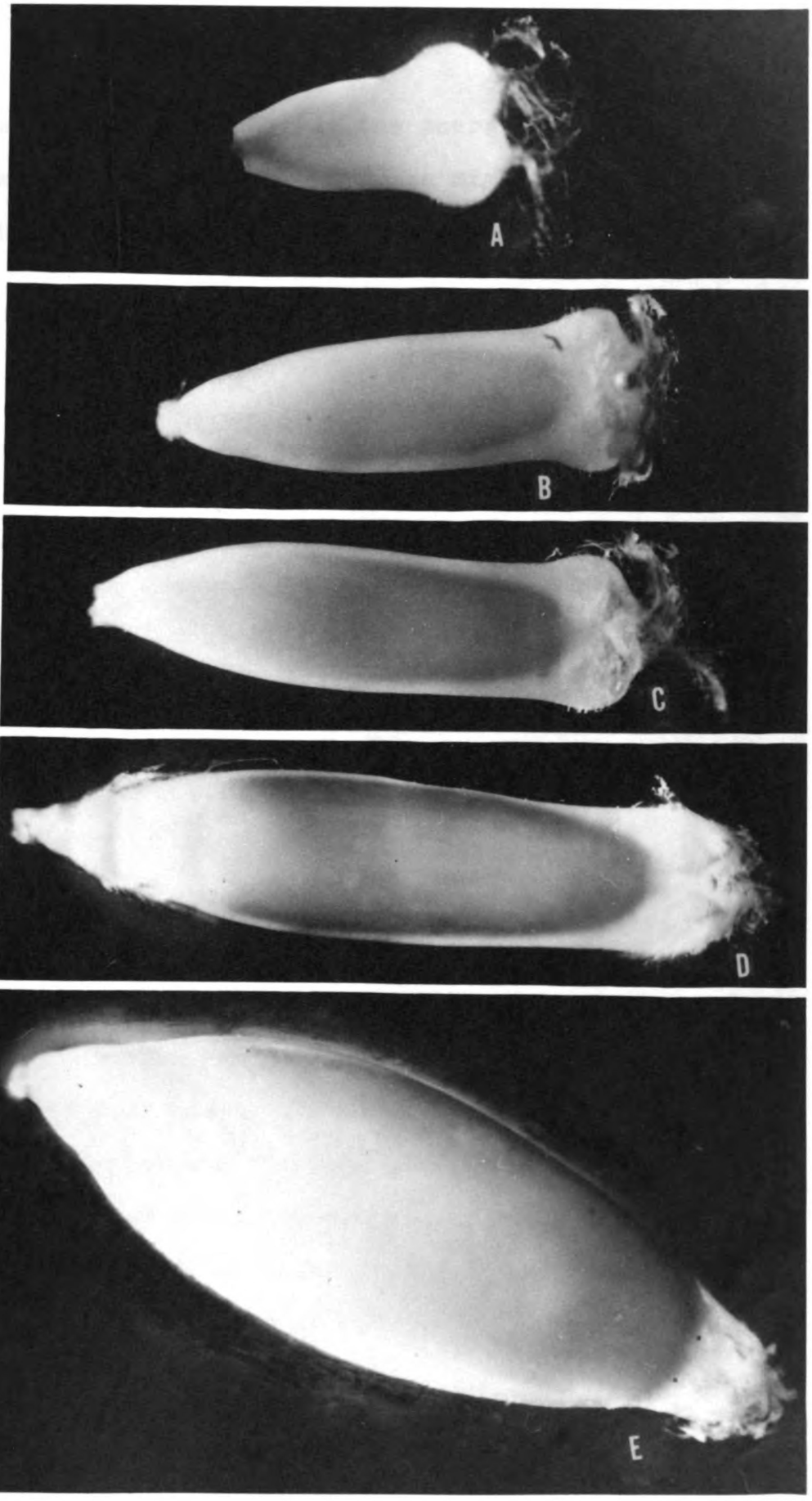
a. Caryopsis, 3.2 mm. long, containing a mid-proembryo, size not determined.

b. Caryopsis, 5.6 mm. long, containing an embryo 0.23 mm. long.

c. Caryopsis, 7.0 mm. long, embryo 0.4 mm. long.

d. Caryopsis 8.8 mm. long, embryo 0.67 mm. long.

e. Caryopsis, 9.7 mm. long, embryo 3.00 mm. long.


A
B
C
D
E

Otherwise one might wonder whether the scars made on the rachis by removing the earlier caryopses might not affect the normal physiology of those remaining above the scars.

In Vitro Study

Sterile culture chambers for this phase of the work were of two types. The first type consisted of small plastic cups placed in petri dishes in the bottom of which were wet filter papers for maintaining a saturated atmosphere (Fig. 2). Plastic cups were sterilized by soaking them in 70 percent alcohol overnight. Chambers of this sort were used for the initial cultures since embryo inoculation could be easily accomplished and measurements of embryos could be accurately made through the petri dish lids with the aid of a calibrated dissecting microscope. After two weeks culture, embryos were transferred to a second type of culture chamber screw cap vials (Fig. 3), since these had more room for upward growth of shoots.

Two kinds of media were used. One was made according to White (1954), while another was prepared by using one part of the above basic medium and nine parts of coconut milk. The first type of medium was used with the screw cap vials and the second type of medium with the petri dishes. Before adding agar, the basic nutrient solution was adjusted to a pH of 5.6 by the addition of 0.1 normal potassium hydroxide. The original pH of the solution ranged from 4.5 to 4.6. Agar, 0.75 percent, was added to the nutrient solution just before autoclaving at 15 lbs. pressure, 240° F, for 20 minutes. Coconut milk was sterilized by using a series of sintered glass

Fig. 2. Culture chamber used during the initial two-week period, plastic cups placed in a petri dish in the bottom of which is wet filter paper for maintaining a saturated atmosphere.

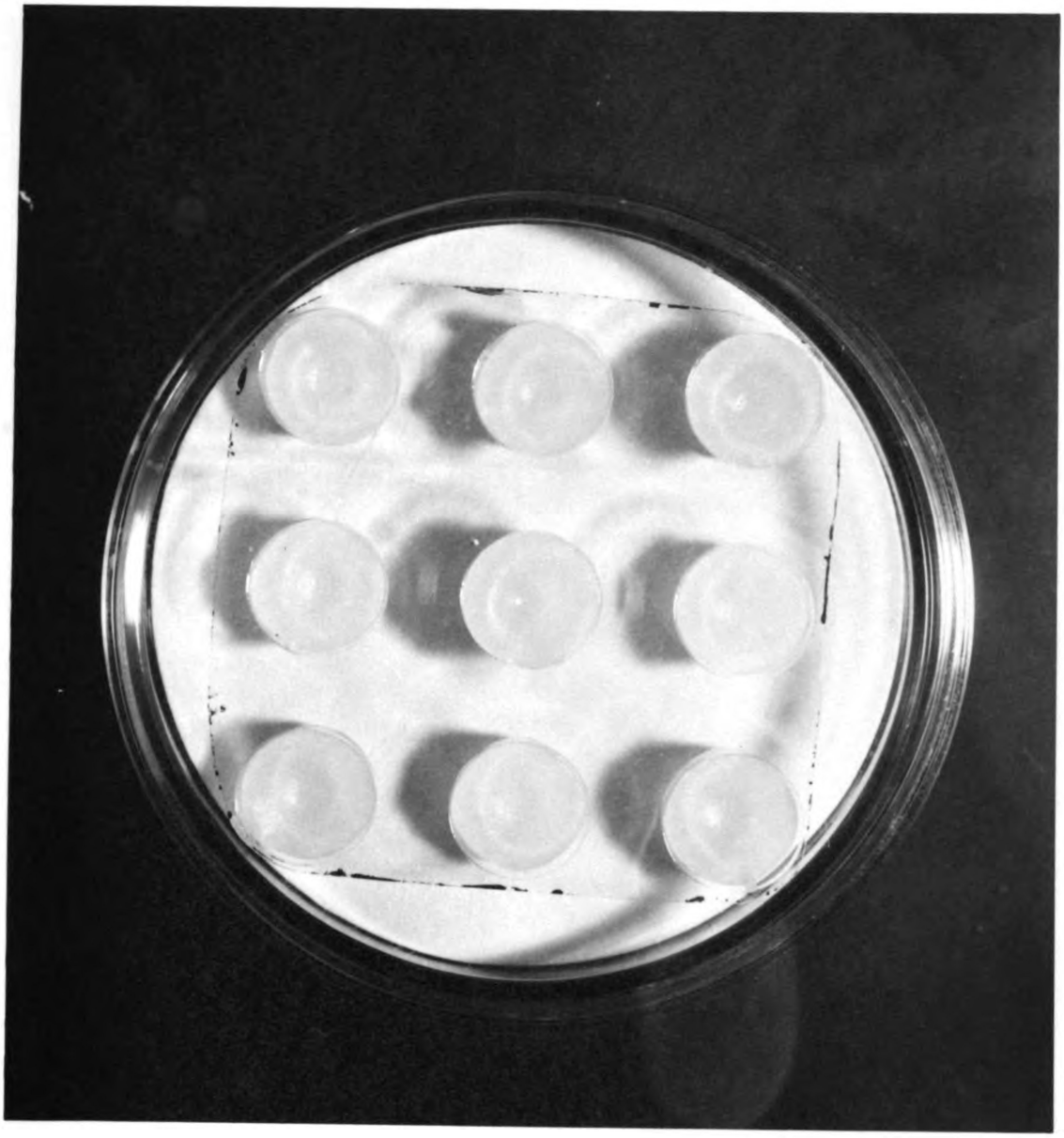
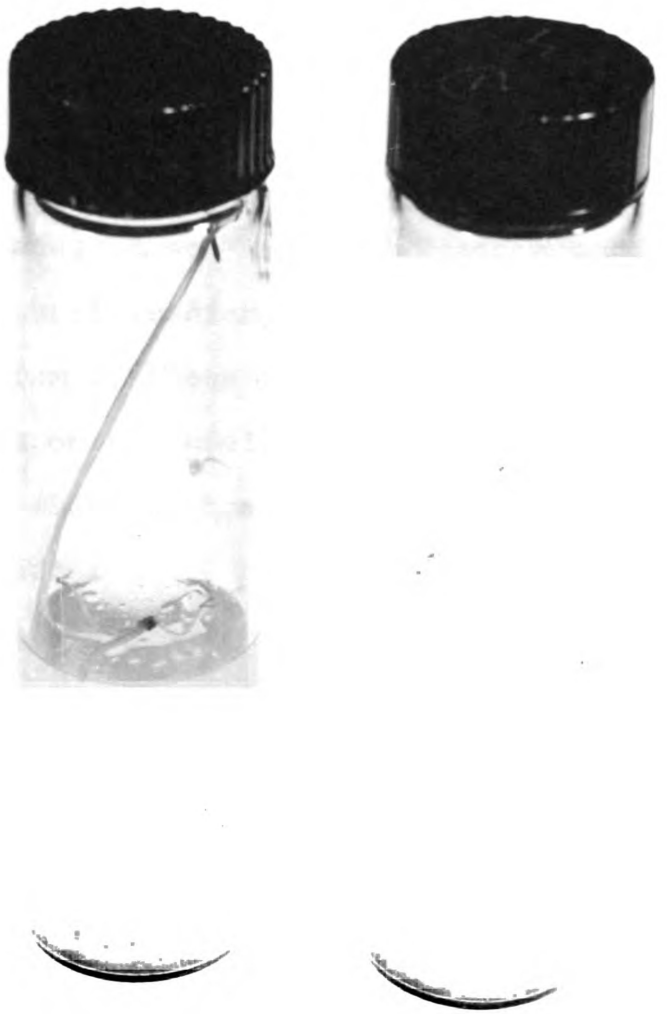


Fig. 3. Plantlets formed from embryos, each 0.70 mm. in initial length and cultured for 19 days.



filters and was added to the autoclaved culture medium aseptically, just prior to gelation, at the time when the medium felt warm to the hand. Since 9 parts of coconut milk were added to 1 part of basic medium, it was hard to have a perfectly uniform medium of these two components. If the medium became gelatinous upon the addition of the coconut milk, it was steamed for as short a time as necessary to obtain a more perfectly mixed medium.

In obtaining embryos for culture, individual young fruits with lemma and palea still intact were transferred through three different rinses of 1 percent each of Kromet.[1] After removing the lemma and palea, the fruits were again sterilized with another solution of 1 percent Kromet for five minutes, then rinsed with three changes of sterile, triple glass-distilled water. All of these procedures were carried out under a glass dust shield in an inoculating room wherein the atmosphere had been previously water sprayed with a hand sprayer. Until the embryos were excised, sterilized fruits were kept in sterile petri dishes which contained a small amount of sterile culture solution. Embryos were aseptically removed from the young fruits with the aid of a dissecting microscope and transferred to the agar medium in the plastic cups. Efforts were made to insure the placement of embryos in such a position that the scutellum was in direct contact

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[1]Trade name of a sodium hypochlorite-detergent compound supplied by the Wyandotte Chemical Company, Detroit, Michigan.

[illegible] supplied by the Wisconsin Dairies Corporation, Portage, Wisconsin.

with the nutrient agar. Because a scutellum had not yet differentiated in the smallest embryos, 0.4 - 0.6 mm. in length, it was difficult but not impossible to orient the excised embryos so that they would have the above position after differentiation. The importance of the position of the young embryos upon the agar medium will be discussed later. It should be pointed out, however, that very small embryos of this type must be excised and placed in culture as quickly as possible after the flowering spikes are obtained from the greenhouse since it was found that with a lapse of time, viability of the embryos in culture was considerably reduced. After the excised embryos were inoculated onto the medium containing coconut milk (the second type of medium as mentioned above) within the plastic cups, the petri dishes were half-sealed lest proper aeration should be hindered and kept in a growth control laboratory in the dark at a 60° F night temperature and a 70° F temperature during the day. At the end of two weeks embryos which were selected to be cultured for an additional length of time were aseptically transferred from the plastic cups to the screw capped vials.

