NASA CONTRACTOR REPORT

NASA CR-303

EFFECT OF WEIGHTLESSNESS AND RADIATION ON THE GROWTH OF THE WHEAT COLEOPTILE FOR THE PURPOSE OF DEFINING AND VERIFYING AN EXPERIMENT SUITABLE FOR USE IN A BIOSATELLITE

by Stephen Gray and Betty F. Edwards

Prepared under Grant No. NsG-521 *by*
EMORY UNIVERSITY
Atlanta, Ga.
for

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • SEPTEMBER 1965

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I - INTRODUCTION

This report concerns our investigation into the influence of gravity and the possible influence of weightlessness on the growth of the wheat seedling.

The influence of gravity as a factor controlling growth is difficult to assess. Response to gravity produces the characteristic orientation of the majority of plants and animals. The force of gravity may be simulated and greatly increased by centrifugation, but it is difficult to simulate a decrease below normal gravity.

It is possible to neutralize the effects of gravity on a growing plant by means of the clinostat, but actual reduction of gravitational force can only be produced for a few seconds in the laboratory or an airplane. Weightlessness, a theoretical zero gravity, can only be achieved in an earth orbiting satellite free from spin. We feel very priviledged, therefore, to have been given the opportunity to prepare an experiment for the first Biosatellite to be launched by the National Aeronautics and Space Administration.

Experimental investigations measuring response to variations of gravity are few compared to those measuring response to other environmental factors.

The earliest reference to an investigation of the effects of gravity in relation to growth and form is that of Thomas Andrew Knight in 1806. He devised a machine to neutralize the force of gravity acting on germinating seeds and young plants by rotating the plant in its container while in a horizontal position and simultaneously rotating the whole plant in an orbit about a central axis by means of water power. He had not eliminated the force of gravity, but had made it omnidirectional. He reported that the epicotyl and radicle grew without geotropic orientation in his device, and surmised that the normal appearance of plants is due to the influence of earth's gravity on the sap. Sachs in 1872 used a similar horizontal clinostat to study plant form and movement. He noted that the plant axis grew always in the direction in which it was

originally placed. It is interesting that Lyon (1962, 1963) is currently conducting experiments on a similar clinostat built on a much larger scale in which he reports that the angle of leaf to stem in several dicotyledonous plants is definitely altered as the tropistic effect of gravity is eliminated on the elongating internodes.

Hertwig, in 1899, appears to have been the first to use low speeds of the centrifuge to observe the effects of an increased gravitational force on growth and development. He used centrifugal forces of 3 to 6 G on developing frog eggs. Slowing of the developmental period was observed, and inhibition of normal pattern resulting in death at a force of 9G. In similar experiments, Gray and Webb (1950) centrifuged developing eggs of the toad at forces from 1.4 to 6 G for varying periods. Animals centrifuged for 10 days at 6 G exhibited abnormal swimming habits, but returned to the normal habit after removal from the centrifuge. No inhibition of development or morphological changes such as reported by Hertwig were observed.

The growth rates of several organisms under increased acceleration have been described by Charles Wunder and his colleagues. Working with fruit fly larvae he demonstrated that their growth rate is accelerated after return to normal gravity from 2200 to 3000 G's for one day and that this response is greater in the smaller larvae (Wunder 1955, 1959, 1960). This accelerated rate of growth after removal from the centrifuge holds true also for mice held at 7 G for eight days and then returned to normal gravity (Wunder, et al 1959, 1960, 1963). Although the mice grew faster, they never exceeded control size. This is explained as

adaptation resulting in a reduced oxygen requirement for growth. (This reduced oxygen requirement was also reported by Miller (1950) for wheat seedlings which consumed less oxygen in spite of additional work growing against 94 G for the first 24 hours.)

In 1962 Wunder (1962) concurred with earlier investigators that younger mice (three weeks) died sooner when exposed to 2 to 10 G continuously, and stated that five week old mice placed at 7 G continuously could live for six months.

Steel (1962) reported that rats grown at 3 G continuously from the 21st to 91st day of life took more food to grow less. He also found that effects of centrifugation are greater on younger animals, agreeing with Britten, et al (1946) and Wunder (1962).

Using plant instead of animal material, there have been many investigators of the "gravity response." Normal gravity is known to orient the stems and roots of plants and possibly the first divisions of plant ova. Boysen-Jensen (1933) substantiated the theory of the control of growth by gravity by measuring a greater quantity of growth substance (auxin) in the lower than in the upper side of a root geotropically induced to bend. Elaborating the geotropic response, Dennison (1961) explained the two effects on mold sporangiophores of gravitational and centrifugal stimuli as (1) a transient bending response, and (2) a long term thickening of the cell wall in reaction to this.

Very high speeds of centrifugation, producing many thousand times gravity, have often been used for analysis and for cellular and

histological effects. Physiological polarity of pollen grains following exposure to 20,000 G was studied by Beams and King (1944). At 3,000 G to 6,000 G Saez (1941) observed stratification according to the density of cellular components in Lathyrus odoratus seeds. He observed mitotic changes attributable to centrifugal action. Camara (1942) reported structural variation in the chromosomes of Triticum induced by high speed centrifugation, with the number of chromosome breaks being greatest 12 hours following return to normal gravity.

Also using plant material and moderate forces, Gray and Edwards (1955, 1956) observed both inhibition and stimulation of centrifuged wheat seedlings. At forces of 25 G applied during the first 24 hours of germination, growth rate of the coleoptile and its roots was stimulated. At higher forces growth rate was still stimulated after removal from the centrifuge, but total coleoptile height attained was somewhat lower. Although a stimulating effect of greater than normal gravity forces was expressed during the first day of the growth period, continuous centrifugation brought general inhibition of growth proportional to the G force applied. With this longer centrifugation, changes in form were noted, such as an increased roundness and thickness in cross section compensating for the stress of increased superposed weight. Compensation failed to keep up with the deforming force, however, when the latter exceeded 100 G. Decrease in height was attributed to the increase in physical work necessary to accomplish growth.

Little as is our knowledge of supranormal gravitational forces, we have even less information of the effect of infranormal forces.

The impossibility of eliminating the normal 1 G on the surface of the earth accounts for the lack of experiments with less than normal gravity until the development of high range rockets, supersonic aircraft and satellites. Such experiments are reported for orbital flights of Discoverer satellites XVII, XVIII, XXIX and XXX by Prince (1962) and Katzberg (1962a) using human Hela cells and chick embryonic hearts (Katzberg and Mori, 1962). The results were equivocal. Katzberg (1962b) reported definitely increased growth from mouse L-cells subcultured from cells recovered from orbited satellites.

At least one Russian satellite (August 19, 1960) carried both tissue cultures (human epithelial tumor cells) and dry seeds (wheat, corn, peas, onion and coriander) as well as two dogs, a number of mice, fruit flies, Tradescantia plants, fungi, algae and bacteria. The orbital flight lasted 27 hours and recovery deceleration did not exceed 10 G. As reported by Haas (1961) the experiments seem to have been designed entirely for determining the genetic effects of radiation in space. The tissue cultures were to be subcultured and the seeds planted for genetic observation. Reports of the results obtained have not been located in the literature.

Russian author Vasily Parin (1962) experimented with rabbits kept for long periods at 2 G to 14 G and related this to circulatory adjustments necessary for cosmonauts. Yuganov (1962) reports on the motor activity of mice under conditions of artificial gravity encountered in parabolic flight, and wonders if the orientation disturbances of weightlessness might be counteracted by placing animals in a centrifuge

designed to produce 0.5 to 1.0 G. A similar arrangement has long been suggested for large orbiting "space stations". Wheel-shaped designs have been proposed which would revolve sufficiently rapidly to produce an effective centrifugal force at their rims in which human beings would live and work.

Some of the information already obtained in our laboratory about the behaviour of wheat seedlings at gravitational forces above normal is summarized in Figure 1. At less than normal gravity almost nothing is known about the responses of any living organism.

Since true weightlessness can only be achieved in an earth orbiting satellite, only in such a vehicle will it be possible to determine the effect of the absence of gravity on normal growth processes. This we propose to do on the first Biosatellite to be launched by the National Aeronautics and Space Administration. The following pages summarize the background experiments performed in anticipation of the Biosatellite experiment.

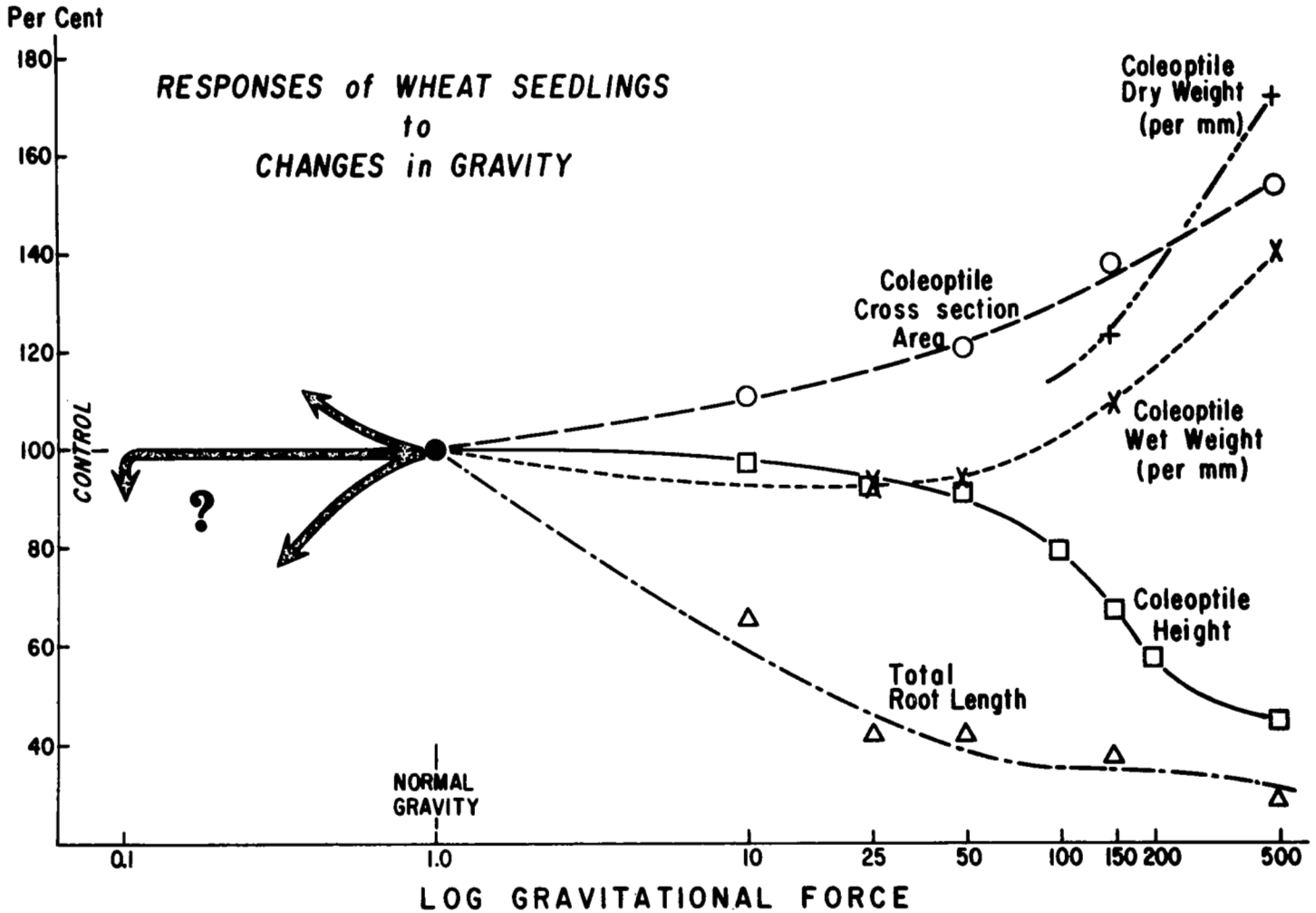
Per Cent
180
160
140
120
100
80
60
40
RESPONSES of WHEAT SEEDLINGS
to
CHANGES in GRAVITY
CONTROL
?
Coleoptile
Dry Weight
(per mm)
Coleoptile
Cross section
Area
Coleoptile
Wet Weight
(per mm)
Coleoptile
Height
Total
Root Length
NORMAL
GRAVITY
0.1
1.0
10
25
50
100
150
200
500
LOG GRAVITATIONAL FORCE

Figure 1

II - MATERIALS

The seedlings of monocotyledonous plants are ideal for gravity response experiments because of their vertical growth, easy germination, and simple shoot and root systems. All of the experimenters had experience with the growth of these seedlings. Three species were originally suggested, corn, oats and wheat.

Corn (Zea) was eliminated because it was larger than the others, and hence fewer seedlings could be used.

Oats (Avena) have had a large number of experimental studies to serve as background material, but their early growth is more curved, and at a slightly slower rate than that of wheat. Per cent germination was appreciably lower than that of the wheat strain finally used.

Wheat (Triticum vulgare) was agreed upon as the experimental plant by all investigators. A great deal is known about its growth under varied gravitational forces (Gray and Edwards, 1955, Edwards and Gray, 1956). The particular strain being used is Georgia 1123 developed at the Georgia Experiment Station (Gore and Stacy, 1961). It is a hexaploid hybrid with a chromosome number of 42 (6n).

III - METHODS

A. SUBSTRATE AND SEED CONTAINER

In earlier experiments (Gray and Edwards, 1955, Edwards and Gray, 1956) the seedlings were planted in centrifuge tubes with sterile Ottawa sand as a substrate. This could not be used for the proposed Biosatellite flight for the following reasons:

1. The weight of the sand makes it difficult to keep in place in any attitude except that of the normal direction of gravity.

2. The sand makes observation of root growth impossible, especially the angles of emergence, as desired by Dr. Lyon.

3. The sand seriously interferes with getting fixative solutions to the roots of the seedlings.

Other substrates such as agar and vermiculite were considered. These had adequate water holding properties, but each had other drawbacks. The seeds often drowned in soft agar, and the roots had some tendency not to penetrate it properly. Vermiculite as a substrate was best at holding moisture and is not dislodged by jarring or shocks such as might occur in Biosatellite flights. In spite of some tendency of the roots not to penetrate it properly, vermiculite in centrifuge tubes has been used for all of the high gravity (centrifugation) experiments.

B. "FLIGHT CHAMBER" DEVELOPMENT

Following the decision to combine our experiment with those of Doctors Lyon, Conrad and Johnson, several other methods of growing seeds were tried.

The earliest method suggested for permitting unconfined growth of the seedlings was to embed them at spaced intervals throughout a cuboidal chamber of vermiculite. This was abandoned in favor of attempting to grow the seedlings in air saturated with water.

Seeds with drilled holes, strung on a wire had been used by Lyon (1961). These seeds were alternated with cotton wicking, but were found to absorb insufficient water. Various schemes for attaching them to a central wick were also tried and discarded.

The final solution was reached when it was discovered that the seed would grow if the end containing the embryo was in the air while the other end was immersed in water. Seedling holders were constructed so that each seed was placed in the end of a 7/32 inch I.D. plastic tube having a rubber diaphragm perforated so that about one-third of the seed with its embryo projected outside while the other two-thirds were within the tube. The tube, called a seed holder, was then filled with vermiculite and water. Details are shown in Figures 2 and 3, and described more fully in Appendix I. By inclining the seed holder downward at an angle of 45 degrees it was possible to grow a seedling whose coleoptile and roots could extend free in water-saturated air without physical restraint in any direction (Figure 3).

Figure 2

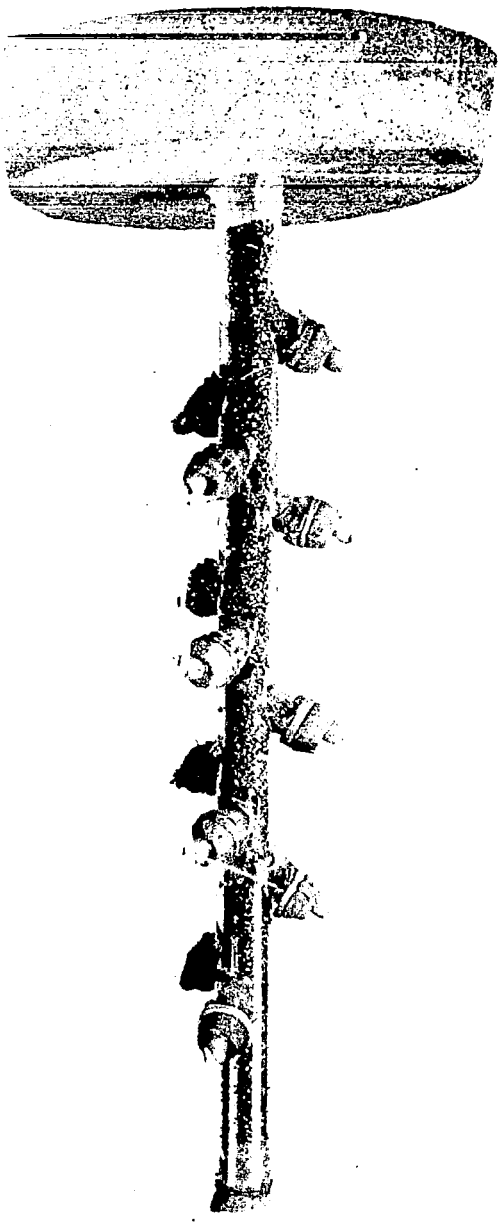

Figure 3

In practice, the seed holders were approximately one-half inch long side-arms in three staggered rows 120 degrees apart, attached to a central stalk of plastic 9/16 inch I.D. and seven inches long. The central stalk and side arms were filled with vermiculite (#4 grade Zonolite) and water added carefully to remove all air bubbles. The stalk with the seeds mounted in the side arms was then placed upright in the center of a closed plastic cylinder (three inches I.D.) containing enough water to provide a saturated atmosphere.

Based upon preliminary experiments, it was concluded that seedlings grown for 72 hours (plus eight hours "hold" time) might be expected to have primary roots (the longest organ) as long as 64 mm. As the direction of growth at zero gravity may be random, the stalk, side arms and enclosing cylinder (called the chamber) were designed to allow freedom from interference by chamber walls and adjacent plants through about two-thirds of the sphere which each plant might possibly occupy. This resulted in seven inch long central stalks containing twelve side arms in staggered rows, the arms in each row being spaced 1-3/16 inches apart vertically, holding the seed 7/16 inch out from the axis of the system. The chamber containing the stalk with side arms was three inches in inside diameter. These dimensions were essentially fixed in June, 1964. A complete assembly is shown in Figure 4.

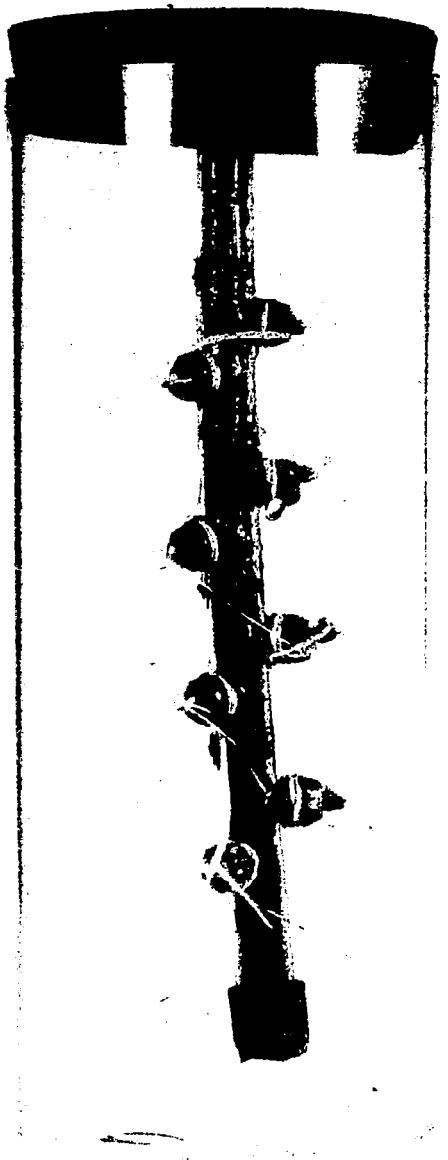

Figure 4

C. TEMPERATURE

The earlier experiments of Gray and Edwards mentioned above had all been conducted in the dark at 25° Centigrade (± 0.5° C.). The original plans for the Biosatellite called for an ambient temperature of 77° Fahrenheit (± 1.0° F.) which is approximately 25° C. When an alteration in overall plan called for a change to 21° C., experiments were then conducted at 2° C. intervals on either side of the optimum of 25° C. (that is, at 21°, 23°, 25°, 27° and 29°). The two degree intervals proved to be too close for significant differences, and consequently the majority of tests were conducted at 21°, 25° and 29°. The seedlings grown at 25° C. were considered the "Controls".

D. CENTRIFUGATION

The influence of higher than normal gravitational fields on the growth of wheat seedlings had been tested in previous work by Gray and Edwards (1955, 1956, 1962 and 1963). Although both inhibition and stimulation of growth rate had resulted from forces of 25 x G to 500 x G, a force of 150 x G was selected for these experiments because it produces a marked effect at 25° C. without causing too much inhibition. This force was produced by continuous centrifugation of the tubes containing the seeds and seedlings at a speed of 990 rpm at a radius of 22 mm in an International Type 2 centrifuge.

E. FIXATION OF THE TISSUES

Fixation of tissues for microscopic study is usually accomplished in the laboratory by cutting the specimen into pieces of suitable size and dropping them into a jar containing at least forty times as much fixative as tissue to be fixed.

Previous work in this laboratory was done with Randolph's (1935) modification of Navashin's chromic acid - acetic acid - formalin fixative (CRAF) which requires two solutions to be mixed just before use. Alternatively, acceptable but less consistent results were obtained by the use of formalin - acetic acid - alcohol mixtures (FAA) which require but one solution.

Immersion of the seedlings in orbit presented two problems. One problem was the bulk of fixative required to flood the chambers; well over a liter of fluid would be required. The second problem was the practical one of transferring liquid from storage to flight chamber in the absence of gravity. Various methods were tried for ensuring removal of air as the chambers were filled with liquid without striking success.

In an attempt to solve both problems at once, the fixative was injected under pressure in the form of a fine mist into the chambers. This required a smaller amount of fixative (between five and ten milliliters), and the seedlings in closed chambers were completely coated by the fixative. To simulate the Biosatellite flight schedule, after spraying with the fixatives the seedlings were left in a closed

container for 24 to 48 hours; then all seedlings were post-fixed for 24 hours in CRAF fixative.