RESULTS AND DISCUSSION

In Vivo Study

Comparisons of embryo sizes (lengths and lateral diameters) and embryo weights (fresh and dry) were made from 378 embryos dissected at two-day intervals from 59 developing barley spikes and are shown in Tables 1 and 2, and Figures 4 and 5. In order to determine changes in embryo sizes and weights (relative to stage of embryo development and caryopsis size) two caryopses borne oppositely on the rachis were sampled at specific times as the spikes developed. One sample was killed and fixed for histological determination of the stage of embryogeny while the other sample was used for size measurements of the caryopsis and for subsequent excision of the embryo so that its size, weight and gross morphology could be determined; 40 pairs of such samples were made and the average of the results obtained are shown in Table 3, beginning with the earliest stage which could be dissected (late proembryo).

Morphological Characteristics of Barley Embryogeny

Patterned after Mericle and Mericle (1957), morphological and histological features of barley embryos, from fertilization time until "maturity" of the embryo as found in the seed, may

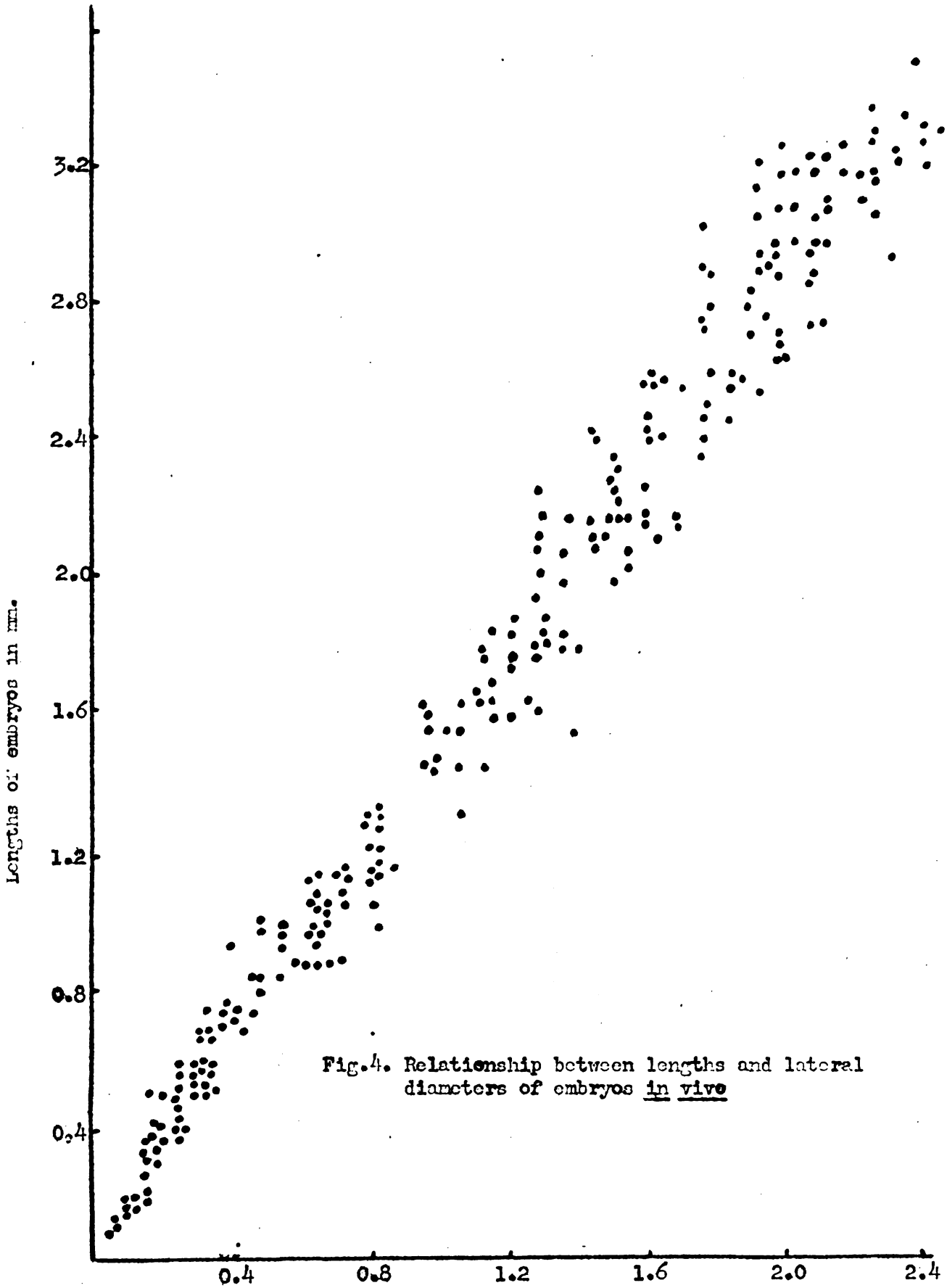
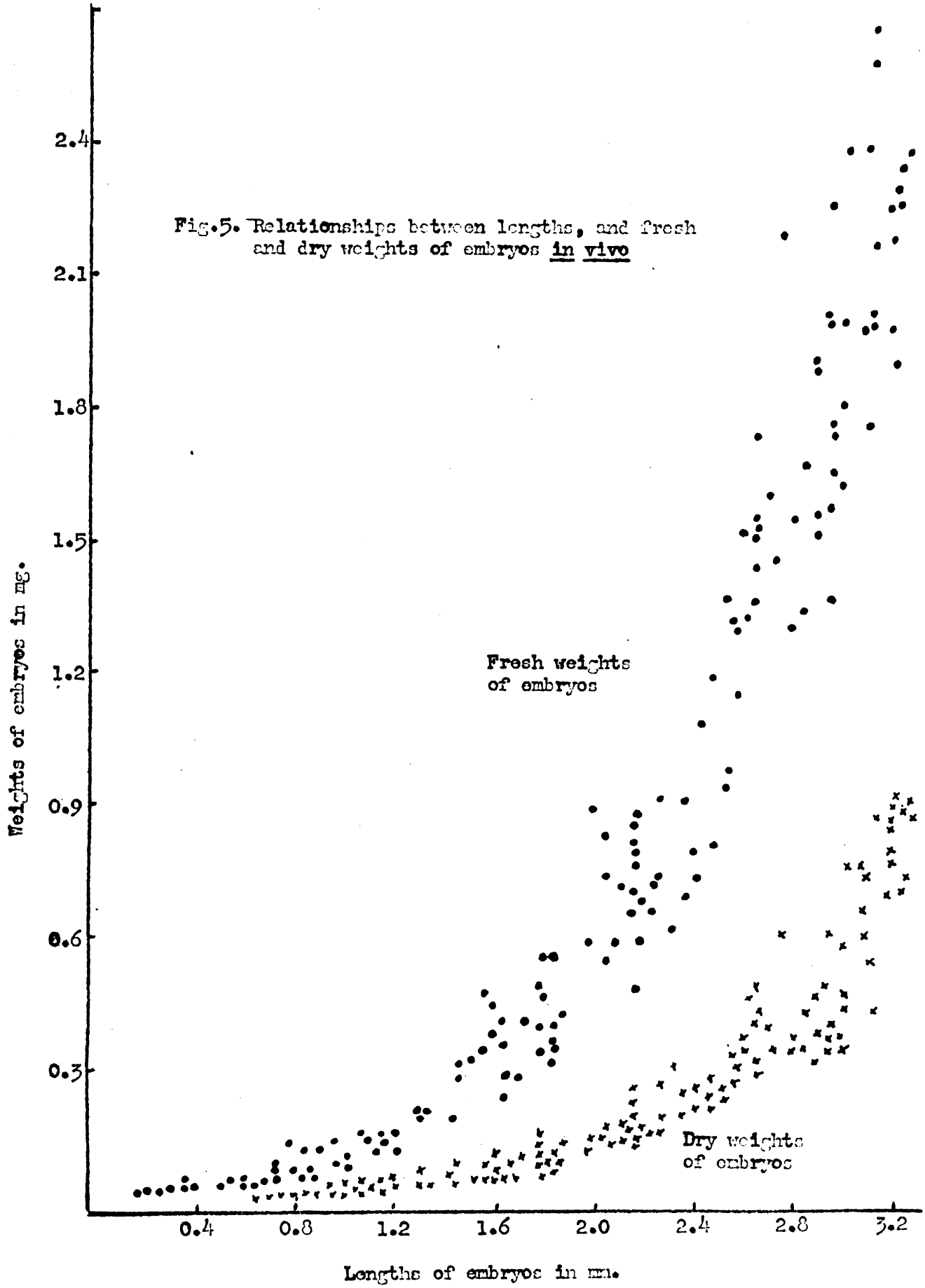


Fig.4. Relationship between lengths and lateral diameters of embryos *in vivo*

Fig.5. Relationships between lengths, and fresh and dry weights of embryos *in vivo*



be divided into two large groups: proembryos and differentiating embryos. The former may be in turn arbitrarily divided into three sub-groups: early, middle, and late proembryos. Early proembryos include developmental stages from the one-celled zygote to the 8-celled stage; middle proembryos from the 16-celled stage to approximately 72 cells; while late proembryos consist of the largest obovate-spheroidal embryos just prior to the initial stages of organogenesis (organ differentiation). Differentiating embryos may be sub-grouped into six stages. The principal feature distinguishing stage 1 is a slight convexity which forms on the abaxial "face" of the embryo surface. In examining this stage three-dimensionally with a dissecting microscope, there is a considerable overlap, however, between this stage and that of a late proembryo, relative to size and gross morphology (Fig. 6, a, b). Stage 2 is characterized by the initiation of the coleoptile, at this time an incomplete circle (collar) of tissue which makes this stage more or less easily recognizable. Little difficulty is experienced, therefore, in distinguishing stage 1 and stage 2. Stage 3 (Fig. 6, b, c) also has clear morphological features: a gradual development of a fan-shaped scutellum, a continued differentiation of the coleoptile (now a complete circle of tissue) and the initiation of the shoot primordium which appears as a "dot" of tissue in the center of the encircling coleoptile when viewed with a dissecting microscope. About this time the

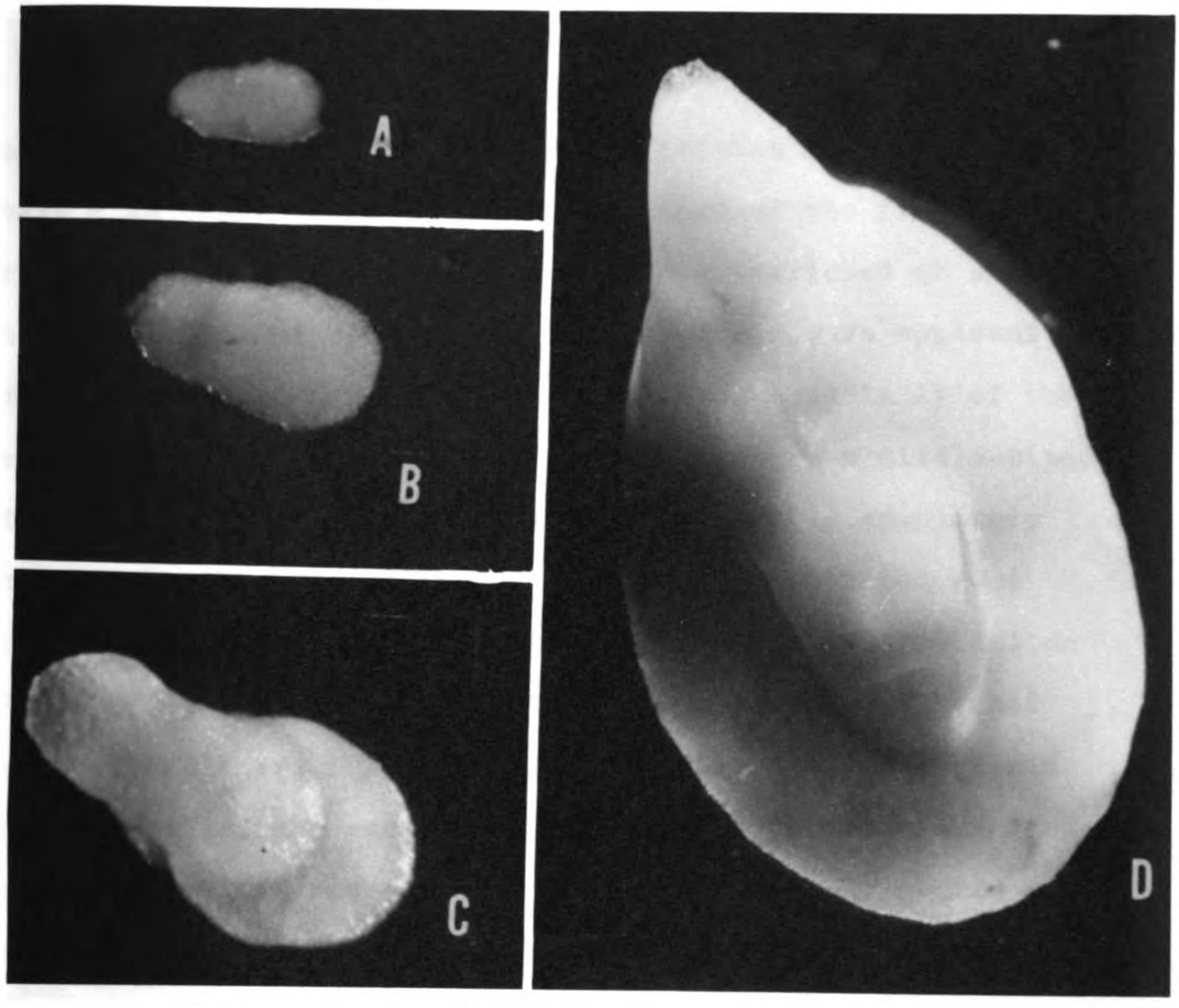
Fig. 6. Representative embryos at different stages of development.

a. Embryo, 0.23 mm. long, at late proembryo stage.

b. Embryo, 0.40 mm. long, at stage 1 (early differentiating embryo).

c. Embryo, 0.67 mm. long, at stage 3 (middle differentiating embryo).

d. Embryo, 3.00 mm. long, at stage 6c, (late differentiating embryo).