A number of different fixatives were tried on the wheat seedling tissues. The composition of these is given in Appendix II. The results produced by the different fixatives are shown in Table 1. Bouin's fixative (picric acid - acetic acid - formalin) and Carnoy's solution (chloroform - acetic acid - alcohol) when post-fixed in CRAF gave very good results, although the penetration was not always uniformly good. A variation of Schaudinn's fixative (mercuric chloride - acetic acid - ethanol, which we named "Wood's" for the technician who developed it) gave the most consistently good results.

At the suggestion of Dr. Conrad, we tried adding dimethyl sulfoxide (DMSO) to the FAA mixture, and this seemed to improve its penetration. After experimenting with the proper concentration of this substance, it was found that 0.2 per cent was enough. This was also added to other fixatives. The results of these experiments are shown in Table 1. The efficiency of the fixatives was, of course, judged by the quality of the sectioned and stained slides following their use.

Figure 5 shows the appearance of sectioned seedlings in three of the earliest fixatives used. It is apparent that Carnoy's produced the best results. However, the chloroform in this fixative melts the plastic of our prototype containers, and the engineers discouraged its use.

Table 1

RESULTS OF VARIOUS FIXATIVES

Sprayed as a Fine Mist

Fixative *	Post-Fixation		
	Time After Spraying	Duration of Post Fixation in CRAF	Results
1. Ethyl Chloride	48 hours	24 hours	very poor
2. Farmer's	48	24	very poor
3. Mercuric Chloride + Acetic Acid	24	24	poor
4. Newcomer's	24	24	fair
	48	24	poor
5. Mercuric Chloride + Acetic Acid + Glutaraldehyde	48	24	fair
6. Mercuric Chloride + Formalin	24	24	fair
	48	24	fair
7. Mercuric Chloride + Alcohol + Acetic Acid + Glutaraldehyde	48	24	fair
8. Bouin's	48	24	good
9. CRAF	24	24	good
10. Carnoy's	24	24	very good
	48	24	very good
11. FAA (Formalin + Acetic Acid + Alcohol)	24	24	fairly good
FAA + DMSO**	24	24	very good
12. Wood's	24	24	good
Wood's + DMSO	24	24	very good

* Composition of fixatives will be found in Appendix II.

** DMSO - Dimethyl-sulfoxide added as a penetrant.

Figure 5

APPEARANCE OF WHEAT COLEOPTILE AND

SHOOT AFTER DIFFERENT FIXATIVES

Top: Carnoy's, H & E Stain, X 40.3

Left: Hematoxylin and Light Green, X 64

Right: Bouin's, Hematoxylin and Fast Green, X 64

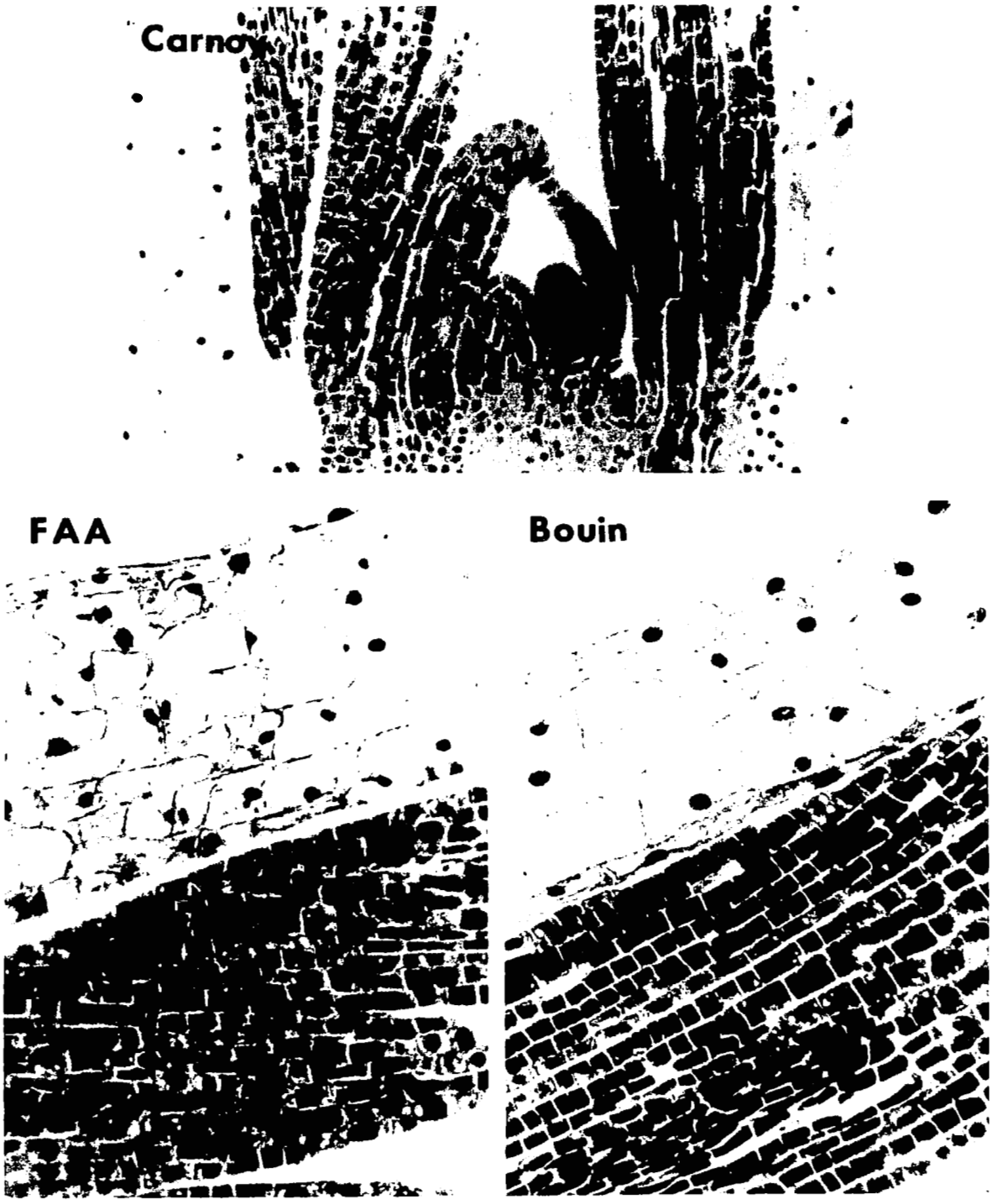

Figure 5

Figure 6 shows the improvement in fixation secured by the addition of dimethyl sulfoxide (DMSO) as a penetrant to FAA and Wood's mercuric chloride mixture. On the basis of many tests either of these fixatives is satisfactory, although Wood's is more consistent in results.

In addition to iron hematoxylin and acid hematoxylin as nuclear stains, eosin, tartrazine, light green and other counter stains were used. The structural components of the seedling were also delineated by the use of the Periodic acid - Schiff technique (PAS) (see Figure 7), and by the removal of pectin from some of the sections prior to staining.

This method of spraying the fixative as a mist, and the use of DMSO as a penetrant has not been reported before to our knowledge. It may prove useful in other space applications.

Figure 6

IMPROVED APPEARANCE OF WHEAT COLEOPTILE

AFTER ADDITION OF DMSO TO FIXATIVES

Top: FAA and DMSO, H & E, X 64

FAA and DMSO, H & E, X 160

Bottom: Wood's Mercuric Chloride Mixture and DMSO

Hematoxylin and Tartrazine, X 40.3

Hematoxylin and Tartrazine, X 160

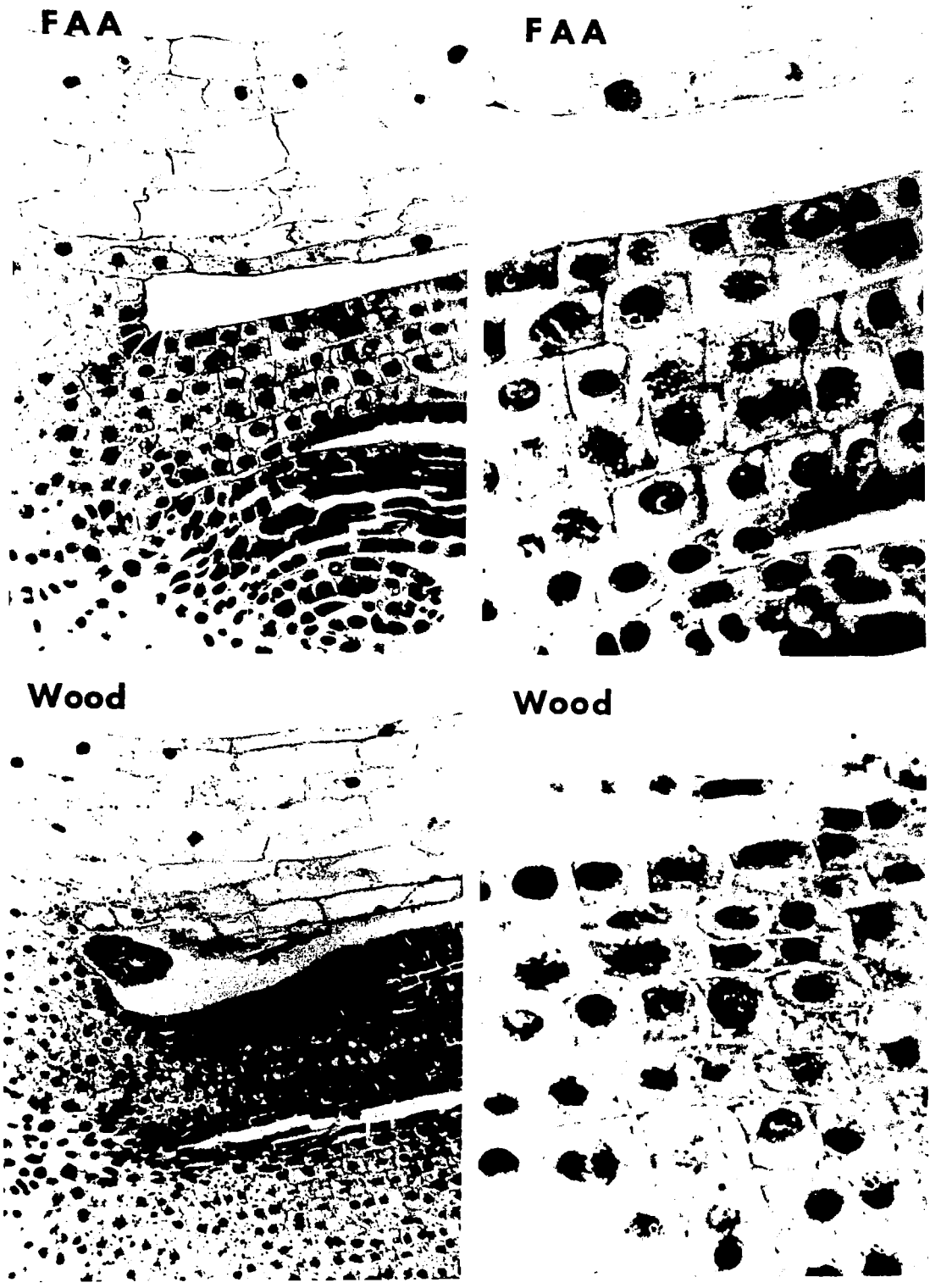

Figure 6

Figure 7

LOCALIZATION OF STARCH GRANULES AND OTHER STRUCTURES IN WHEAT SHOOT AND COLEOPTILE

PAS Technique

Counterstained with Fast Green

Top: X 40

Bottom: X 100

Figure 7

IV - RESULTS

A. GROWTH IN "FLIGHT CHAMBERS"

Growth of seedlings in the experimental "flight chambers" is limited by the water available to the seedlings. There is considerable variation in growth from seedling to seedling within the same chamber, with a slight tendency for growth to be better in seedlings located toward the bottom of the chamber. This may be due to hydrostatic pressure in the central stalk. An example of the results from a single chamber containing twelve seeds and grown at 25° C. for 81 hours is shown in Table 2.