A
B
C
D

root (radicle) primordium is being initiated internally. Stage 4 is characterized by the formation of the first leaf primordium in addition to the structures developed at stage 3. Characteristics of stage 5 and stage 6 are not as apparent externally except for an increase in size, especially of the scutellum. Internally, however, stage 5 shows a middle-sized differentiating embryo with two leaf primordia, completely enclosed by the coleoptile and a well differentiated root primordium. In stage 6 the embryo completes differentiation (Fig. 6, d). The scutellum becomes full sized, additional leaf primordia are formed (usually a total of 3 - 4) within the coleoptile, the radicle is fully formed and seminal root primordia are differentiated (usually 3-4 in this strain of barley). As this study progressed, it was found necessary to subdivide stage 6 into three groups of "maturing" embryos: early (6a); middle (6b); and late (6c). These three groups are morphologically and histologically very similar, but differ markedly in their rates of increase in size and weight, as will be shown later.

Relationship between Caryopses and Embryos Based upon Developing Stages (Fig. 7)

The lengths and lateral diameters of developing embryos are directly proportional to each other (as shown in Fig. 4), therefore these two entities may be spoken of collectively as "embryo size." On the other hand, the lengths and widths of

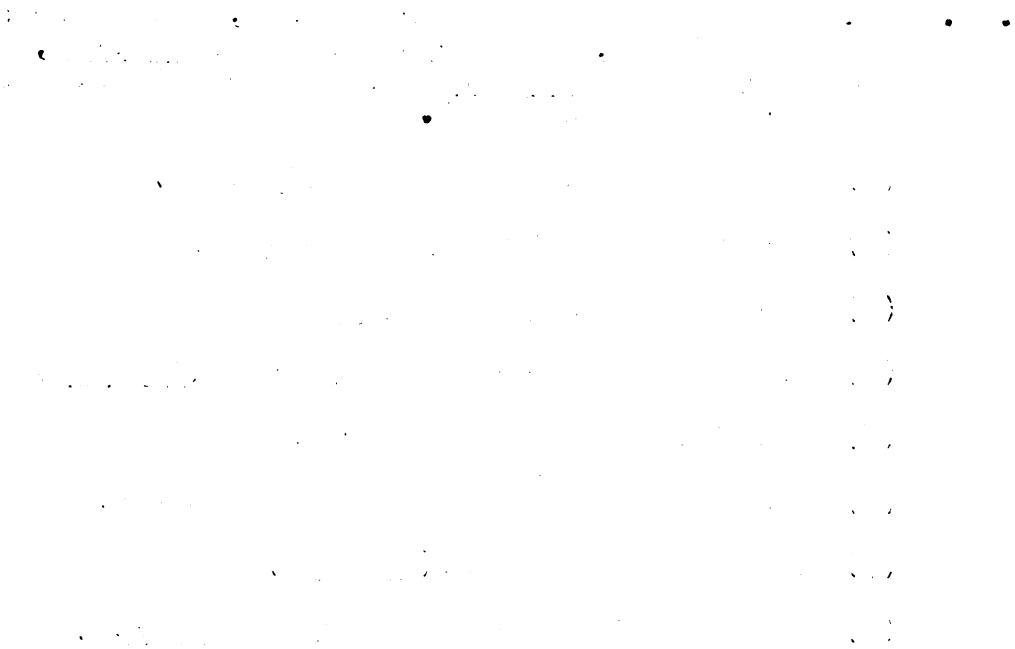


Fig. 7. Relationships between embryo sizes, embryo fresh and dry weights, and caryopsis sizes *in vivo*, and embryo sizes *in vitro* as compared to stages of embryonic development.

(1) Fresh weights of embryos (*in vivo*)

(2) Dry weights of embryos (*in vivo*)

(3) Lengths of caryopses (*in vivo*)

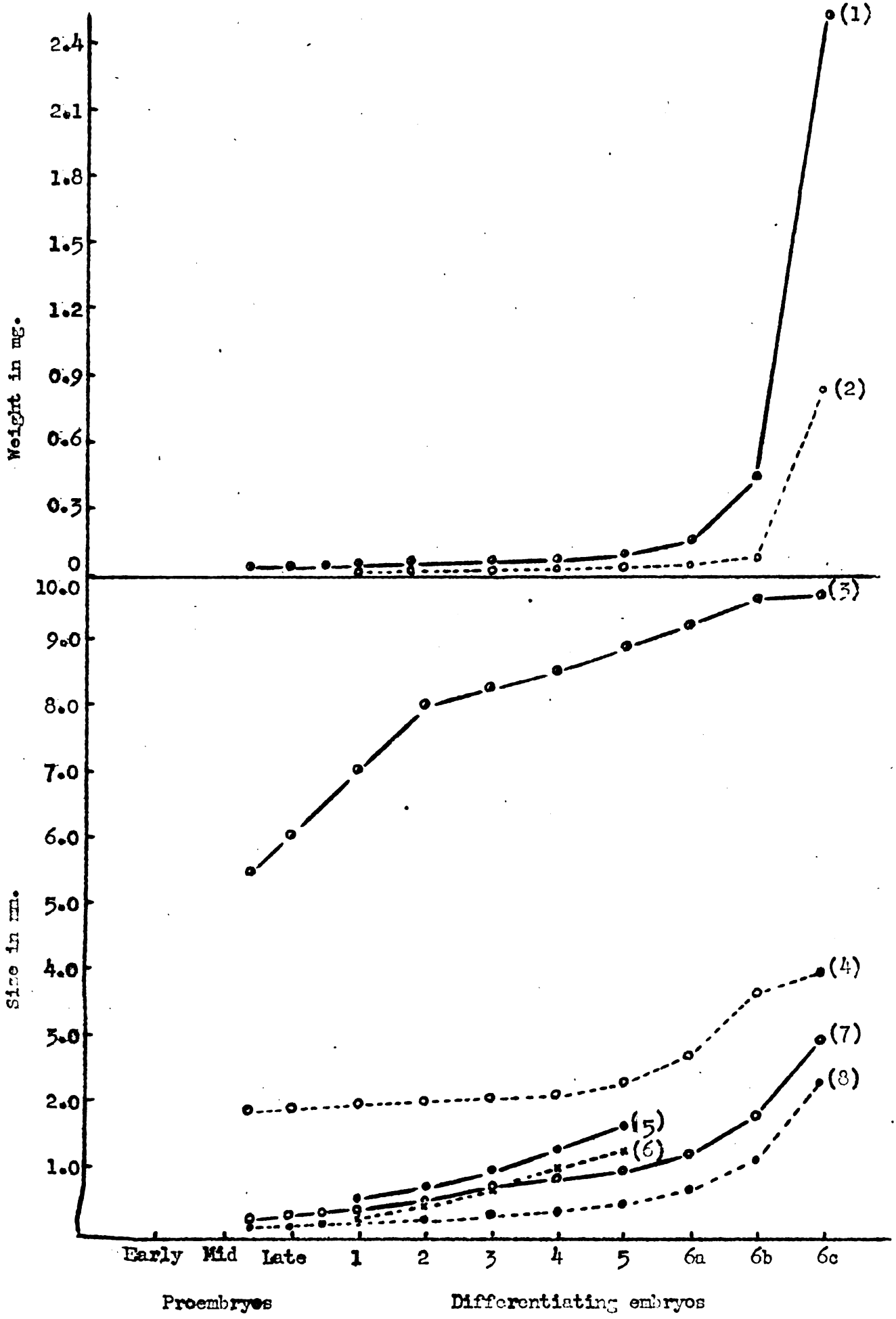
(4) Lateral diameters of caryopses (*in vivo*)

(5) Lengths of cultured embryos

(6) Lateral diameters of cultured embryos

(7) Lengths of embryos (*in vivo*)

(8) Lateral diameters of embryos (*in vivo*)


Weight in mg.
2.4
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0
(1)
(2)
Size in mm.
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(3)
(4)
(7)
(8)
(5)
(6)
Early
Mid
Late
1
2
3
4
5
6a
6b
6c
Proembryos
Differentiating embryos

developing caryopses are not well correlated (Fig. 8) so that these two entities must be considered separately. From the time in late proembryo development (when measurements could first be made) until stage 2, only the lengths of the caryopses showed a rapid increase, with the caryopses attaining 82 percent of their final length by stage 2, while the lateral diameters of the embryos undergo little change in measurements during this time. After stage 2, the increase in lengths of the caryopses gradually slow down until the middle of stage 6 is reached. The lateral diameters of the caryopses and the lengths and lateral diameters of the embryos, on the other hand, gradually increase in rate of growth. Just prior to stage 5, the lateral diameters of the caryopses undergo rapid increases in size while the embryos increase in size at a somewhat lesser rate. By the middle of stage 6, the lengths of the caryopses become almost constant while the lateral diameters are still increasing, but at a much diminished rate. The embryos, however, are at this time undergoing their most rapid growth, in terms of increases in length and lateral diameter.

According to Merry (1941) it takes approximately 7 days after pollination for the developing embryo to reach 0.2 - 0.3 mm. in length (late proembryo stage); 8 days to reach 0.5 mm. in length (first differentiating embryo stage); 10 days to become 0.6 mm. long (stage 3); and 12 days to reach a length of 1.1 mm. (stage 6a). The results obtained in this study agree essentially with those of Merry, as can be seen

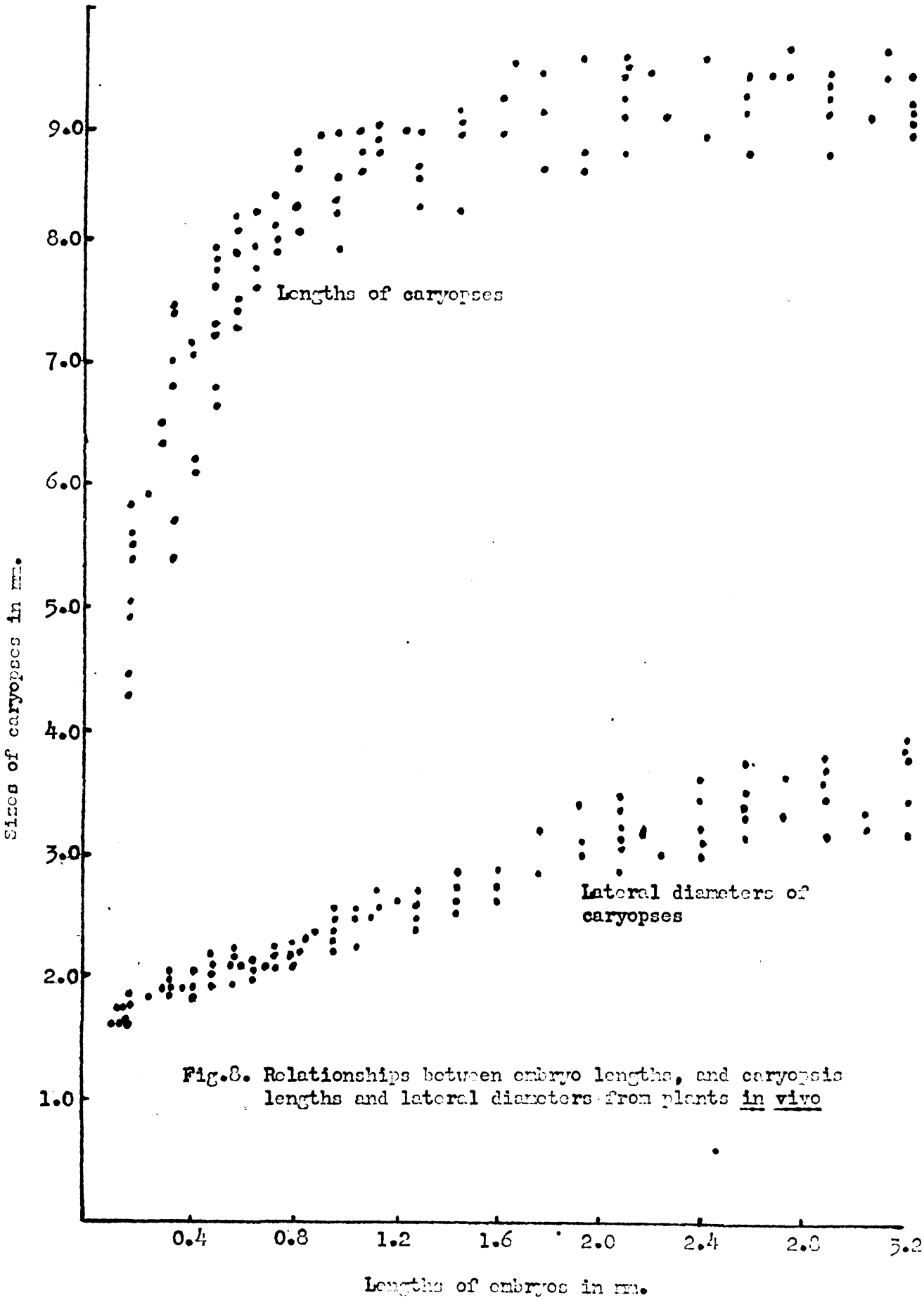


Fig.8. Relationships between embryo lengths, and caryopsis lengths and lateral diameters from plants *in vivo*

in Table 3. It should be pointed out, however, that although the variety of barley used by Merry was Alpha rather than Hannchen, it was a two-rowed variety and was grown under greenhouse conditions.

The results of this study are not in agreement, however, with those reported earlier by Harlan (1926) using the same variety of barley (Hannchen) but grown under field conditions. Harlan found that a caryopsis 8 mm. long contained an embryo which was 0.12 mm. in length. In the present work, a caryopsis of this length contains an embryo 0.5 mm. long. In addition to this, Harlan reported that a differentiating embryo (stage 1) is found in a caryopsis which is 8.8 mm. long, while in this study, this stage of embryonic development was present in a caryopsis averaging 7.0 mm. in length, whereas a caryopsis of 8.8 mm. contains an embryo, not in stage 1, but rather in stage 5-6. In other words, Harlan's material consisted of caryopses of much larger size, relative to stages of embryogeny, or embryos which were much smaller than those found in the present study.

Relationships between Embryo Sizes and Embryo Fresh and Dry Weights

As presented in Figures 5 and 7, the fresh and dry weights of embryos show an ever-increasing relationship to embryo size (length and lateral diameter). At least a part of the sudden upsurgence of weights beginning with the middle

of stage 6 is believed to be a reflection of an increase in the embryos' dorsi-ventral diameters. Accurate measurements of the dorsi-ventral diameters were not practicable because of the errors which would probably be induced by shrinkage during the time consumed in positioning the embryos on the edges of their fan-shaped scutella. If embryo volume, however, could have been determined, there would probably have been a more perfect correlation between embryo size and weight. When fresh and dry weights of embryos are compared (Fig. 5) it may be seen that approximately 2/3 of the embryos' fresh weight is due to water. This water content is relatively constant throughout all periods of embryogeny investigated in this study.

Embryos In Vivo as Controls for Those In Vitro

Not only was the in vivo portion of this study undertaken to learn more about normal embryo development per se, but also to serve as a standard of comparison for embryos developing under culture conditions, so that it might be determined to what extent embryos developing in vitro approximate normal embryogeny. In order to achieve this end, embryos of the in vivo study must include initial stages which are as early in development and of a size equal to or less than the smallest ones which can be excised and placed in culture. Furthermore, if embryos are to be maintained in culture for a two-week period, then those developing in vivo must be

observed throughout a corresponding period of time. It therefore becomes most important to ascertain the extent of development, both in regard to size and stage achieved by embryos *in vivo* during this two-week period.

The smallest embryos which could be excised and placed in culture, with the equipment at hand, averaged 0.5 x 0.3 mm. (ranged from 0.4 - 0.6 mm. in length) initially, and were in a late proembryo stage of development. Of a total of 59 spikes from which embryos were excised for the *in vivo* study, 4 spikes contained embryos which ranged from 0.50 x 0.25 to 0.60 x 0.30 mm. initially, the average of which was found to be 0.55 x 0.30 mm. Their average final size at the end of two weeks was 3.15 x 2.38 mm. which, according to Tables 3 and 4, corresponds well with the average size of embryos which are in stage 6c (3.00 x 2.30 mm.). Comparison of the initial length and the final length results in an increase of 472 percent or a final length which is 5.7 times the initial length, attained at an average rate of 0.37 mm. per two-day intervals. Comparison of the initial and final lateral diameters shows an increase of 693 percent or a final diameter which is 7.9 times the initial, reached at an average rate of 0.30 mm. per two-day interval. The development of these embryos (from the four spikes) provides the basis for comparison of embryos in the *in vitro* study with those growing *in vivo*.

In Vitro Study

As shown in Table 5, Group 1 consisted of 150 embryos cultured for two weeks in plastic cups on the medium containing 1 part of White's basic medium and 9 parts of coconut milk. These embryos were retained on this medium without transfer even after the two week period in order to see whether shoots or roots might be initiated.

Series 1 of Group 1 was comprised of 30 embryos ranging in size, initially, from 0.3 - 0.4 mm. in length, and 0.15 - 0.20 mm. in width in either a late proembryo stage, or stage 1, of development. Of these embryos, 7 showed growth during the two-week period while the others did not enlarge during this time but gradually turned brown. Of the embryos showing growth, the average initial size was 0.35 x 0.20 mm. and the final length and lateral diameter attained during the two weeks, was 0.7 x 0.50 mm., resulting in a doubling of size. Two of these embryos were definitely proembryos (initially) with no suggestion of differentiation when observed with a dissecting microscope. In culture they each developed into a ball-shaped mass of cells and maintained a good white color. One was 0.35 x 0.20 mm. initially, and 0.85 x 0.65 mm. after two weeks, the length increasing by a factor of 2.4 and the lateral diameter, 3.2. The other proembryo was 0.35 x 0.25 mm. initially, and 0.90 x 0.60 mm. after two weeks, length increasing 2 times and lateral diameter 2.4 times. In

both cases, therefore, a greater relative increase occurred laterally than longitudinally.

In series 2 of Group 1, 64 embryos were placed in culture, ranging in initial size from 0.45 x 0.20 mm. to 0.60 x 0.35 mm. These embryos were in stage 1 - 2 of embryogeny. Of these, 36 embryos grew, increasing in size for two weeks and attained an average final size of 1.0 x 0.75 mm., after an initial average size of 0.50 x 0.30 mm. Thus, the final average length increase was 2 times the initial, and the final average lateral diameter was 2.5 times the initial.

Series 3 of Groupll consisted of 56 embryos in stage 3 - 5, and ranged in size from 0.70 - 0.90 mm. Of these, 26 were apparently growing at the end of two weeks. Their average initial length was 0.80 mm. and final average was 1.55 mm., representing a growth increase of 1.9 times in length, while in lateral diameter an increase of 2.3 times was obtained (0.45 mm., initially, and 1.05 mm., finally).

Throughout the three series of cultures described above, none of the embryos gave rise to shoots or roots even though they remained in culture for longer periods of time than two weeks. The low increase in size and the failure of shoots and roots to appear (suggesting a lack of internal differentiation) would certainly indicate that the goal of inducing normal embryogeny had not been attained. In other words, satisfactory environment was not being supplied by

these culture conditions. Therefore, growth rates of these embryos at two-day intervals are not listed individually but are summarized as averages in Table 5. The main purpose in mentioning these results is to call attention to the fact that two proembryos showed increase in size in culture.

As shown in Table 6 and summarized in Table 7, Group 2 consisted of 88 embryos cultured in plastic cups on 1 part of White's basic medium and 9 parts of coconut milk for a period of two weeks, then transferred to vials containing the basic medium only, where they remained for an indefinite period of time or until roots or shoots appeared.

Series 1 of Group 2 consisted of 36 embryos, ranging in size from 0.30 - 0.40 mm. in length and 0.15 - 0.20 mm. in lateral diameter. Most of these embryos were in stage 1 or were late proembryos. After two weeks, 1 embryo of this series was growing well, and after being transferred to the basic medium without coconut milk at the end of this time, continued to develop and eventually (one month from initial placement in culture) produced a normal appearing root (Fig. 9, a, b). This embryo (No. 8 - 6 in Table 6) initially did not show any noticeable sign of the indentation which characterizes, morphologically, the beginning of differentiation. Therefore, this embryo was either a late proembryo or a very early stage 1 and measured 0.40 x 0.25 mm. At the end of the two-week period, this embryo reached a size of 0.85 x 0.65 mm.

Fig. 9. Representative embryos which produced shoots and roots, after being transferred into screw-cap vials.

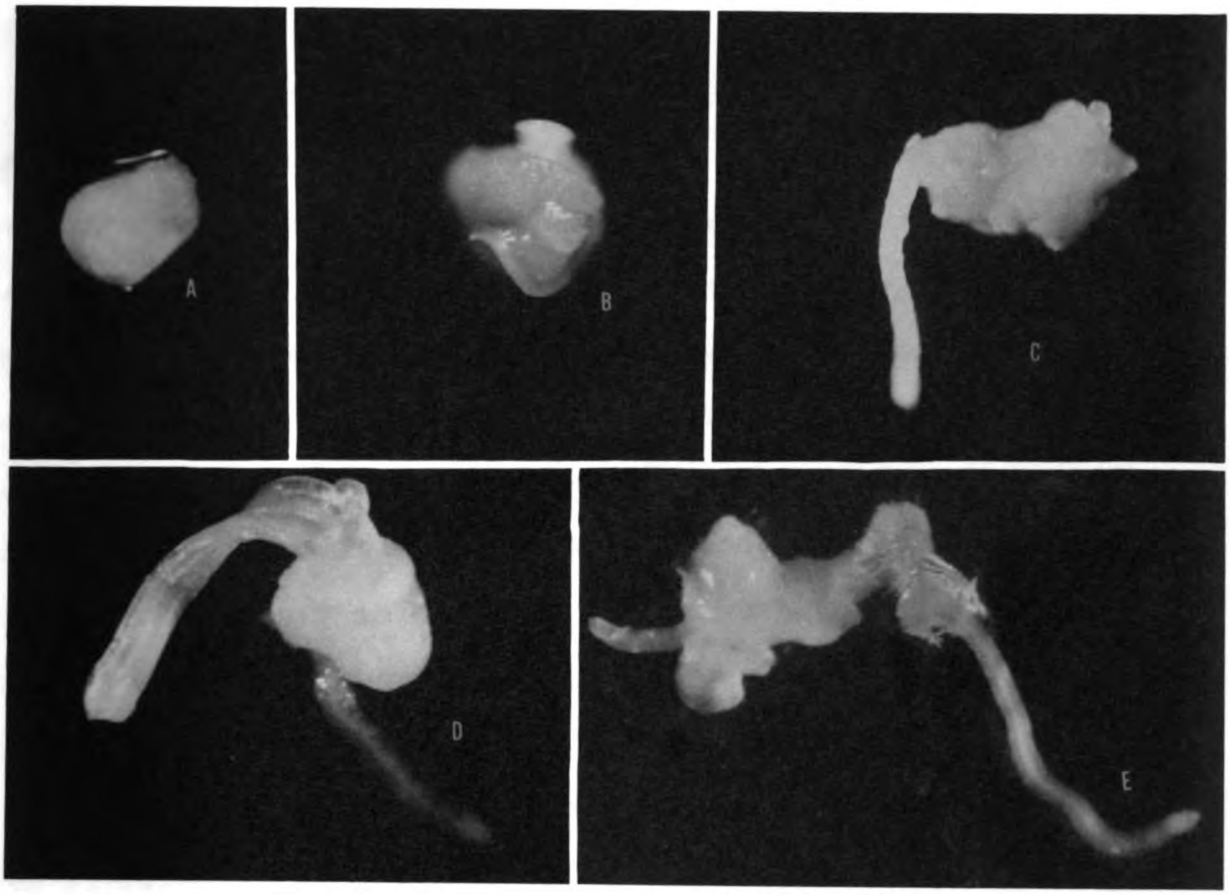
a. Embryo after 29 days in culture. Initial size was 0.40 x 0.20 mm. and final size just before root was formed was 1.80 x 1.35 mm.

b. Embryo No. 8-6 which was the same size as (a) above initially and produced a normal appearing root after 30 days in culture.

c. Embryo which was 0.5 mm. in initial length and formed normal shoot root after 28 days in culture.

d. Embryo with normal shoot and root which were produced after 18 days in culture. The original length of this embryo was 0.70 mm.

e. Excellent normal shoot and root which arose from an embryo 0.55 mm. in initial length after 21 days in culture.


A
B
C
D
E

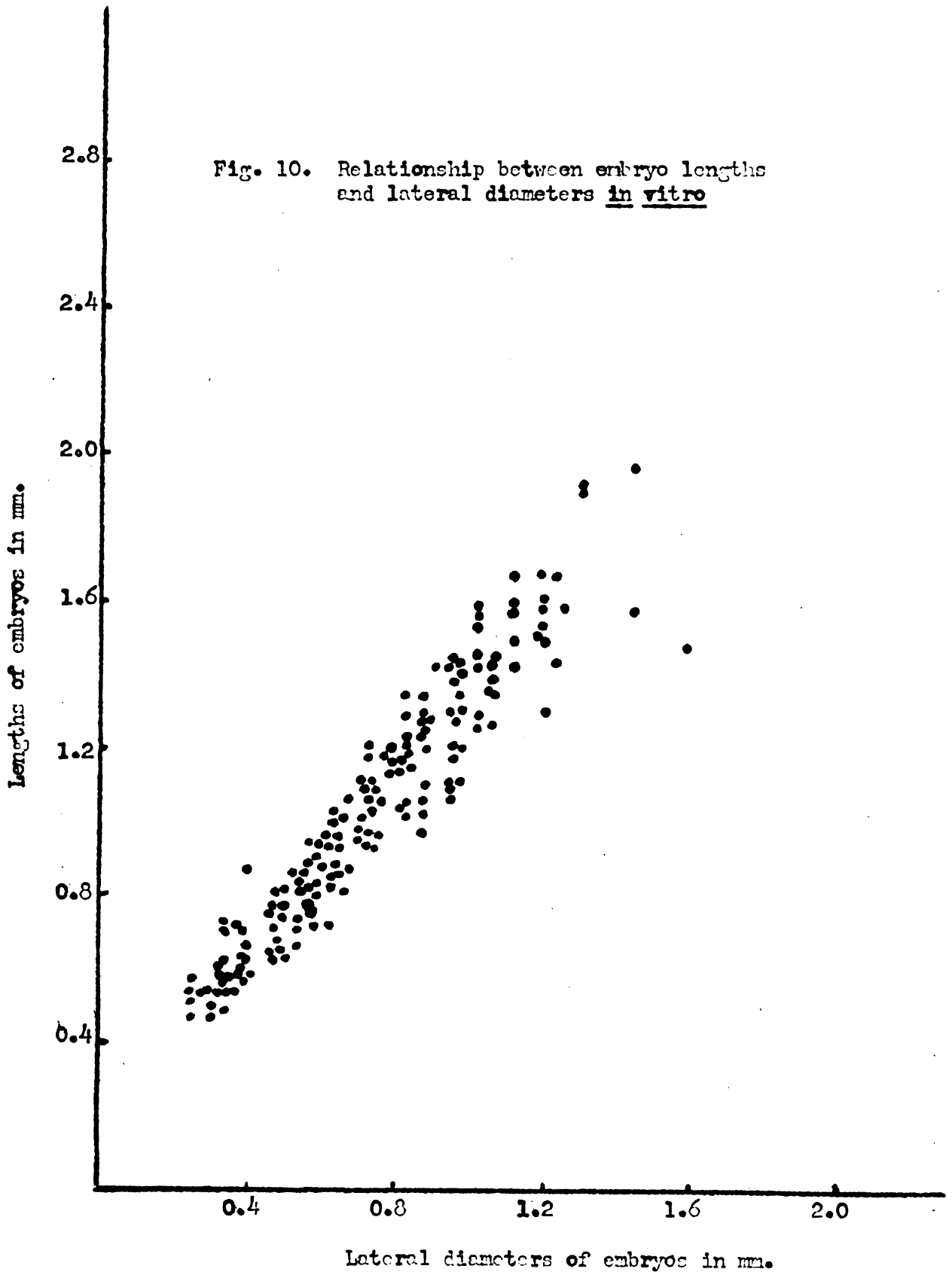
and attained a size of 1.80 x 1.35 mm. just prior to the appearance of the root, giving an increase of 2.1 times in length and 2.6 times in lateral diameter by the end of two weeks, and an increase of 4.5 times in length and 5.4 times in lateral diameter just prior to root formation. Because this embryo was the smallest one to undergo differentiation under culture conditions, it was selected as the representative embryo for evaluation of morphological stages of embryos in culture and for comparison with embryos developing in vivo (as will be discussed later).