The growth of seedlings in the "flight chambers" is shown in Figure 8 with the growth of "tube-grown" control seedlings for comparison. The average length of all organs of seedlings grown in the "flight chambers" is less than that of comparable organs of seedlings grown on water saturated vermiculite in closed tubes. The difference is attributed to a lower rate of water uptake of plants in "flight chambers".

Not all of the seedlings seem to be water deprived at 80 hours. The largest seedlings at 25° have coleoptiles, primary roots and secondary roots which compare favorably with those of the average tube-grown control plant. The four tallest coleoptiles in Table 2 average 23.9 mm, while tube-grown coleoptiles average 24.2 mm at 25° (Table 3). Three plants have primary roots longer than the average

Table 2

GROWTH OF SEEDLINGS IN A "FLIGHT CHAMBER"

Run XIX, 81 Hours

Seedling	Coleoptile	Primary Root	Secondary Root
#1	4.5 mm	8 mm	13 mm
2	5.0	37	17
3	22.5	24	23
4	4.5	23	24
5	7.5	12	24
6	26.5	27	36
7	13.5	39	23
8	6.5	28	14
9	22.0	38	30
10	8.5	29	18
11	19.0	25	29
12	24.5	27	21
Average	13.7	26.4	22.7

25° C., Angled side arms, vermiculite, soaked two hours in aerated water.

Table 3

AVERAGE LENGTH OF WHEAT SEEDLING ORGANS

Grown in Tubes on Vermiculite at

Different Temperatures

TIME	TEMPERATURE		
Coleoptile	21° C.	25° C.	29° C.
48 hours	3.15 mm	5.14 mm	6.88 mm
72	9.71	19.89	25.85
80	14.51	24.16	28.92
96	25.58	40.31	32.53
Primary Root			
48 hours	9.30 mm	11.9 mm	14.76
72	22.40	21.99	30.14
80	28.80	36.00	35.90
96	29.10	38.34	33.47
Secondary Root			
48 hours	5.7 mm	9.0 mm	11.3 mm
72	15.6	20.1	26.7
80	22.1	26.8	41.4
96	28.9	35.5	28.6

length of those organs (36.0 mm) in the tube-grown control plants, and six plants have secondary roots about the same size as those of the controls at 80 hours.

By 96 hours all of the 25° plants in the "flight chambers" are water deprived. Only the longest primary roots have kept up with those of the tube-grown controls; the coleoptiles and secondary roots have fallen behind (Table 4).

It is noteworthy that of the three organs, it is the primary root that most nearly achieves its normal growth. When the average of all plants grown in "flight chambers" at 96 hours is taken, the primary root is 68.1 per cent of the average for tube-grown controls while the coleoptile and secondary roots reached only 36.2 per cent and 56.1 per cent respectively.

Table 4 and Figure 8 imply that a few of the seedlings in each of the "flight chambers" receive enough water to grow normally up to 80 hours, but beyond that time growth of even the longest coleoptiles and secondary roots is inhibited, and only the longest primary roots achieve normal length. The primary root may thus be considered a "preferred organ". This may be expressed by measuring the coleoptile and secondary roots as percentages of their own primary roots (Table 5).

Seedlings grown in experimental "flight chambers" showed characteristic responses to temperature differences (Figure 8). The variation between seedlings and the effects of water deprivation mentioned above make their actual measurements less suitable for comparison of temperature effects than the measurements of tube-grown seedlings, to be discussed later.

Table 4

LENGTH OF SEEDLING ORGANS IN "FLIGHT CHAMBERS"

AND IN VERMICULITE TUBES AT 96 HOURS

25° C.	Average of Plants in Vermiculite-Filled Tubes	Average of Plants in "Flight Chambers"	Average of Longest Organ in Each of Seven Chambers
Coleoptile	40.3 mm	14.6 mm	30.1 mm
Primary Root	38.3	26.1	40.3
Secondary Root	35.5	19.9	28.9

Table 5

WHEAT SEEDLING ORGAN LENGTHS

AS PERCENTAGE OF PRIMARY ROOT LENGTH

96-101 Hours, 25° C.	Coleoptile	Secondary Root
Tube-Grown Controls	105.2%	92.7%
"Flight Chambers":		
Largest Seedlings	74.7%	71.7%
Average Seedlings	55.9%	76.2%

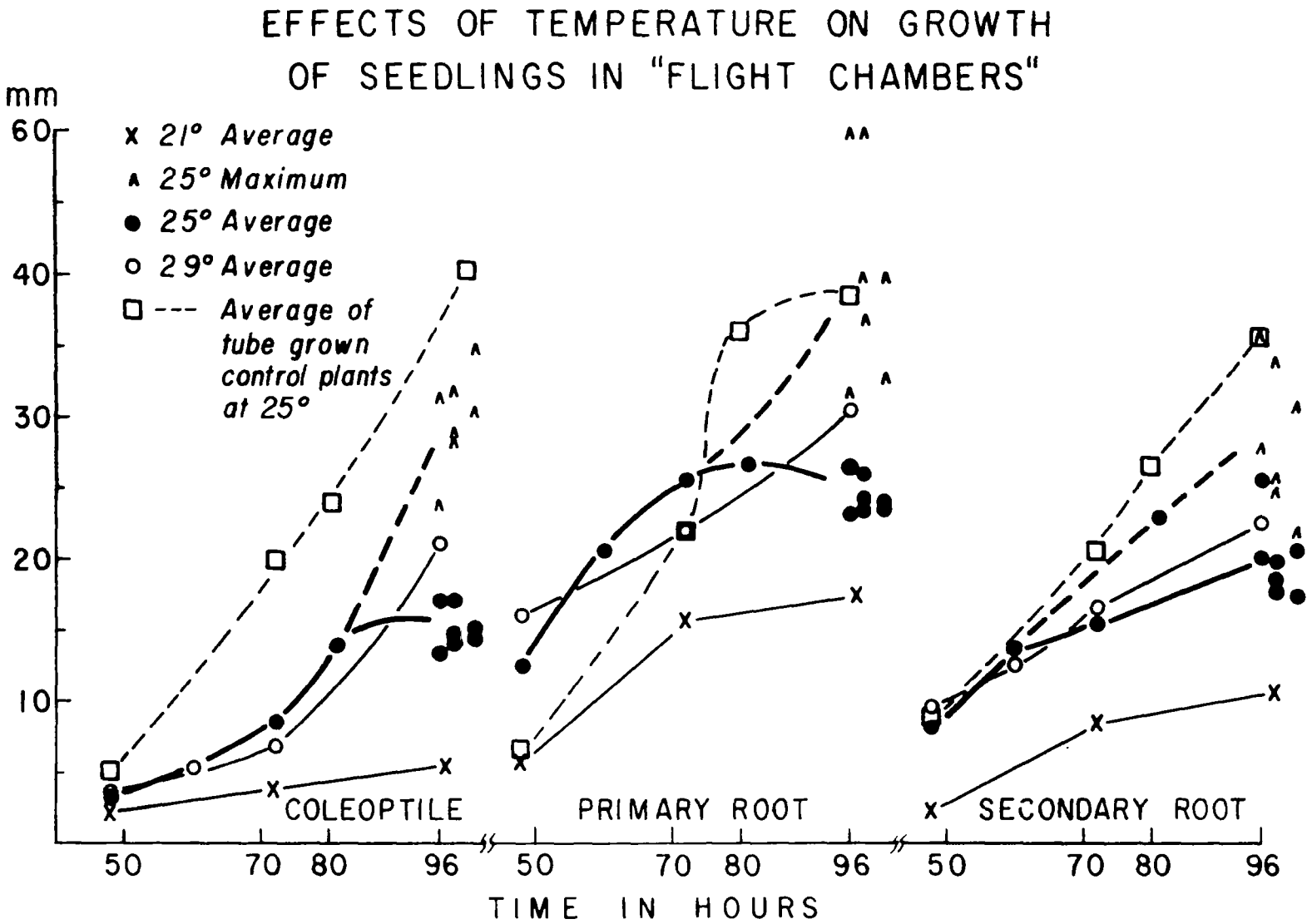
EFFECTS OF TEMPERATURE ON GROWTH
OF SEEDLINGS IN "FLIGHT CHAMBERS"
mm
60
40
30
20
10
x 21° Average
▲ 25° Maximum
● 25° Average
○ 29° Average
□ --- Average of tube grown control plants at 25°
COLEOPTILE
PRIMARY ROOT
SECONDARY ROOT
50
70
80
96
TIME IN HOURS

Figure 8

B. EFFECTS OF TEMPERATURE

Wheat seedlings grown in stoppered tubes on water-soaked vermiculite for four days at 25° C. are taller and have longer roots than those grown at 21° C. or at 29° C. This cannot, however, be taken to indicate that 25° seedlings grow consistently faster than do others for the entire four days. There are marked differences in the rate of growth at different times and at different temperatures. These are summarized in Table 6 and shown graphically in Figure 9.

Coleoptiles: For the first three days the growth rate of the coleoptile increases with the temperature, as might be expected. At 72 hours coleoptiles grown at 21° are 9.7 mm tall, those grown at 25° are 19.9 mm tall, and those grown at 29° are 25.9 mm tall. From 72 to 80 hours the coleoptiles grown at 25° maintain their earlier growth rate, and between 80 and 96 hours they nearly double it. Those grown at 21° also increase their rate of growth from 72 hours onward, while the growth rate of those grown at 29° declines from 72 to 80 hours, and drops still more during the 80-96 hour period. The result is that 25° coleoptiles, which were shorter than the 29° coleoptiles at 72 and even at 80 hours, are by 96 hours much taller. Similarly, 21° coleoptiles, which were only half the height of 29° coleoptiles at 80 hours, are three-quarters as tall by 96 hours (Table 6 and Figures 10 and 11).

Roots: Growth of roots of the 29° seedlings was faster than those at lower temperatures during the first three days, but there was little difference between roots of seedlings grown at 21° and 25°.