Series 2 of Group 2 was comprised of 38 embryos which initially measured 0.45 - 0.60 mm. in length and 0.20 x 0.30 mm. in width, and were in stage 1 - 2 of development. At the end of two weeks, 18 of these embryos were growing and nine of them gave rise to roots or shoots (Table 7). The average initial size of embryos in this series was 0.50 x 0.30 mm. and 1.20 x 0.90 mm. at the end of two weeks; therefore, final length showed an increase of 2.4 times the initial length and lateral diameter increased 3 times. If the initial average size of the cultured embryos is compared with the average size of these embryos one day before the appearance of shoots or roots (the average size attained being 1.80 x 1.40) this gives an increase of 3.6 times in length and 4.6 times in lateral diameter. The average two-day increment in size was a length increase of 0.10 mm. and a lateral diameter increase

of 0.09 mm. The relationships between length and lateral diameter (width) of these embryos, graphed in Figure 10, show essentially a straight line relationship, the length being proportional to width during the two-week culture period. Since a number of these embryos were initially rather small, yet grew well and differentiated in culture, it is assumed that the culture conditions used with the Group 2 embryos were more satisfactory than those used with Group 1. Therefore, the results obtained in series 2 of Group 2 were considered to be good enough in approaching the original aim of the study to warrant comparison with those of the in vivo study.

Series 3 of Group 2 consisted of 14 embryos, ranging in size from 0.70 - 0.90 mm. in length and 0.40 - 0.50 mm. in lateral diameter. Since these embryo sizes were initially too large to consider for the purposes of this work, the results obtained in this series were neglected.

Fig. 10. Relationship between embryo lengths and lateral diameters in vitro



Development of Embryos *In Vitro* with Those *In Vivo*

Morphological Comparison

In order to compare the embryos developing in culture with those developing *in vivo* it is necessary not only to compare the size increases of the embryos in each case, but also to determine the morphological stages and the phases of differentiation through which the cultured embryos have passed. While the morphological features of the various stages of embryogeny are rather specific in the case of embryos developing *in vivo*, the same cannot be said for those in culture. Embryos developing *in vitro* are, in general, not as consistent in their growth patterns as those which develop *in vivo*, and further, depending upon critical environmental conditions such as nutritional and/or atmospheric factors, or perhaps as a result of the mere mechanics of culture techniques, the growth patterns of cultured embryos may vary slightly from one set of cultures to another. This is particularly true of those embryos which are not placed on the agar in such a way that the developing scutellum is in contact with the medium. Thus, orientation of the embryos on the agar medium may be said to have implications in terms of developmental morphology.

The following description of morphology of embryos in culture is based upon observations of *in vitro* embryos in general and embryo No. 8-6 (Table 6), in particular, which as mentioned before, was the smallest embryo to undergo

differentiation in culture. One of the most characteristic features of embryos which develop in culture is that of greater, or precocious, increase in dorsi-ventral diameter. While *in vivo* embryos at stage 1 or state 2 all have a greater length than lateral diameter and little dorsi-ventral diameter, such embryos after being placed in culture show rapid increase in dorsi-ventral diameter even while still in these stages. This increase may be caused by the fact that excised embryos are removed from whatever mechanical restrictions might be otherwise imposed by the caryopsis coat and endosperm tissue. The appearance of the slight indentation, which is characteristic of the first stage (stage 1) of differentiation of the *in vivo* embryos, was never observed in any of the embryos which were placed in culture prior to the differentiation of stage 1. Stage 2 *in vitro* is characterized by the initiation of the coleoptile as an incomplete collar of tissue, as in stage 2, *in vivo*. Stages 3, 4 and 5 of embryos in culture exhibited exceptionally poor differentiation of the scutellum which resulted in the development of a ball-shaped embryo during differentiation stages rather than the typical fan-shaped embryo which develops *in vivo*. In addition, all cultured embryos showed an anomalous formation of the coleoptile, caused by a failure of the lower "lip" (that portion of the coleoptile circle most distant from the scutellum) to elongate at the same rate as the rest of the structure, thereby

resulting in a greatly enlarged coleoptile "pore" through which the shoot emerged prematurely. Norstog (1955) also reported the formation of abnormal coleoptiles in cultured barley embryos. Finally, stage 6 of differentiation, as seen in the *in vivo* embryos, was never found in any of the embryos placed in culture at any stage prior to that stage: instead, embryos in stage 5 *in vitro* sooner or later directly gave rise to shoots or roots.

Rate of Growth, Size and Stage Comparisons

As has already been mentioned, Van Overbeek, *et al* (1941) were the first ones successful in producing seedlings from proembryos in culture (in this case with *Datura*, a dicot). Norstog (1955), has been the most successful thus far in culturing monocot embryos of very small size. He induced small embryos (one as small as 0.16 mm. in length) to form leaves and roots in culture. However, because the smallest embryo also had a number of anomalies, it was suggested by him that this development might actually represent regeneration from callus tissue. It is significant that the small embryos cultured by Norstog are within the size range of the proembryos of the present study. While Norstog mentioned that his smallest embryos did not show outward signs of differentiation, the fact that he did not compare his cultured material to any *in vivo* stages, and only recorded growth increases of cultured embryos at the end of a one-week period,

makes it almost impossible to evaluate his results in the light of the present study. Further, Norstog did not use the same barley variety as was used in this investigation; neither did he describe his growing conditions, nor did he make size-stage relationships so that stages of cultured embryos could be accurately determined. If they are similar to those of Merry (1941, 1942) or to the present study, then Norstog's youngest embryos were definitely proembryos; on the other hand, if the embryo sizes correspond more closely to those of Harlan (1926), then it is more probable that Norstog's youngest embryos were late proembryos or very early stage 1, and, therefore, would be comparable to the smallest embryos successfully cultured in the present work.

Kent and Brink (1947) and Ziebur and Brink (1951), using a six-rowed variety of barley, reported limited success in the culture of a few "proembryos" showing no outward signs of differentiation and measuring 0.3 mm. in length initially. No details of morphological development or growth rates were given, and no comparisons were made with <u>in vivo</u> embryos of the same age.

The work most similar in nature to the present study was that of Merry (1942) who compared growth rates of embryos in culture with those of the same aged embryos <u>in vivo</u>. The only embryos, however, with which Merry had any success in culture were, initially in the mid-stages of differentiation

at the time of placement in culture; therefore his results have only a very limited bearing on the present work.

If the sizes and growth rates of the 38 embryos *in vitro* of Series 2, Group 2 (Table 7) are compared with the 28 embryos *in vivo* of the four spikes (Table 4) which were chosen as the controls for the cultured embryos, the following results are obtained. The *in vitro* embryos averaged 0.50 x 0.30 mm., initially, and attained an average size of 1.2 x 0.9 mm. at the end of two weeks, giving an average length increase of 2.4 times and a lateral diameter increase of 3 times at an average increment per two day interval of 0.10 and 0.09 mm., respectively. The *in vivo* (control) embryos in the same stage of development averaged 0.55 x 0.30 mm., initially, and attained an average size of 3.15 x 2.38 mm. by the end of two weeks, giving an average increase in length of 5.7 times and lateral diameter increase of 7.9 times at an average increment per two-day interval of 0.37 and 0.30 mm., respectively. When the ratios of average final length to average final width are compared, they are seen to be identical (1.3 : 1.0) in both *in vitro* and *in vivo* embryos. The *in vitro* embryos just prior to the appearance of shoots and roots attained an average size of 1.8 x 1.4 mm. and, when compared to the size of *in vivo* embryos at the end of two weeks (by which time full differentiation has occurred), it is seen that again the same ratio of length to width (1.3 : 1.0)

is obtained. Therefore, it may be said that *in vitro* embryos maintain the same length to width relationships (for a two-week period and through as much differentiation as occurs in culture) as takes place *in vivo* (during the same two-week period and throughout differentiation), with merely an overall lack of size increase in the case of the cultured embryos. These relationships are also shown graphically in Figures 4 and 10.

Stages of development of cultured embryos are determined by comparing embryo sizes of the 38 *in vitro* embryos (Series 2 of Group 2) to the morphological development of embryo No. 8-6 as shown in Table 6. Justification for this comparison may be found in the fact that the average final size just before the emergence of shoots or roots of those embryos of the 38 which produced shoots and/or roots was 1.8 x 1.4 mm., while in the case of embryo No. 8-6, it was 1.8 x 1.35 mm. On the basis, then, of sizes and of Table 8, the embryos of Series 2, Group 2, are assumed to have reached stage 3 by the end of two weeks. About 30 days from initial placement in culture, they reached stage 5 and gave rise directly to shoots and/or roots. *In vivo* embryos, on the other hand, although starting out at the same size and stage of development as the cultured embryos, completed differentiation to the end of stage 6 during the same period of time (two weeks) in which *in vitro* embryos were only reaching stage 3.

Embryos, therefore, differentiate more slowly in culture than *in vivo*, taking a longer period of time to pass through each stage and never attain an actual stage 6, morphologically.

Keeping in mind that cultured embryos during the two-week period attain a size which is comparable to stage 6a *in vivo*, yet a morphological stage of only stage 3, it can be said that cultured embryos at any given stage must be larger than the *in vivo* embryos of a comparable stage. Further support for this conclusion can also be found in the fact that cultured embryos just prior to the appearance of shoots and roots attain a size which is comparable to stage 6b *in vivo*, while only reaching stage 5, morphologically. From this, it then follows that the culture techniques used in the present study must actually induce more embryonic growth per stage than occurs *in vivo*. In fact, the length of stage 3 *in vitro* is approximately 1.4 times that of the same stage *in vivo*, and stage 5 *in vitro* is 1.8 times that of stage 5 *in vivo*.

In an effort to discover whether this increased size is due to an increase in cell size or in cell number, histological comparisons were made of embryos of the same size *in vitro* and *in vivo*. When the number of cells per microscope field was determined for *in vitro* and *in vivo* embryos, the cell sizes of the *in vitro* embryos was found, in general, to

be 1.5 times those of the *in vivo* embryos. Cell size differences could, therefore, account for much of the difference in total embryo size, yet there must also be an increase to some extent in the number of cells of cultured embryos. It may be concluded that the nutritive and atmospheric conditions used in the present culture techniques promote both cell enlargement and cell division in cultured embryos over and above that normally occurring *in vivo*. While it is difficult to pinpoint the specific factors in the culture technique which might be responsible for these differences, the fact that embryos cultured on the basic medium plus coconut milk, failed to undergo differentiation, would suggest that the addition of coconut milk to the basic medium was at least one of the critical factors. However, considerably more work is needed along these lines before any more definite conclusions can be reached as to the specific role of an "embryo factor" such as coconut milk.

In conclusion, embryos developing in culture maintain length/width relationships which are identical with, and attain sizes which approach those of *in vivo* embryos. *In vitro* embryos differ, however, from those developing *in vivo* in that cultured embryos are larger at any given morphological stage, are slower in the rate at which they pass through the various stages, show a number of morphological deviations from normal embryogeny, and never attain the morphological development of stage 6.

SUMMARY

1. The growth and development of barley embryos *in vitro* were studied by comparing them to embryos *in vivo* in the following ways: (a) by comparing their morphological development, (b) by comparing their relative sizes and rates of growth, and (c) by relating their growth to stages of embryonic development.

2. The lengths and lateral diameters of developing embryos *in vivo* and *in vitro* are directly proportional to each other.

3. The lengths and lateral diameters of developing caryopses *in vivo* are not well correlated.

4. From the time in late proembryo development until stage 2, only the lengths of the caryopses show a rapid increase, while the lateral diameters of the caryopses and the sizes of embryos *in vivo* undergo little change in measurements during this time. After stage 2, the increase in lengths of the caryopses gradually slow down until the middle of stage 6 is reached. Just prior to stage 5, the lateral diameters of the caryopses undergo rapid increases in size while the embryos increase in size at a somewhat lesser rate. By the middle of stage 6, the lengths of the caryopses become almost constant, while the lateral diameters are still increasing,

but at a much diminished rate. The sizes of embryos within the caryopses, however, are at this time undergoing their most rapid growth.

5. Fresh and dry weights of embryos *in vivo* show an ever increasing relationship to embryo size. A sudden upsurgence of weights begins with the middle of stage 6. Approximately 2/3 of the embryos' fresh weight is due to water, this water content being relatively constant throughout all periods of embryogeny.

6. The *in vivo* embryos, which averaged 0.55 x 0.30 mm., initially, attained an average size of 3.15 x 2.38 mm. by the end of two weeks, giving an average increase in length of 5.7 times and lateral diameter increase of 7.9 times.

7. Cultured embryos, which initially measured 0.45 - 0.60 mm. in length and 0.20 - 0.30 mm. in width were still growing at the end of two weeks and some of them gave rise to roots or shoots. The average final size of these embryos was 1.20 x 0.90 mm. at the end of two weeks; therefore, final length showed an increase of 2.4 times the initial length and the lateral diameter increased 3 times. If the initial average size of these cultured embryos is compared with their average size one day before the appearance of shoots or roots, this gives an increase of 3.6 times in length and 4.6 times in lateral diameter.