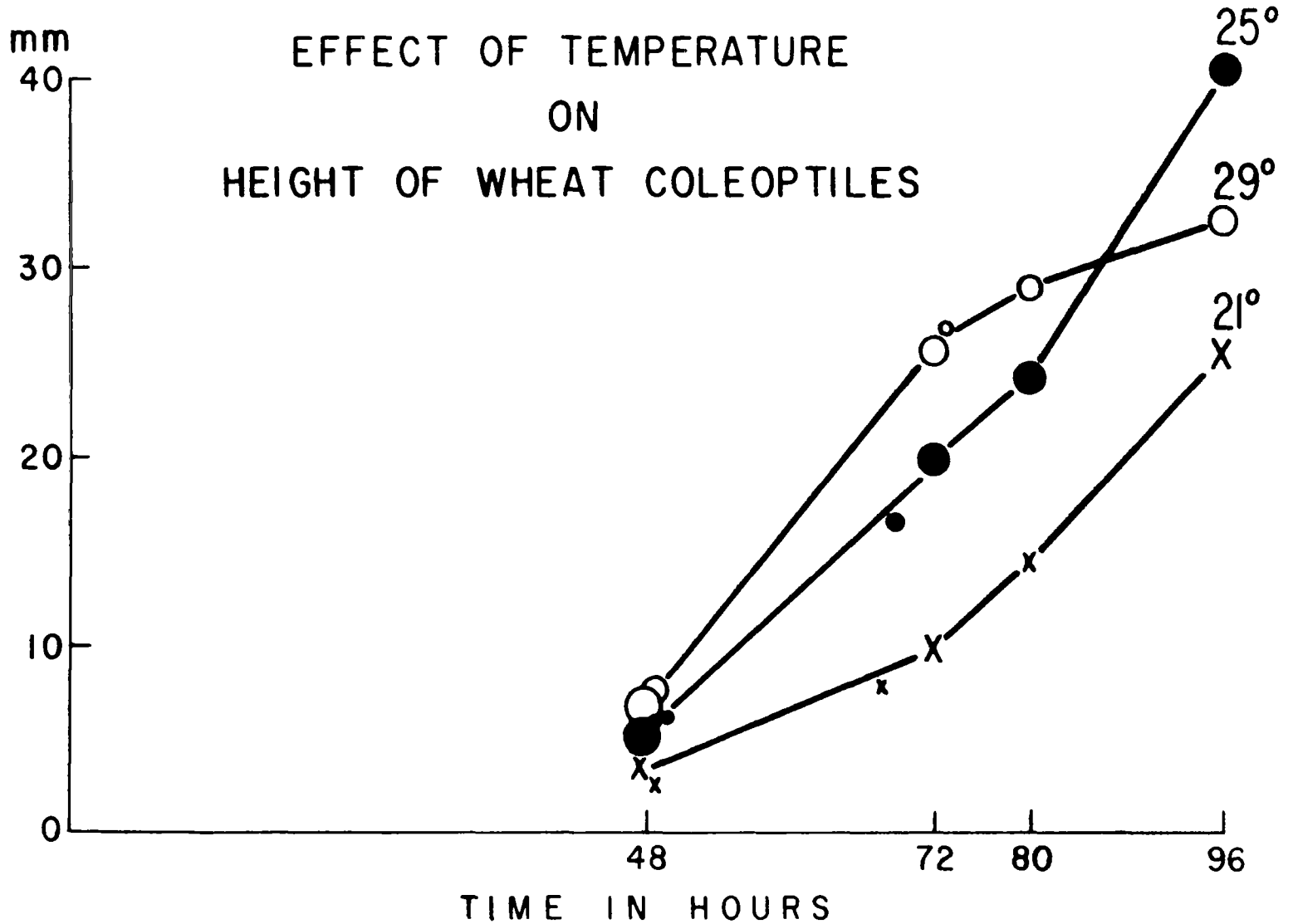
EFFECT OF TEMPERATURE
ON
HEIGHT OF WHEAT COLEOPTILES
mm
40
30
20
10
0
48
72
80
96
TIME IN HOURS
25°
29°
21°

Figure 9

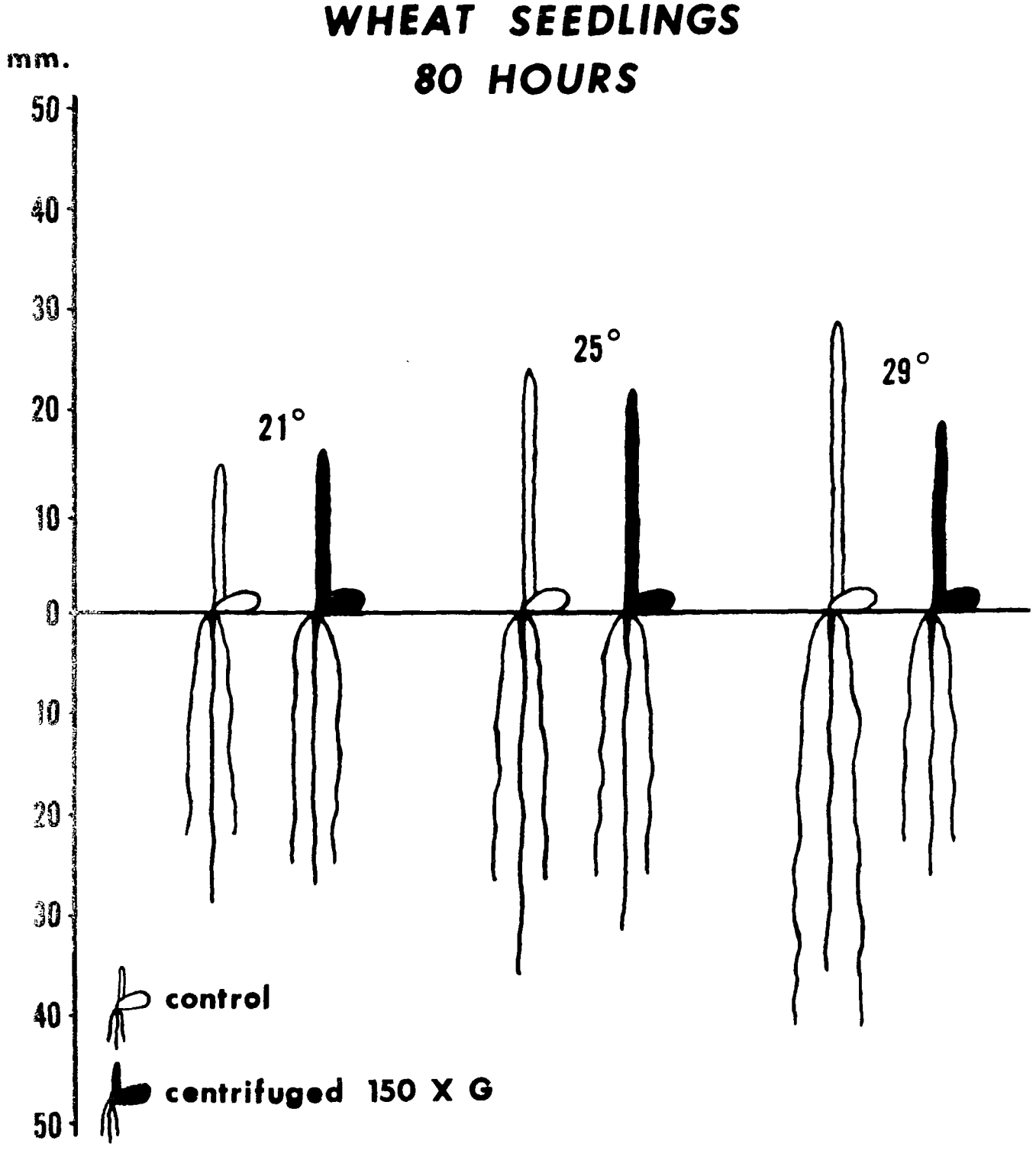
WHEAT SEEDLINGS
80 HOURS
mm.
50
40
30
20
10
0
10
20
30
40
50
21°
25°
29°
control
centrifuged 150 X G

Figure 10

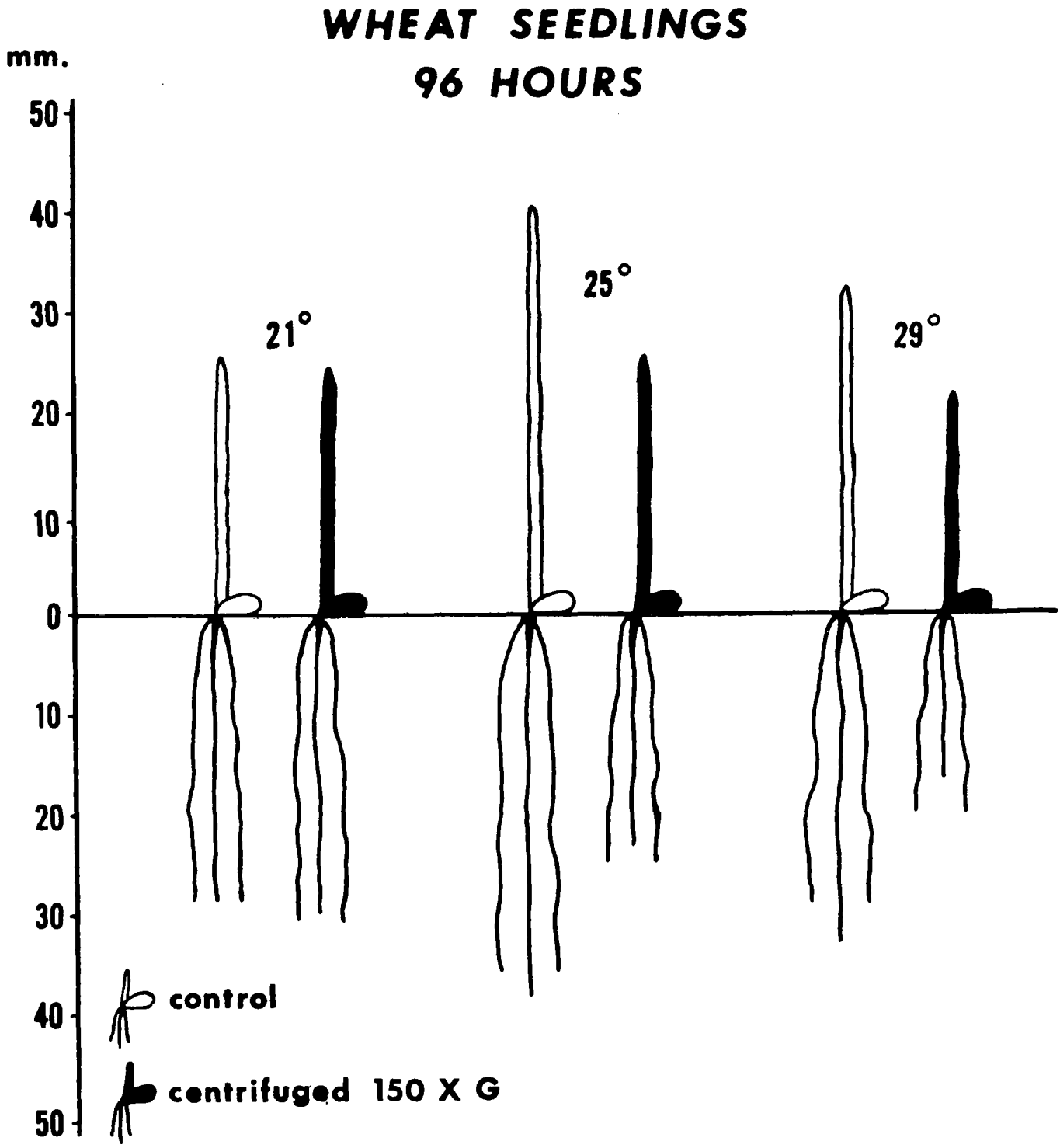
WHEAT SEEDLINGS
96 HOURS
mm.
50
40
30
20
10
0
10
20
30
40
50
21°
25°
29°
control
centrifuged 150 X G

Figure 11

Table 6

GROWTH RATES OF SEEDLING ORGANS AT DIFFERENT TEMPERATURES

PERIOD	TEMPERATURE		
Coleoptiles	21° C.	25° C.	29° C.
48-72 hours	0.275 mm/hr	0.622 mm/hr	0.794 mm/hr
72-80	0.600	0.534	0.394
80-96	0.692	1.009	0.226
Primary Roots			
48-72 hours	0.546 mm/hr	0.420 mm/hr	0.641 mm/hr
72-80	0.800	1.751	0.720
80-96	-0-	0.146	-0-
Secondary Roots			
48-72 hours	0.413 mm/hr	0.461 mm/hr	0.644 mm/hr
72-80	0.813	0.842	1.833 (?)
80-96	0.425	0.546	-0- (?)

From 72 to 80 hours the growth rate of roots of 21° seedlings almost doubles, while the primary root of 25° seedlings quadruples its growth rate. From 80 to 96 hours there is a sharp decline in growth rate of the primary root at all temperatures, while the secondary roots continue to elongate in all but 29° seedlings. The lengths attained by the roots of seedlings at 80 and 96 hours are graphically shown in Figures 10 and 11, and growth rates are shown in Table 6. (The values for growth rate of 29° seedlings between 72 and 96 hours are less reliable than values at other hours.)

Thus, throughout the growth period there is a changing ratio between the length of coleoptile, primary root and secondary root. These may be expressed by measuring the coleoptile against its own primary root (Table 7).

C. EFFECTS OF CENTRIFUGATION

Seedlings were also grown on water-saturated vermiculite in stoppered tubes and centrifuged in the dark at the different temperatures. For these experiments a force equal to 150 x G was used.

The seedlings resulting from this centrifugation at 80 and 96 hours are shown with comparable control seedlings in Figures 10 and 11. Growth curves are shown graphically in Figure 12, which should be compared with Figure 9.

The lengths of the seedling organs of the centrifuged plants may be expressed in percentage of the length of organs of control plants at similar temperatures and times. It will be seen in Table 8 (and graphically in Figure 13) that centrifugation may produce acceleration

Table 7

COLEOPTILE HEIGHT AT VARIOUS AGES

AND TEMPERATURES AS

PERCENTAGE OF PRIMARY ROOT LENGTH

TEMPERATURE	HOURS OF GROWTH		
	72 hours	80 hours	96 hours
21° C.	43.4%	50.3%	88.0%
25° C.	90.5%	66.9%	105.2%
29° C.	86.8%	80.5%	97.0%

Table 8

PERCENTAGE OF CONTROL SIZE ACHIEVED BY

SEEDLINGS CENTRIFUGED AT 150 X GRAVITY

	72 hours	80 hours	96 hours
21° C.			
Coleoptile	122.2	110.3	96.1*
Primary Root	101.3*	93.8*	102.1*
Secondary Root	143.0	113.1	104.5*
25° C.			
Coleoptile	80.1	91.7	62.0
Primary Root		87.8	60.6
Secondary Root		96.6	69.3
29° C.			
Coleoptile	64.9	64.9	67.1
Primary Root	71.7	73.3	48.7
Secondary Root	78.7	49.0	68.2

* Difference from control not significant.

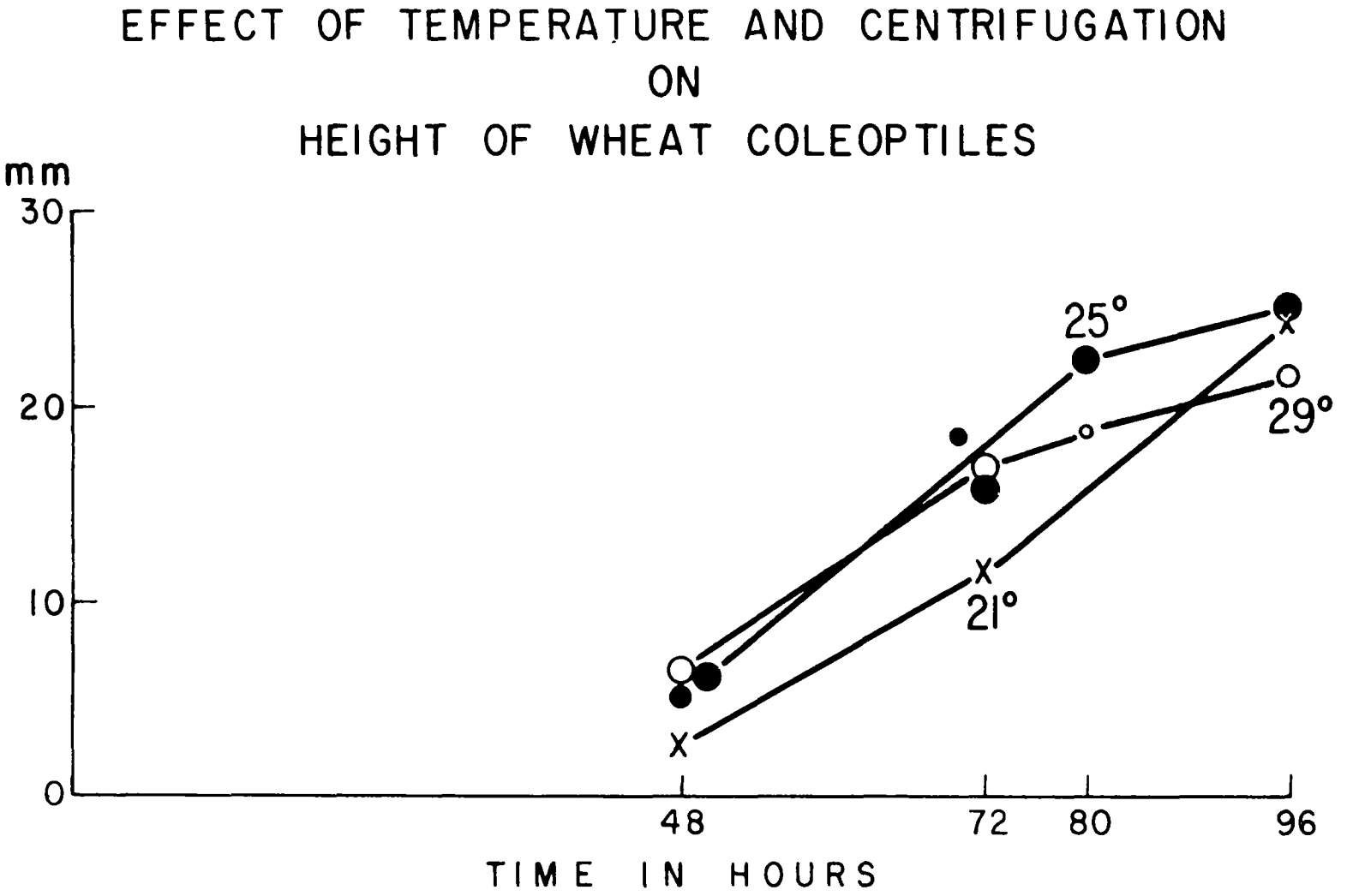
EFFECT OF TEMPERATURE AND CENTRIFUGATION
ON
HEIGHT OF WHEAT COLEOPTILES
mm
30
20
10
0
25°
29°
21°
48
72
80
96
TIME IN HOURS

Figure 12

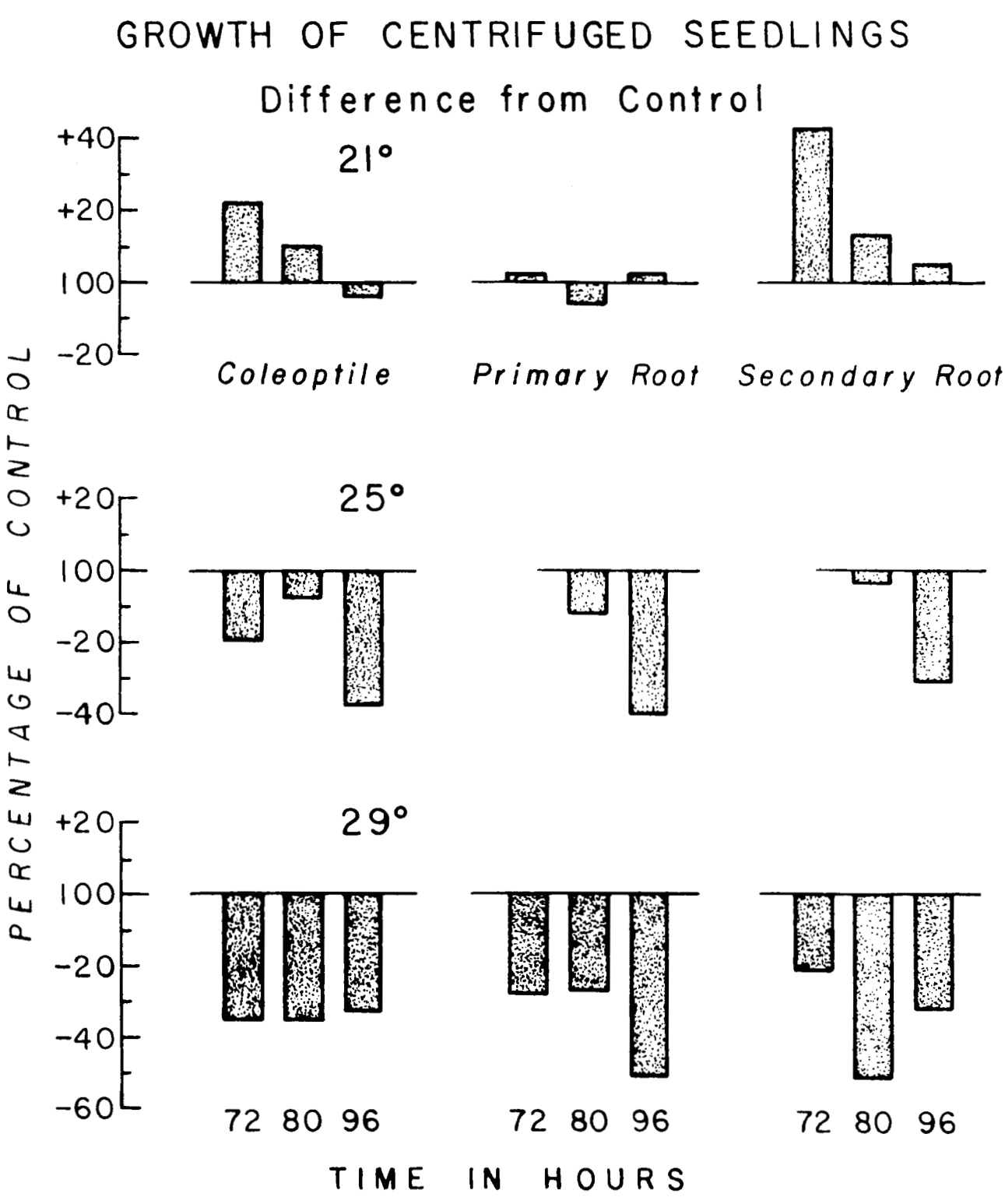
GROWTH OF CENTRIFUGED SEEDLINGS
Difference from Control
21°
25°
29°
+40
+20
100
-20
-40
-60
Coleoptile
Primary Root
Secondary Root
PERCENTAGE OF CONTROL
72 80 96
72 80 96
72 80 96
TIME IN HOURS

Figure 13

or depression of growth depending on (1) the temperature at which the seedling is grown, (2) the time at which it is measured, and (3) the specific seedling organ under consideration.

21° Seedlings Centrifuged: At 72 hours both the coleoptiles and the secondary roots are significantly taller than those grown at 21° without centrifugation. At 80 hours they are still slightly larger, but by 96 hours the centrifuged plants are no longer significantly different from the control plants. The relative length of the primary root does not change.

25° Seedlings Centrifuged: Growth inhibition at 80 hours was small, but growth of all organs between 80 and 96 hours was markedly inhibited by continuous centrifugation during the entire growth period.