8. When the ratios of average final length to average final width are compared, they are seen to be identical (1.3 : 1.0) in both *in vitro* and *in vivo* embryos at the end of two weeks. In addition to this, if the sizes of the *in vitro* embryos just prior to the appearance of shoots and roots are compared to those of *in vivo* embryos at the end of two weeks, it is seen that again the same ratio of length to width (1.3 : 1.0) is obtained. Therefore, it may be said that *in vitro* embryos maintain the same length to width relationships as take place *in vivo*, with merely an overall lack of size increase in the case of the cultured embryos.

9. While the morphological features of the various stages of embryogeny are rather specific in the case of embryos developing *in vivo*, embryos growing *in vitro*, are, in general, not as consistent in their growth patterns.

10. Embryos developing in culture maintain length/width relationships which are identical with, and attain sizes which approach those of *in vivo* embryos. *In vitro* embryos differ, however, from those developing *in vivo* in that cultured embryos are larger at any given morphological stage, are slower in the rate at which they pass through the various stages, show a number of morphological deviations from normal embryogeny, and never attain the morphological development of stage 6.

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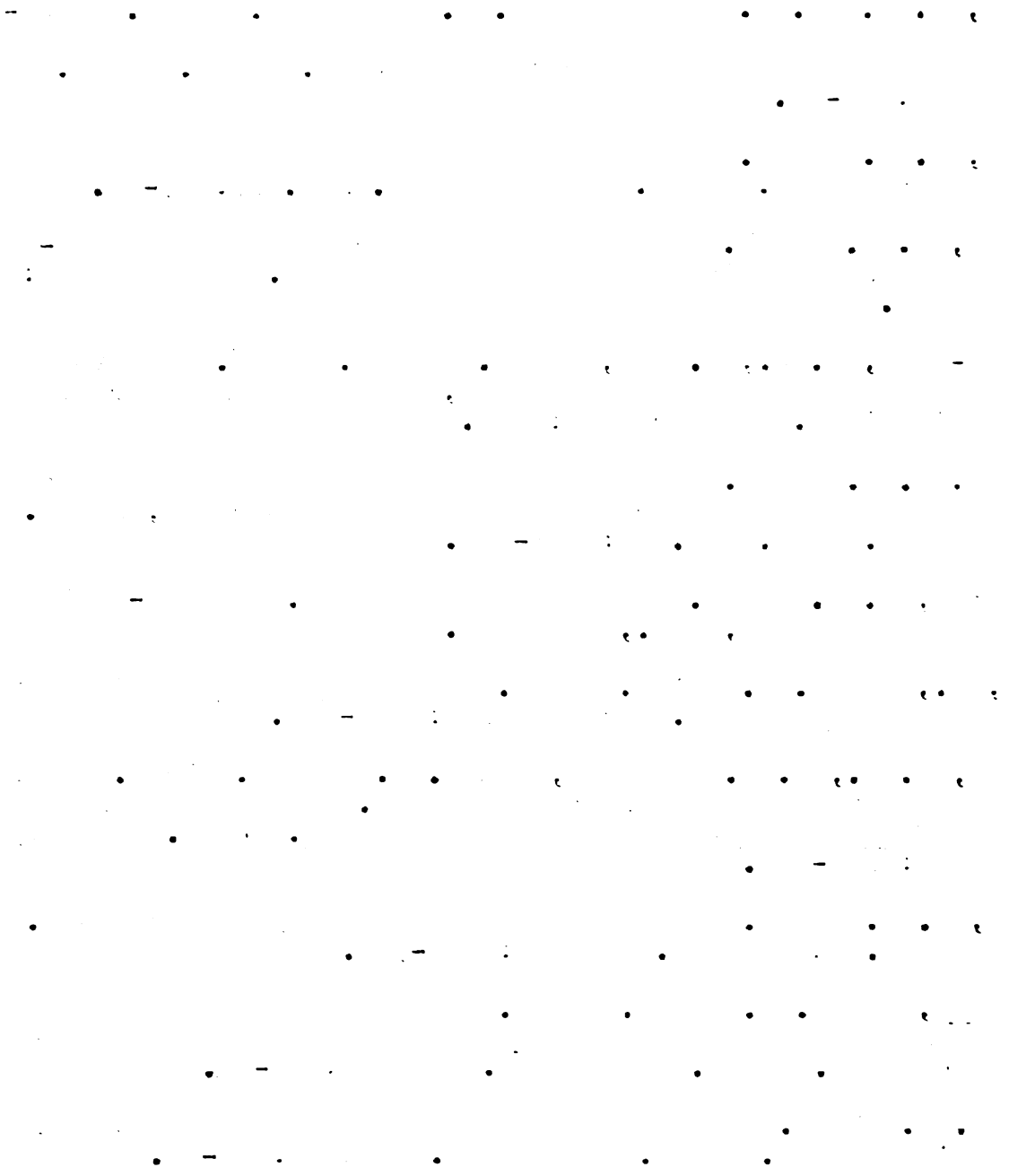

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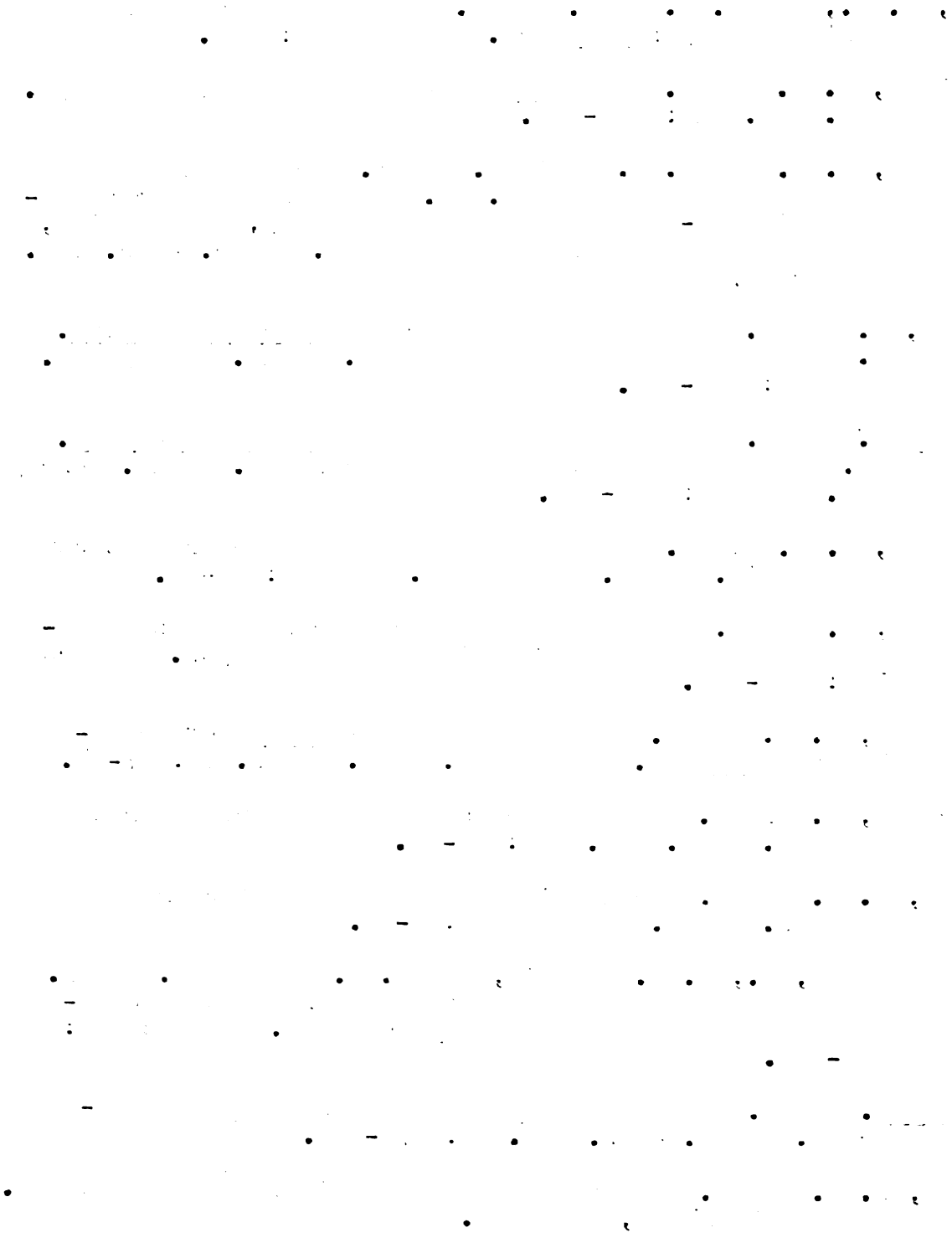

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APPENDIX

Table 1. Individual length, width, fresh weight, and dry weight measurements made at two-day intervals of immature barley embryos developing *in vivo*. (Four basal and four terminal grains of each head were not included.)

Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.
Head No. 1			
0.75	0.35	0.030	-----
0.90	0.65	0.110	0.025
1.75	1.20	0.270	0.055
2.70	1.90	1.515	0.340
3.20	2.30	1.980	0.760
3.35	2.40	2.275	0.880
Head No. 2			
0.55	0.30	0.015	-----
1.15	0.65	0.120	0.040
1.70	1.15	0.230	0.070
2.60	1.85	1.370	0.250
3.00	2.15	1.890	0.390
3.05	2.40	2.260	0.400
Head No. 3			
0.85	0.45	0.040	0.015
1.20	0.90	0.120	0.045
2.10	1.60	0.550	0.155
3.10	2.10	1.805	0.460
Head No. 4			
0.20	0.15	-----	-----
Head No. 5			
0.95	0.55	0.045	0.015
1.00	0.90	0.135	0.025
Head No. 6			
0.25	0.15	0.005	-----
0.75	0.40	0.050	0.020
1.05	0.60	0.080	0.020
1.85	1.40	0.550	0.160
2.45	1.55	0.700	0.255
Head No. 7			
0.20	0.15	0.009	-----
0.95	0.40	0.049	0.025
1.10	0.75	0.150	0.040
1.65	1.20	0.445	0.095
2.90	1.95	1.300	0.350
3.05	2.00	1.735	0.665
3.20	2.10	2.395	0.765
Head No. 8			
0.50	0.25	0.014	-----
0.90	0.75	0.055	0.020
1.50	1.10	0.310	0.045
2.95	2.15	1.675	0.430
Head No. 9			
0.35	0.15	0.028	-----
0.90	0.70	0.050	0.015
1.70	1.20	0.350	0.075
2.40	1.55	0.610	0.319
3.00	2.05	1.560	0.470
3.30	2.25	2.305	0.625
3.65	2.45	3.195	1.080
Head No. 10			
0.35	0.20	0.030	-----
0.90	0.60	-----	-----
1.70	1.15	0.380	0.080
2.35	1.55	0.730	0.190
3.05	2.00	1.375	0.475
3.35	2.15	2.320	0.715
3.40	2.35	2.990	0.910
Head No. 11			
0.35	0.15	0.009	-----
0.80	0.50	0.055	0.015
1.55	1.05	0.305	0.050
2.25	1.50	0.480	0.120
2.65	2.00	1.305	0.270
3.30	2.30	2.260	0.700
3.40	2.55	3.030	0.870
Head No. 12			
0.95	0.65	-----	-----
1.90	1.25	0.350	0.090
2.25	1.65	0.595	0.140
3.20	2.05	1.760	0.610
3.30	2.05	2.190	0.800
Head No. 13			
0.30	0.15	0.005	-----
1.20	0.85	0.120	0.035
1.90	1.40	0.550	0.090
2.10	1.60	0.830	0.140

Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.
Head No. 14			
0.55	0.30	-----	-----
1.15	0.65	0.135	0.025
2.35	1.55	0.735	0.265
Head No. 15			
0.25	0.15	0.005	-----
0.40	0.25	-----	-----
----	----	-----	-----
1.70	1.30	0.360	0.060
2.85	2.00	1.280	0.450
Head No. 16			
0.50	0.25	-----	-----
1.80	1.25	0.370	0.145
2.45	1.80	0.800	0.290
Head No. 17			
0.20	0.15	0.002	-----
0.70	0.35	0.040	0.015
Head No. 18			
0.85	0.55	0.110	0.015
Head No. 19			
0.45	0.25	0.010	-----
----	----	-----	-----
2.25	1.75	0.700	0.255
----	----	-----	-----
3.50	2.45	2.760	0.880
4.25	2.50	3.710	-----
Head No. 20			
0.45	0.25	0.010	-----
----	----	-----	-----
2.25	1.75	0.700	0.255
----	----	-----	-----
3.50	2.45	2.760	0.880
4.25	2.50	3.710	-----
Head No. 21			
1.85	1.30	0.335	0.060
2.50	1.70	0.795	0.210
3.30	2.10	1.990	0.770
3.45	2.35	2.950	0.840
3.70	2.60	3.695	0.940
Head No. 22			
0.20	0.15	0.005	-----
0.60	0.30	0.025	0.005
1.60	1.15	0.340	-----
2.25	1.55	0.760	0.195
2.55	1.90	1.190	0.285
Head No. 23			
0.20	0.15	0.005	-----
0.65	0.35	0.020	0.010
1.35	0.85	0.185	0.035
1.85	1.40	0.490	0.122
----	----	-----	-----
3.25	2.15	2.170	0.870
3.55	2.35	3.070	-----
Head No. 24			
0.85	0.45	0.050	0.020
----	----	-----	-----
2.15	1.60	0.580	0.125
2.70	1.85	1.320	0.380
3.05	2.15	2.010	0.495
3.35	2.20	3.110	0.895
3.40	2.25	3.160	0.900
Head No. 25			
1.05	0.70	0.075	0.025
1.60	1.45	0.500	0.090
Head No. 26			
1.70	1.10	0.285	0.060
2.35	1.65	0.910	0.170
2.75	2.05	1.430	0.295
Head No. 27			
0.65	0.30	0.050	-----
0.75	0.45	0.075	0.015
1.90	1.20	0.360	0.130
2.55	1.75	0.810	0.215
2.90	1.85	1.545	0.375
3.05	2.05	2.055	0.605
Head No. 28			
0.55	0.35	0.035	-----
0.95	0.55	0.055	0.010
1.95	1.35	0.420	0.135
2.45	1.55	0.910	0.195
3.05	2.00	1.575	0.350
3.20	2.10	1.995	0.550
3.30	2.35	2.840	0.750

Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.
Head No. 29			
0.60	0.30	0.060	----
1.00	0.65	-----	-----
1.65	1.25	0.375	0.050
2.05	1.55	0.590	0.110
2.80	1.95	1.605	0.445
3.30	2.35	2.740	0.725
3.35	2.40	3.180	0.850
Head No. 30			
0.35	0.20	0.040	-----
0.75	0.40	0.055	0.015
1.35	0.85	0.200	0.040
2.30	1.55	0.660	0.155
2.95	1.95	1.340	0.350
3.20	2.20	2.055	0.445
Head No. 31			
0.35	0.20	-----	-----
0.95	0.55	0.070	0.030
1.50	1.10	0.280	0.090
2.25	1.60	0.810	0.155
3.05	2.00	1.650	0.380
3.20	2.20	2.585	0.730
3.45	2.50	2.850	0.950
3.70	2.50	3.905	0.970
Head No. 32			
0.12	0.06	-----	-----
0.50	0.25	-----	-----
0.85	0.55	0.045	0.020
1.85	1.30	0.400	0.085
2.25	1.75	0.875	0.175
3.00	2.05	1.505	0.320
Head No. 33			
0.20	0.11	-----	-----
0.65	0.30	0.040	0.010
1.20	0.85	0.155	0.030
1.60	1.10	0.300	0.055
2.05	1.40	0.895	0.140
Head No. 34			
0.35	0.20	-----	-----
0.55	0.30	0.065	0.010
1.20	0.85	0.125	0.025
2.20	1.70	0.705	0.170
3.10	2.10	1.625	0.355
Head No. 35			
0.75	0.40	0.025	-----
----	----	-----	-----
2.55	1.80	0.930	0.225
2.70	1.95	1.395	0.465
3.40	2.05	2.355	0.735
Head No. 36			
0.35	0.20	0.020	-----
1.00	0.55	0.085	0.025
1.45	1.00	0.180	0.050
2.25	1.65	0.800	0.210
2.65	1.90	1.295	0.320
2.75	2.05	1.565	0.485
Head No. 37			
0.27	0.15	0.005	-----
0.60	0.30	0.020	0.005
1.25	0.85	0.105	0.035
1.90	1.35	0.400	0.085
2.55	1.80	0.985	0.230
3.05	2.05	1.710	0.365
Head No. 38			
0.50	0.25	0.020	-----
0.90	0.60	0.050	0.015
1.70	1.20	0.315	0.085
2.25	1.75	0.865	0.205
2.70	1.90	1.355	0.320
2.75	2.05	1.755	0.415
2.85	2.20	2.195	0.615
Head No. 39			
0.35	0.20	0.045	-----
1.05	0.70	0.085	0.045
1.35	1.10	0.195	0.065
2.25	1.65	0.690	0.155
2.50	1.85	1.080	0.260
3.10	2.05	2.000	0.530
Head No. 40			
0.50	0.25	0.040	0.010
0.90	0.70	0.065	0.020
1.80	1.25	0.400	0.095
2.30	1.75	0.720	0.230
2.80	2.05	1.615	0.400

Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.
Head No. 41			
1.95	1.25	0.225	0.080
3.15	1.85	1.135	0.480
3.30	2.15	1.920	0.910
Head No. 42			
1.15	0.75	0.165	0.030
2.15	1.40	0.505	0.110
2.65	1.75	1.150	0.320
3.00	2.00	1.900	0.480
3.10	2.20	2.395	0.595
Head No. 43			
0.25	0.15	-----	-----
0.65	0.35	0.010	0.015
1.30	0.85	0.070	0.050
1.90	1.35	0.395	0.085
2.20	1.65	0.635	0.145
2.80	2.05	1.455	0.345
Head No. 44			
1.00	0.65	0.050	-----
1.85	1.45	0.475	0.120
----	----	-----	-----
----	----	-----	-----
3.20	2.35	2.565	0.660

Table 2. Individual length, width, fresh weight, and dry weight measurements made at two-day intervals of barley embryos and caryopses developing *in vivo*. (Four basal and four terminal grains of each head were not included.)

	Embryos				Caryopses	
	Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.	Length in mm.	Width in mm.
Head No. 1						
	0.16	0.08	-----	-----	5.92	1.76
	0.16	0.08	-----	-----	5.60	1.76
	0.16	0.08	-----	-----	5.60	1.76
	0.56	0.32	0.025	-----	8.60	2.08
	0.48	0.32	0.020	-----	8.00	2.08
	0.56	0.32	0.020	-----	8.16	2.24
	1.12	0.80	0.140	0.025	8.96	2.56
	1.04	0.80	0.125	0.020	8.96	2.56
	1.12	0.80	0.150	0.030	8.96	2.72
	2.00	1.28	0.620	0.110	9.76	4.16
Head No. 2						
	----	----	-----	-----	4.64	1.68
	----	----	-----	-----	4.64	1.68
	----	----	-----	-----	4.64	1.60
	0.35	0.18	0.010	-----	7.36	2.08
	0.35	0.16	0.010	-----	7.36	2.08
	0.40	0.18	0.015	-----	7.20	2.08
	0.32	0.16	0.010	-----	7.36	2.08
	1.12	0.72	0.110	0.025	8.48	2.72
	1.12	0.72	0.100	0.030	8.48	2.72
	0.96	0.64	0.095	0.020	8.48	2.56
	1.60	1.28	0.400	0.075	9.60	3.20
	1.92	1.28	0.625	0.090	9.60	3.36
	2.88	1.92	1.635	0.335	9.60	3.84
	3.04	1.92	1.910	0.370	9.60	4.00
	2.88	1.92	1.350	0.310	9.60	3.84
Head No. 3						
	0.27	0.18	-----	-----	6.40	1.92
	0.27	0.18	-----	-----	6.40	1.92
	0.27	0.18	-----	-----	6.56	1.92
	0.64	0.32	0.035	-----	8.16	2.08
	0.72	0.40	0.035	-----	8.96	2.24
	0.64	0.32	0.040	-----	8.64	2.24
	1.44	1.12	0.220	0.035	9.12	3.00
	1.44	0.96	0.210	0.030	9.28	3.00
	1.44	0.96	0.250	0.035	9.28	3.20
	2.40	1.60	1.015	0.165	9.60	3.50
	3.04	2.08	1.875	0.495	9.60	3.84

	Embryos				Caryopses	
	Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.	Length in mm.	Width in mm.
Head No. 4						
	0.56	0.32	0.030	-----	8.00	2.24
	0.65	0.32	0.035	-----	8.48	2.24
	0.48	0.24	0.015	-----	7.84	2.08
	0.64	0.32	0.030	-----	7.84	2.08
	0.48	0.32	0.015	-----	8.00	2.08
	1.28	0.80	0.185	0.035	8.64	2.56
	1.28	0.80	0.175	0.030	8.64	2.56
	1.12	0.80	0.145	0.025	8.64	2.56
	2.08	1.28	0.625	0.090	9.60	3.52
	2.72	1.76	1.285	0.245	9.76	3.68
Head No. 5						
	0.80	0.48	0.095	-----	8.80	2.24
	0.80	0.40	0.075	-----	8.64	2.24
	1.44	0.96	0.210	0.030	9.12	2.88
	1.44	0.96	0.240	0.035	8.96	2.72
	2.24	1.28	0.610	0.110	9.44	3.00
	2.23	1.29	0.620	0.125	9.44	3.00
	2.72	1.76	1.385	0.270	9.44	3.36
	2.89	2.08	2.170	0.500	9.44	3.84
	3.20	2.40	3.260	0.970	9.44	4.00
Head No. 6						
	0.32	0.16	0.010	-----	6.88	1.92
	0.32	0.16	0.015	-----	6.88	1.92
	0.32	0.16	0.015	-----	7.04	1.92
	0.88	0.64	0.095	0.020	8.96	2.43
	0.96	0.64	0.100	0.020	8.96	2.40
	0.96	0.64	0.100	0.030	8.96	2.45
	1.76	1.12	0.445	0.070	9.44	3.00
Head No. 7						
	1.28	0.88	0.155	0.025	8.64	2.72
	1.12	0.80	0.145	0.030	8.80	2.72
	1.92	1.28	0.450	0.090	8.64	3.00
	2.08	1.44	0.580	0.100	8.80	3.20
	2.56	1.12	1.275	0.305	8.80	3.36
	2.88	1.12	1.800	0.350	8.80	3.52
	3.20	2.08	2.798	0.828	8.96	3.84
	3.20	2.24	3.350	1.075	9.12	4.00
Head No. 8						
	0.06	0.03	-----	-----	3.68	1.60
	0.08	0.05	-----	-----	4.48	1.12
	0.08	0.05	-----	-----	4.48	1.12
	0.48	0.24	0.020	0.010	7.68	2.08
	0.48	0.24	0.030	0.010	7.68	2.24
	0.64	0.32	0.030	0.015	8.16	2.21
	1.20	0.80	0.155	0.030	8.96	2.57

Embryos				Caryopses	
Length in mm.	Width in mm.	Fresh Wt. in mg.	Dry Wt. in mg.	Length in mm.	Width in mm.
Head No. 8 (continued)					
0.96	0.66	0.100	0.025	8.64	2.40
2.23	1.44	0.630	0.100	9.28	3.20
Head No. 9					
0.64	0.32	0.035	-----	8.32	2.08
0.64	0.32	0.030	-----	8.32	2.08
1.04	0.72	0.115	-----	8.64	2.56
1.04	0.64	0.115	-----	8.64	2.40
1.44	0.96	0.245	0.050	9.12	2.56
1.60	1.12	0.290	0.075	9.28	2.88
2.56	1.60	1.100	0.255	9.44	3.52
2.88	1.92	1.920	0.460	9.44	3.84
Head No. 10					
0.11	0.01	-----	-----	4.00	1.60
0.13	0.01	-----	-----	4.48	1.60
0.13	0.01	-----	-----	4.96	1.12
0.56	0.24	0.015	-----	7.52	2.08
0.64	0.32	0.035	-----	7.52	2.24
0.56	0.32	0.020	-----	7.68	2.08
1.04	0.64	0.115	0.035	8.80	2.24
1.04	0.64	0.130	0.030	8.80	2.24
Head No. 11					
0.64	0.32	0.040	-----	7.84	2.23
0.72	0.40	0.045	-----	8.16	2.23
1.04	0.64	0.115	0.020	8.96	2.56
1.12	0.72	0.110	0.020	8.96	2.56
1.76	1.12	0.345	0.080	9.12	3.20
1.76	1.28	0.365	0.085	9.12	3.20
2.56	1.60	0.990	0.240	9.28	3.36
2.88	2.23	2.410	0.450	9.28	3.50
Head No. 12					
----	----	-----	-----	3.52	1.60
----	----	-----	-----	3.52	1.60
----	----	-----	-----	3.84	1.60
0.48	1.60	0.025	-----	7.36	2.24
0.36	1.60	0.020	-----	7.20	2.08
0.40	1.60	0.025	-----	7.20	1.92
0.96	0.64	0.100	-----	8.48	2.56
1.04	0.64	0.110	-----	8.48	2.72
2.08	1.44	0.655	0.100	9.76	3.68
1.92	1.28	0.495	0.080	9.76	3.68

Table 3. Average size, fresh and dry weights of embryos (from Table 1 and Table 2) and average size of caryopses (from Table 2) related to histologically determined stages of embryonic development.

Stage of embryo	Size of embryo	Fresh weight of embryo	Dry weight of embryo	Size of caryopsis
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
D	-	-	-	-
E	-	-	-	-
F	0.25 x 0.15	0.005	-	5.5 x 1.9
G	0.30 x 0.15	0.005	-	6.0 x 1.9
G-1	0.35 x 0.20	0.015	-	6.0 x 1.9
1	0.40 x 0.20 (0.45 x 0.20)	0.025	0.005	7.0 x 2.0
2	0.50 x 0.25 (0.60 x 0.30)	0.035	0.010	8.0 x 2.0
3	0.70 x 0.35	0.045	0.010	8.3 x 2.1
4	0.80 x 0.40	0.055	0.015	8.5 x 2.2
5	0.90 x 0.50	0.075	0.020	8.6 x 2.3
6a	1.20 x 0.80	0.150	0.045	9.2 x 2.7
6b	1.80 x 1.20	0.450	0.075	9.6 x 3.7
6c	3.00 x 2.30	2.500	0.850	9.7 x 3.9

Table 4. Individual length and width measurements of barley embryos developing *in vivo* made at two-day intervals from four representative spikes.

Embryos of Spikes	Days *in vivo* 0	2	4	6	8	10	12
1	0.55	1.15	1.70	2.60	3.00	3.05	3.10
	0.30	0.65	1.15	1.85	2.15	2.40	2.60
2	0.55	0.95	1.95	2.45	3.05	3.20	3.30
	0.35	0.55	1.35	1.55	2.00	2.10	2.35
3	0.60	1.00	1.65	2.05	2.80	3.30	3.35
	0.30	0.65	1.25	1.55	1.95	2.35	2.40
4	0.50	0.90	1.70	2.25	2.70	2.75	2.85
	0.25	0.60	1.20	1.75	1.90	2.05	2.25
Average	0.55	1.00	1.75	2.33	2.88	3.07	3.15
	0.30	0.60	1.23	1.67	2.00	2.22	2.38

Table 5. Culture group 1. Average growth rate of embryos cultured continuously on basic medium plus coconut milk (no culture transfers).