29° Seedlings Centrifuged: The growth of all organs was inhibited at all of the hours at which they were measured. The greatest effect is seen in the primary root which reaches only half the length of that of the control plant at 96 hours.

The height attained in any particular period by the coleoptile subjected to centrifugation appears to be inversely proportional to the normal rate of growth during the period. This effect is marked in coleoptiles grown at 21° and 25°. It is almost absent at 29° (Figure 14). That growth rate alone is not the only factor involved is shown by the fact that the temperature curves are independent of one another. Where growth rates are comparable between two temperature curves, the seedlings are not of the same age.

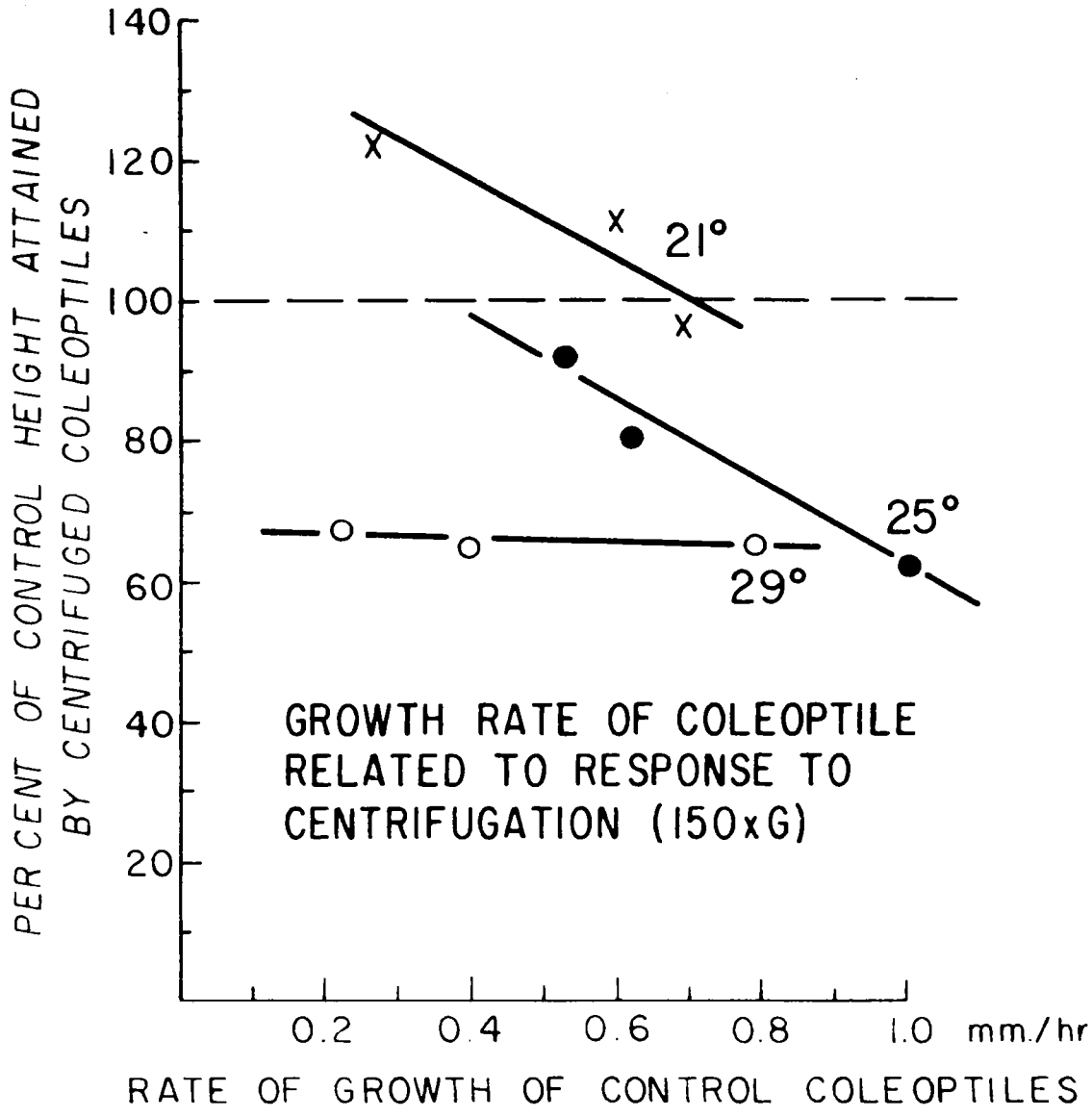
140
120
100
80
60
40
20
PER CENT OF CONTROL HEIGHT ATTAINED BY CENTRIFUGED COLEOPTILES
21°
25°
29°
GROWTH RATE OF COLEOPTILE RELATED TO RESPONSE TO CENTRIFUGATION (150xG)
0.2
0.4
0.6
0.8
1.0 mm./hr
RATE OF GROWTH OF CONTROL COLEOPTILES

Figure 14

This relation between gravitational sensitivity and rate of growth can be demonstrated clearly only for the coleoptiles. The root curves, although not ready to present at this time, will probably not resemble those of the coleoptile.

D. EFFECTS OF RADIATION PLUS CENTRIFUCATION

In December, 1963, through the Biosatellite Project Manager, we were urged to drop the radiation aspect of our experiment since it was not compatible with the other radiation experiments chosen for the first Biosatellite, but was otherwise flightworthy. Therefore the emphasis in our later experiments was directed toward the effects of temperature and centrifugation on wheat seedlings grown in simulated satellite conditions. Nevertheless, we feel it important to include herewith our earlier results showing the effects of radiation and centrifugation on the growth of the wheat seedling parts. Figures 15 and 16 show the effects of X-radiation doses up to 160,000 roentgens applied to seeds which had been soaked for two hours prior to a single dose of X-radiation and then grown in an incubator or in the centrifuge at 150 times gravity at a temperature of 25^{o} C.

Caldecott (1954) had claimed an inverse relationship between the water content of seeds and their susceptibility to X-rays. It seems possible that the high doses tolerated by our seeds are due to the short initial soaking period which permitted neither leaching of important compounds (Kamra *et al*, 1960) nor damage from free radicals persisting in the irradiated seeds.