	Series 1	Series 2	Series 3
No. of embryos cultured	30	64	56
No. of embryos growing at the end of two weeks	7	36	26
Average initial size in mm.	0.35 x 0.20	0.50 x 0.30	0.80 x 0.45
Average final size in mm.	0.70 x 0.50	1.00 x 0.75	1.55 x 1.05
Percent increase in size	100 x 150	100 x 150	94 x 133

Table 6. Culture group 2. Ratio of length/width (in mm.) of immature barley embryos developing in culture, including total days in culture (d) and day (d) on which shoot or root first appeared. One part basic medium (White, 1954) plus nine parts coconut milk was used as the culture medium for two weeks, after which embryos were transferred to basic medium only (without coconut milk).

	Days in culture								
Embryo	0	2	4	6	8	10	12	Total	Remarks
Culture No. 1									
1	0.50	0.65	0.85	0.90	1.00	1.15	1.30	1.65	(26d) Shoot
	0.35	0.45	0.60	0.65	0.75	0.90	0.95	1.20	(28d)
2	0.70	0.75	0.90	1.05	1.15	1.25	1.40	1.75	(26d) Shoot
	0.40	0.45	0.65	0.70	0.80	0.90	1.00	1.30	(28d)
3	0.55	0.70	0.85	0.90	1.00	1.10	1.20	1.40	(20d)
	0.25	0.40	0.55	0.60	0.65	0.75	0.85	1.00	
4	0.70	0.75	0.90	0.95	1.00	1.05	1.15	2.00	(26d) Shoot
	0.35	0.45	0.55	0.65	0.70	0.75	0.85	1.30	(28d)
5	0.25	0.30	—	—	—	—	—	—	
	0.15	0.15							
6	—	—	—	—	—	—	—	—	
7	0.50	0.60	0.70	0.80	0.90	0.95	—	—	
	0.30	0.40	0.50	0.55	0.60	0.65			
8	0.90	1.10	1.25	1.30	1.45	1.55	1.75	1.90	(20d) Root
	0.50	0.70	0.95	1.10	1.25	1.35	1.50	1.60	(22d)
9	0.95	1.30	1.60	1.80	1.95	2.05	2.40	2.55	(23d)
	0.70	0.90	1.65	1.30	1.40	1.50	1.60	1.65	
Culture No. 2									
1	0.45	0.55	0.65	0.70	0.80	0.85	1.00	1.10	(18d)
	0.30	0.35	0.50	0.55	0.60	0.65	0.75	0.95	
2	0.65	0.80	0.95	1.10	1.25	1.40	1.45	1.65	(21d) Shoot
	0.35	0.45	0.60	0.65	0.75	0.85	0.95	1.25	(26d)
3	0.25	0.25	—	—	—	—	—	—	
	1.15	0.20							
4	0.95	1.20	1.40	1.55	1.75	1.90	2.00	2.20	(21d) Shoot
	0.65	0.75	0.95	1.10	1.25	1.40	1.50	1.70	(23d)
5	0.45	0.65	0.80	0.90	1.00	1.10	—	—	
	0.30	0.40	0.50	0.55	0.65	0.70			
6	0.30	—	—	—	—	—	—	—	
	0.15								
7	0.70	0.80	0.90	0.95	1.00	1.05	1.15	1.40	(23d) Shoot
	0.35	0.45	0.55	0.65	0.75	0.80	0.90	1.20	(26d)
8	0.40	0.40	—	—	—	—	—	—	
	0.20	0.20							
9	0.25	0.30	—	—	—	—	—	—	
	0.15	0.20							

Embryo	Days in culture 0	2	4	6	8	10	12	Total	Remarks
Culture No. 3									
1	0.25	—	—	—	—	—	—	—	
	0.15								
2	0.55	0.60	0.65	0.65	0.70	0.75	0.90	—	
	0.35	0.35	0.40	0.45	0.50	0.55	0.60		
3	0.65	0.85	1.00	1.10	1.15	1.25	1.40	—	
	0.35	0.50	0.70	0.75	0.80	0.85	0.85		
4	0.60	0.80	0.90	0.95	1.15	1.25	1.30	—	
	0.40	0.50	0.60	0.65	0.75	0.80	0.85		
5	0.90	1.10	1.30	1.60	1.85	2.00	2.10	—	
	0.55	0.70	0.90	1.05	1.15	1.20	1.30		
6	0.60	0.75	0.90	1.00	1.15	1.35	1.50	—	
	0.35	0.35	0.60	0.65	0.75	0.90	1.00		
7	0.55	0.65	0.80	0.90	0.95	1.05	1.15	1.60	(27d)
	0.25	0.40	0.50	0.55	0.60	0.65	0.75	1.10	
8	0.60	0.70	0.85	0.90	1.00	1.05	1.50	—	
	0.25	0.45	0.60	0.70	0.80	0.90	1.00		
9	0.90	0.12	1.40	1.50	1.65	1.85	2.00	2.20	(18) Root
	0.55	0.70	0.85	0.95	1.00	1.10	1.40	2.00	(20d)
Culture No. 4									
1	0.60	0.80	0.90	0.95	1.05	1.15	1.25	1.55	(30d) Shoot
	0.40	0.45	0.55	0.60	0.65	0.75	0.85	1.65	(32d)
2	0.25	—	—	—	—	—	—	—	
	0.15								
3	0.50	—	—	—	—	—	—	—	
	0.25								
4	0.35	0.45	0.45	0.50	—	—	—	—	
	0.25	0.40	0.45	0.45					
5	0.95	1.20	1.35	1.50	—	—	—	—	
	0.55	0.75	0.85	0.90					
6	0.60	0.70	0.80	0.85	—	—	—	—	
	0.30	0.40	0.55	0.60					
7	0.40	0.55	0.65	0.70	—	—	—	—	
	0.25	0.40	0.50	0.55					
8	0.55	0.70	0.90	1.00	1.15	—	—	—	
	0.35	0.40	0.55	0.65	0.75				
Culture No. 5									
1	0.90	1.20	—	—	—	—	—	—	
	0.60	0.75							
2	0.55	0.55	—	—	—	—	—	—	
	0.25	0.30							
3	0.95	1.20	1.45	1.60	—	—	—	—	
	0.65	0.80	1.05	1.20					
4	0.90	0.65	—	—	—	—	—	—	
	0.20	0.40							
5	0.60	0.75	0.90	—	—	—	—	—	
	0.35	0.45	0.50						
6	0.50	0.55	0.65	—	—	—	—	—	
	0.30	0.30	0.40						

Embryo	Days in culture 0	2	4	6	8	10	12	Total	Remarks
Culture No. 6									
1	0.45 / 0.25	—	—	—	—	—	—	—	
2	0.50 / 0.30	—	—	—	—	—	—	—	
3	0.55 / 0.30	0.75 / 0.40	0.90 / 0.55	0.95 / 0.65	1.05 / 0.70	1.20 / 0.75	1.30 / 0.85	—	
4	0.30 / 0.20	—	—	—	—	—	—	—	
5	0.40 / 0.25	—	—	—	—	—	—	—	
6	0.30 / 0.20	—	—	—	—	—	—	—	
Culture No. 7									
1	0.80 / 0.35	1.05 / 0.65	1.20 / 0.80	1.35 / 1.00	1.45 / 1.00	1.55 / 1.05	1.60 / 1.10	—	
2	1.00 / 0.60	1.20 / 0.75	1.40 / 0.90	1.60 / 1.05	—	—	—	—	
3	0.70 / 0.35	0.90 / 0.50	1.00 / 0.60	1.05 / 0.65	1.05 / 0.65	1.05 / 0.70	1.10 / 0.70	—	
4	0.30 / 0.15	—	—	—	—	—	—	—	
5	0.65 / 0.35	0.80 / 0.45	0.90 / 0.55	0.95 / 0.65	1.05 / 0.65	1.15 / 0.70	1.20 / 0.80	—	
Culture No. 8									
1	0.35 / 0.20	0.45 / 0.20	0.45 / 0.25	0.50 / 0.35	0.50 / 0.45	—	—	—	
2	0.35 / 0.20	0.35 / 0.25	0.35 / 0.25	0.40 / 0.25	—	—	—	—	
3	0.30 / 0.15	0.35 / 0.15	—	—	—	—	—	—	
4	0.15 / 0.10	—	—	—	—	—	—	—	
5	—	—	—	—	—	—	—	—	
6	0.40 / 0.25	0.60 / 0.35	0.60 / 0.40	0.65 / 0.50	0.75 / 0.55	0.80 / 0.60	0.85 / 0.65	1.80 / 1.35	(30d) Root (32d)
7	0.40 / 0.25	0.60 / 0.35	0.70 / 0.45	—	—	—	—	—	
8	0.40 / 0.20	0.50 / 0.25	0.55 / 0.35	0.60 / 0.45	0.65 / 0.50	—	—	—	
9	0.30 / 0.20	0.60 / 0.35	0.70 / 0.40	0.75 / 0.40	—	—	—	—	

Embryo	Days in culture 0	2	4	6	8	10	12	Total	Remarks
Culture No. 9									
1	0.25	0.25	—	—	—	—	—	—	
	0.20	0.20							
2	0.55	0.75	0.85	0.90	0.90	0.95	0.95	1.05	(31d) Shoot
	0.30	0.40	0.55	0.60	0.65	0.65	0.70	0.70	(33d)
3	0.35	0.40	0.45	—	—	—	—	—	
	0.25	0,25	0.25						
4	0.35	0.35	0.45	—	—	—	—	—	
	0.25	0.25	0.30						
5	0.40	0.55	0.65	—	—	—	—	—	
	0.25	0.30	0.40						
6	0.45	0.60	0.75	0.85	0.95	1.00	—	—	
	0.25	0.35	0.55	0.55	0.65	0.75			
7	0.40	0.45	0.55	—	—	—	—	—	
	0.20	0.20	0.25						
8	0.35	0.35	0.40	—	—	—	—	—	
	0.20	0.20	0.20						
Culture No. 10									
1	0.50	0.50	0.50	—	—	—	—	—	
	0.25	0.30	0.30						
2	0.45	0.55	0.65	—	—	—	—	—	
	0.25	0.30	0.40						
3	0.45	0.55	0.65	0.70	—	—	—	—	
	0.20	0.35	0.45	0.50					
4	0.40	0.45	0.50	0.60	0.75	—	—	—	
	0.25	0.25	0.35	0.45	0.55				
5	0.50	0.75	0.90	0.90	0.95	1.00	1.10	2.45	(28d) Root
	0.30	0.40	0.40	0.60	0.75	0.90	0.95	1.25	(30d)
6	0.35	0.40	0.40	—	—	—	—	—	
	0.20	0.20	0.25						
7	0.55	0.80	0.90	1.00	1.10	1.25	1.40	1.95	(21d) Root
	0.35	0.50	0.65	0.75	0.85	0.95	1.00	1.35	(23d)
Culture No. 11									
1	0.30	0.35	0.40	—	—	—	—	—	
	0.20	0.20	0.20						
2	0.40	0.50	0.50	—	—	—	—	—	
	0.20	0.30	0.35						
3	0.45	0.45	0.50	—	—	—	—	—	
	0.20	0.25	0.25						
4	0.35	0.50	0.55	0.60	—	—	—	—	
	0.20	0.35	0.35	0.40					
5	0.45	0.60	0.65	—	—	—	—	—	
	0.30	0.35	0.35						
6	0.35	—	—	—	—	—	—	—	
	0.25								

Embryo	Days in culture 0	2	4	6	8	10	12	Total	Remarks
Culture No. 12									
1	0.45 / 0.25	—	—	—	—	—	—	—	
2	0.45 / 0.25	—	—	—	—	—	—	—	
3	0.35 / 0.20	—	—	—	—	—	—	—	
4	0.40 / 0.25	0.55 / 0.35	0.65 / 0.35	0.70 / 0.45	0.80 / 0.55	—	—	—	
5	0.50 / 0.30	0.75 / 0.35	0.85 / 0.45	0.85 / 0.60	0.95 / 0.65	1.00 / 0.75	1.05 / 0.85	1.65 / 1.50	(31d) Shoot (33d)
6	0.55 / 0.30	—	—	—	—	—	—	—	
7	0.40 / 0.20	—	—	—	—	—	—	—	
8	0.25 / 0.20	—	—	—	—	—	—	—	

Table 7. Summary of three series (based upon sizes) from table 6.

	Series 1	Series 2	Series 3
No. of embryos cultured	36	38	14
No. of embryos grown for two weeks	1	18	10
No. of embryos formed shoots or roots	1	9	6
Average initial size (length x width) in mm.	0.35 x 0.20	0.50 x 0.30	0.80 x 0.45
Average final size (length x width) in mm.	0.85 x 0.65 (for two weeks)	1.20 x 0.90 (for two weeks)	1.82 x 1.12 (for two weeks)
	1.80 x 1.35 (for total days)	1.80 x 1.40 (for total days)	2.20 x 1.85 (for total days)
	112 x 225 (for two weeks)	140 x 300 (for two weeks)	127 x 148 (for two weeks)
Percent increase in size	350 x 575 (for total days)	260 x 366 (for total days)	175 x 311 (for total days)

Table 8. Growth characteristics of a "typical" immature barley embryo (No. 8-6 of Table 6) in culture, at two-day intervals, for 30 days.

Days in culture	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	
Average size in mm. (length x width)	0.40 0.25	0.60 0.35	0.60 0.40	0.65 0.50	0.75 0.55	0.80 0.60	0.85 0.65	0.85 0.65	0.90 0.70	1.00 0.80	1.25 0.90	1.30 1.00	1.40 1.10	1.45 1.15	1.50 1.12	1.80 1.35	root
Stage	stage - 1				stage - 2		stage - 3					stage - 4			stage - 5		
Duration of stage	6 days				4 days		10 days					6 days			4 days		
Overall average size in mm. (length/ width)	0.56 0.37				0.78 0.58		0.97 0.74					1.38 1.07			1.65 1.23		