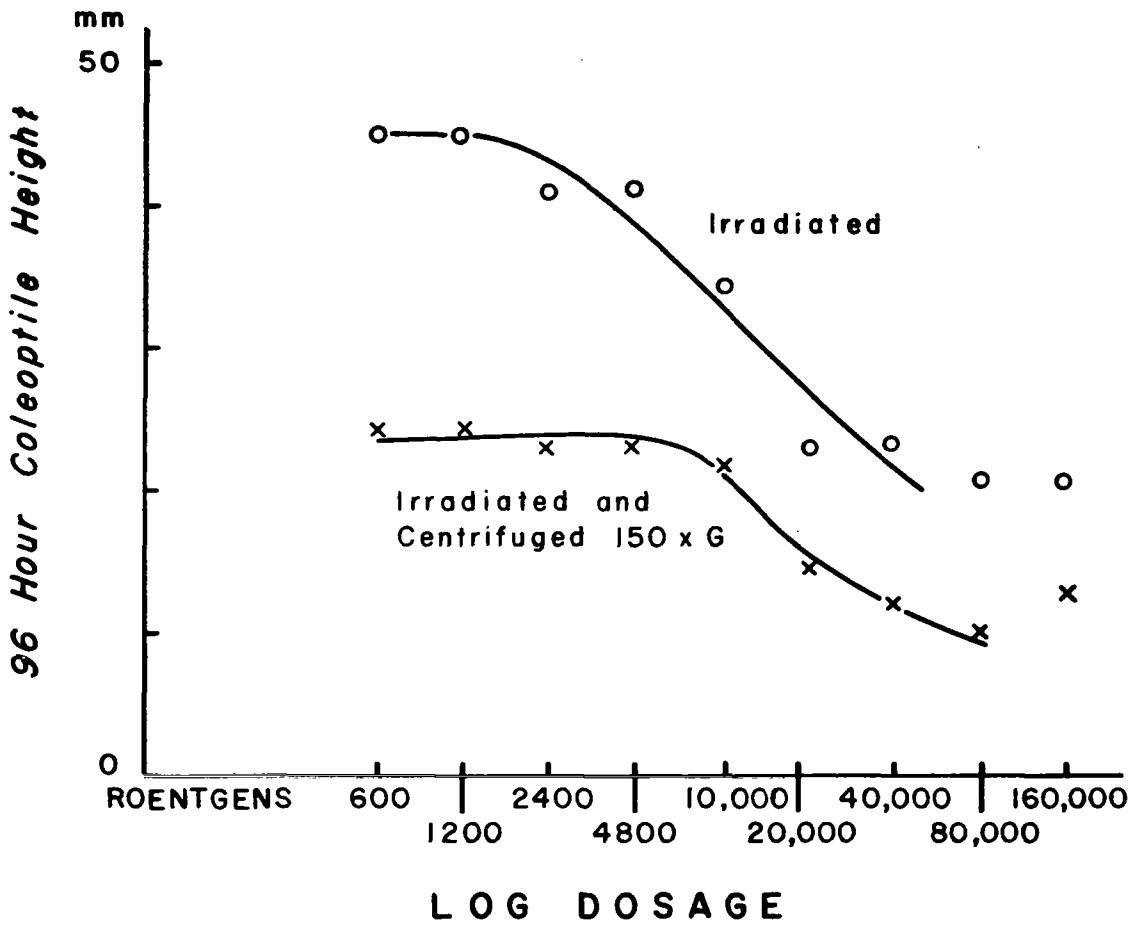

Figure 15. CHANGES IN COLEOPTILE HEIGHT UNDER EXPERIMENTAL CONDITIONS

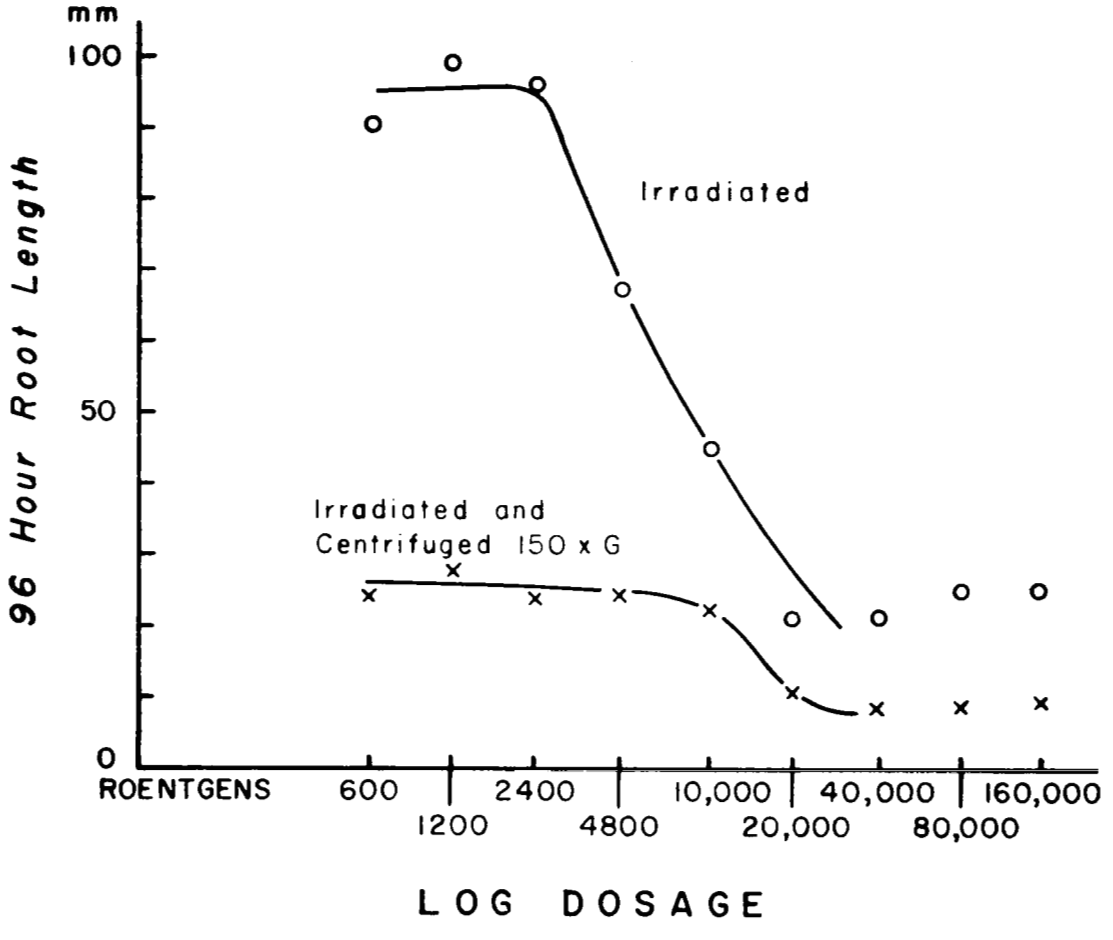

Figure 16. CHANGES IN ROOT LENGTH UNDER EXPERIMENTAL CONDITIONS

The coleoptile height of plants centrifuged at 150 X G is approximately 50 per cent of the height of control coleoptiles. The dose of X-radiation which inhibits coleoptile height to the same extent is near 40,000 r, as seen in Figure 15. If plants centrifuged only are compared to others both centrifuged and irradiated, the dose necessary to reduce this height by 50 per cent is also 40,000 r. Thus it is obvious that the inhibition of growth produced by irradiation and centrifugation is not additive. Plants which were centrifuged after receiving 10,000 r measured 49.4 per cent of the controls, yet this represents 90.7 per cent of the growth of plants centrifuged but not irradiated.

The average root length of seedlings is affected by a lower dose of X-radiation than is coleoptile height. (See Figure 16.) Fifty per cent inhibition of total root length in plants irradiated only is close to 8,000 r. Root growth of plants centrifuged at 150 G is reduced to 30 per cent of control root length. The radiation dose necessary to reduce roots in centrifuged plants by a further 50 per cent lies near 15,000 r, or close to twice the dosage required for a similar inhibition in plants irradiated only.

Thus, although increased radiation dose affects the total growth of both coleoptile and root, the responses are not parallel. It will be noted, also, that while a decrease in height or root length appears to be proportional to the dose administered between 2,400 r and 20,000 r, above 40,000 r and even at 160,000 r there is no increased effect. This phenomenon has been noted by others following irradiation of wheat and other cereals (Sicard and Schwartz, 1959, and others).

The manner in which these effects are produced within the seedling are being investigated with histochemical tests and histological studies.

Remembering that the cell division phase of seedling growth occurs before it is 8mm high (Avery and Engel, 1954) and that the greatest effect on the seedlings was produced when they were irradiated at 24 hours (approximately 1.5 mm tall) the mitotic process of the seedlings was undoubtedly affected. Cell elongation in plants is less affected by radiation, and may account for continued upward growth after lethal doses, those cells present at the beginning having simply elongated. Moutschen (1958) felt that cell elongation may even increase with radiation dose, and that a growth factor may even be stimulated by radiation. Quastler et al (1952) found that the mean final root length in bean seedlings is a constant, and that inhibition of linear growth was poorly correlated with radiation dose.

Nothing is known of the possible radiation effects producing malignancy or mutations. Had our seedlings grown to maturity instead of for a few days, they might have been observed. It is interesting to note, however, that Bozzini and others (1962) in Caldecott's laboratory noted an inverse relation between seedling height and genetic injury in barley following X-radiation.

Of more importance than the radiation effects on actual linear growth of the seedling parts, however, is the apparent modification of radiation effects on seedlings grown at higher than normal gravity. It is obvious that the irradiated seeds grown at 150 X G suffered less from comparable radiation doses than seeds similarly irradiated, but grown at normal gravity.

This alteration of radiation sensitivity by centrifugation has little background in the literature. Sax (1943) found no increase in the number of aberrations caused by radiation at 400 X G, and variable results were reported by Wolff and von Borstel (1954). Yeargers (1962) found fewer aberrations from a dose of 400 r on Tradescantia inflorescences irradiated and centrifuged from 5,000 to 10,000 X G, and a greater number of chromosome breaks from the same dose at lower G forces.

The decreased response to radiation found in our centrifuged wheat seedlings remains to be explained. Certainly the implied synergism between gravitational forces and radiation effects makes it imperative that the effects of similar doses of radiation be studied under conditions of less than normal gravity. We hope that this aspect of our experiment may be included in a later Biosatellite flight.

V - SUMMARY AND CONCLUSIONS

There are many difficulties in assessing the separate roles of factors which regulate a process as complex as growth in plants. The manufacture and distribution of auxins and other growth substances, as well as the metabolic rate of the tissues on which they act, create the environment which in turn responds to external factors such as gravity, temperature and radiation. Stimulation of growth may result from changes in these physical factors. Inhibition of growth, on the other hand, may result from alterations in the rate of other life-sustaining processes at the expense of growth processes, as suggested by Gaunt (1954). In such a manner centrifugation may influence growth after radiation of the wheat seedlings in our experiments. This may similarly explain why the early high growth rate of wheat seedlings grown at high temperatures is not sustained.

As is not unusual with living organisms, the conditions for optimal growth are also the conditions at which the greatest inhibition of growth may be produced by a single unfavorable environmental factor. In the present experiments, growth at the optimal 25° C. is greatly affected by centrifugation at 150 X G. Interestingly, the centrifugation appears to be unfavorable to growth only at the optimal temperature and above. At the lowest temperature used (21° C.) the initial effect of 150 X G was to stimulate growth in all organs except the primary root.

Because gravity in excess of normal produces the effects we have described on the normal growth of wheat seedlings, it is probable that

decreased gravitational forces will show effects somewhat consistent with those of forces above normal. When environmental gravity becomes zero, or close to zero, the results may be very different because gravity has a qualitative effect on plant orientation as well as the quantitative effect with which the present experiments are concerned. It is this qualitative effect of gravity that the Biosatellite experiments are uniquely designed to evaluate.

PUBLICATIONS

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Edwards, B. F. and Stephen W. Gray, April, 1964
Opportunity for studying the effects of weightlessness. Ga. Acad. of Sci. XXII:5 (Abstract)

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Appendix I

METHOD OF MAKING PROTOTYPE SEED HOLDERS

We have developed a stalk for a 12-seed holder which may be placed in a growing chamber of appropriate size. You will note that we have achieved the 45 degree angle for the seed holder as required by Dr. Lyon. Since we modified the needle cover by beveling it on the sander in our shop, the growing coleoptiles have not touched the holders.

These seed holders were devised by R. Whipple and C. Wickliffe, our summer medical students. Their stepwise procedure for making them is as follows:

1. Bevel top edge of each needle cover from disposable needle (B.D. Yale #20, 1½" long).
2. Place needle cover upright in vise.
3. Place square (approx. 1") of rubber on top (see Fig. A). (Cut from sterilized rubber glove. Glove is better than dental dam.)
4. After placing a #0 orthodontic rubber band <u>doubled</u> over the end of long tweezers, slip it over the end of a #5 cork borer, then gently place cork borer over top of needle cover (see Fig. A). Slip band from cork borer over collar of needle cover.

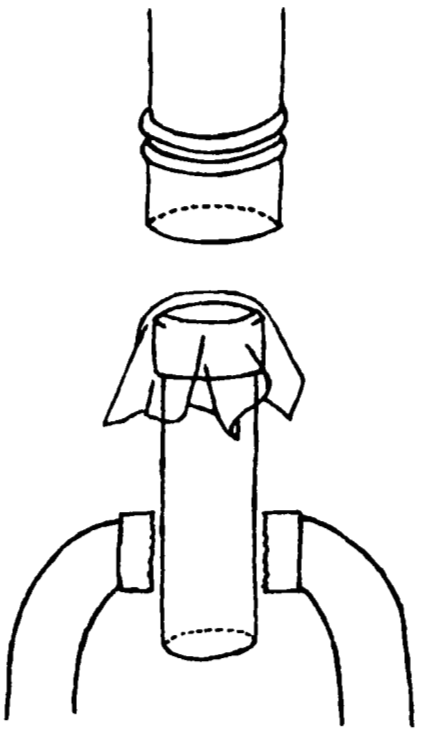

Figure A

5. Trim excess rubber below rubber band, as in B.

6. Cut needle cover (now a seed holder) at 45 degree angle, as indicated in Fig. B.

Figure B

7. Glue seed holder to stalk with small amount of Eastman 910 adhesive applied with wooden applicator stick, making sure that holder does not project into the lumen of the stalk.

8. Pour vermiculite into stalk with attached seed holders, ramming it down with narrow probe or wooden applicator stick.

9. Add water carefully, being sure to remove air bubbles.

10. Make small hole in rubber diaphragm near upper edge with needle point, and enlarge with one point of fine tipped forceps.

11. Place seed in holder with seed oriented so that axis of embryo is in line with the stalk and root end is downward.

12. Add water as necessary so that back of each seed is covered with a film of water, and no air bubbles remain within the stalk or the individual holders to keep water from circulating freely within the system.

Appendix II

COMPOSITION OF FIXATIVES

(As Listed in Table 1)

1.	Ethyl chloride	
2.	Farmer's:	
	Absolute alcohol	75 parts
	Acetic acid	25 parts
3.	Mercuric chloride	95 parts
	Acetic acid	5 parts
4.	Newcomer's:	
	Isopropyl alcohol	6 parts
	Propionic acid	3 parts
	Ether	1 part
	Acetone	1 part
	Dioxane	1 part
5.	Mercuric chloride	75 parts
	Acetic acid	5 parts
	Glutaraldehyde	40 parts
6.	Mercuric chloride	90 parts
	Formalin (40% formaldehyde)	10 parts
7.	Mercuric chloride	75 parts
	Absolute alcohol	25 parts
	Acetic acid	5 parts
	Glutaraldehyde	20 parts
8.	Bouin's:	
	Picric acid (saturated aqueous sol.)	75 parts
	Formalin	25 parts
	Glacial acetic	5 parts

9. CRAF:

Solution A:	
Chromium trioxide	1.0 cc
Glacial acetic	7.0 cc
Distilled water	92.0 cc
Solution B:	
Formalin (40% formaldehyde)	30.0 cc
Distilled water	70.0 cc

Mix solutions ½ and ½ just before fixing.

10. Carnoy's:

Absolute alcohol	60 parts
Chloroform	30 parts
Acetic acid	10 parts

11. FAA:

Formalin (40% formaldehyde)	10 parts
Glacial acetic acid	7 parts
95% Ethanol	50 parts
Distilled water	33 parts
Add: Dimethyl sulfoxide	0.2 parts

12. Wood's:

Mercuric chloride (saturated aqueous sol.)	60.0 cc
Absolute alcohol	40.0 cc
Acetic acid	5.0 cc
Add: Dimethyl sulfoxide	0.2 cc